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REVIEW ARTICLE

Recent Advancements in Green Synthesis of Nanoparticles for Improvement of Bioactivities: A Review

Vinay Deep Punetha[1], Sunil Dhali[1], Anita Rana[1], Neha Karki[1], Himani Tiwari[1], Pushpa Negi[2], Souvik Basak[3,*] and Nanda Gopal Sahoo[1,*]

[1]*Department of Chemistry, Prof. Rajendra Singh Nanoscience and Nanotechnology Centre, DSB Campus, Kumaun University, Nainital, Uttarakhand, India;* [2]*Department of Chemistry, Graphic Era Hill University, Bhimtal Campus, Nainital, Uttarakhand, India;* [3]*Dr. B.C. Roy College of Pharmacy & Allied Health Sciences, Durgapur, WB, India*

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Abstract: Natural products have widely been used in applications ranging from antibacterial, antiviral, antifungal, and various other medicinal applications. The use of these natural products was recognized way before the establishment of basic chemistry behind the disease and the chemistry of plant metabolites. After the establishment of plant chemistry, various new horizons evolved, and the application of natural products breached the orthodox limitations. In one such interdisciplinary area, the use of plant materials in the synthesis of nanoparticles (NPs) has exponentially emerged. This advancement has offered various environment-friendly methods where hazardous chemicals are completely replaced by natural products in the sophisticated and hectic synthesis processes. This review is an attempt to understand the mechanism of metal nano particles synthesis using plant materials. It includes details on the role of the plant's secondary metabolites in the synthesis of nanoparticles including the mechanism of action. In addition, the use of these nanomaterials has widely been discussed along with the possible mechanism behind their antimicrobial and catalytic action.

Keywords: NPs, green synthesis, natural products, secondary metabolites, antimicrobial, catalyst.

1. INTRODUCTION

After the evolution of nanoscience and nanotechnology, various interdisciplinary areas of research have emerged. Synthesis of Nanoparticles (NPs) and their applications in various areas have largely been investigated. The most crucial aspect of these NPs is their size, which reflects in the quantum size effect and large surface energies. This makes control of the size of these particles imperative for better exploitation [1]. NPs exhibit entirely different properties when their size is controlled with desired measures. Restricting the size of NPs within certain limits changes many properties like lowering of melting point, imparting unique optical properties, high catalytic activity, and unusual mechanical properties. These nanoparticles have been employed in various applications ranging from energy conversion and storage to biomedical applications. The most researched areas are their antimicrobial potential, as catalysts in chemical reactions, antifungal, antioxidant, anticancer activities, and drug delivery [2-11].

Nanotechnology deals with the manufacture of novel materials having a range between 1 and 100 nm. Various physical and chemical methods have been used for the synthesis of various nanoparticles having attractive shapes [12-14]. In recent years, methods of NPs synthesis have shaped themselves with utmost perfection. Various green technologies were introduced, and the fundamentals of green chemistry have immensely influenced scientists in finding out green and eco-friendly routes for the synthesis of these NPs. The superiority of green synthesis can explicitly be elucidated as it includes simple procedures and suitable plant materials in comparison to that of the earlier used sophisticated instrumentations, hectic separation procedures, and the use of toxic chemicals. In other techniques where NPs are synthesized by applying force, in order to break them into smaller particles requires more energy. These energy-consuming techniques are again inextricably associated with irregularity in the shape of the NPs. In general, it has been observed that NPs synthesized by applying these forces are of different morphologies and sizes which immediately affect and inhibit the potential application of these NPs in various applications [15]. Green reducing agents in plants (flavonoids, sugars, *etc.*) have eliminated hazardous reducing agents such as $NaBH_4$, elemental hydrogen, DMF from the hazardous synthesis procedure of NPs synthesis. In addition to this, in most of the green methods, no additional stabilizing agents are required, as hydroxyl group containing natural product moieties could stabilize these NPs. Using the natural reducing agent and solventless synthetic processes in place of hazardous reducing agents makes these greener techniques more cost-effective and efficient in comparison to that of earlier methods.

*Address correspondence to these authors at the Department of Chemistry, Prof. Rajendra Singh Nanoscience and Nanotechnology Centre, DSB Campus, Kumaun University, Nainital, Uttarakhand, India and Dr. B.C. Roy College of Pharmacy & Allied Health Sciences, Durgapur, WB, India;
E-mail: ngsahoo@yahoo.co.in; souvik_basak1@yahoo.com

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Simplicity in the preparation methods through green techniques makes these nanoparticles more commercially viable and turn out to be cost-effective than previously used techniques. In addition, some of the workers have reported the use of extremely invasive terrestrial plants for the synthesis. Aqueous extracts of one such weed were found to reduce the metal ions of Ag and Au cations. In addition, this weed plant was reported to have an exceptionally high capacity to stabilize these nanoparticles [16]. The core objective of these green approaches is to produce moieties with particles in the size range of approximately 1 to 100 nm. The green techniques are reported to be very consistent in the production of NPs with altered characteristics in comparison to that of their macroaggregates. Kumarasamyraja *et al.,* reported AgNPs with even less than 1 nm size (0.5nm) by taking *Acalypha indica* plant material as a reducing agent. The AgNPs synthesized showed exceptional antimicrobial activity against tested pathogens [17]. The NPs obtained using green techniques are reported to be of different shapes such as triangles, pentagons, hexagons, spherical, triangular, quasilinear, *etc*. In addition to the plant materials, there are many other natural resources such as fungi, algae, *etc.*, used along with plant extracts for the synthesis of NPs. Honey is also another natural product that has been reported in recent papers to efficiently catalyse NPs synthesis through relevant activities (details are given in a subsequent section). Though in some of the cases, the use of biological natural resources turned out to be efficient, the use of plant material is favoured over these other resources due to ease in handling and availability [18]. However, the green synthesis of NPs using their bio activities can reduce many effects of physical and chemical techniques. The green synthesis of NPs includes the biosynthesis of NPs at optimum pH, pressure, and temperature and does not involve any toxic or hazardous [19-22]. The following segment of the review paper highlights recent reports on this developing horizon of natural products and NPs along with the possible role of secondary metabolites in the synthesis.

2. GREEN SYNTHESIS AND ROLE OF PLANT METABOLITES

The use of plant materials for the synthesis of the NPs has been adopted by a wider community of scientists, as nature has blessed plants with the capacity to produce bioactive secondary metabolites. A secondary metabolite is a unique class of organic compounds found in plant species. These organic compounds are not crucial for the survival of plants unlike primary metabolites like glucose, nucleic acids, proteins, *etc.* [23]. These secondary metabolites cover a rich source of functional moieties such as hydrocarbons, aldehydes, ketones, alcohols, esters, ethers, lactones, oxides, and peroxides. In principle, these secondary metabolites have been categorized into three parts. They are flavonoids which include phenolic and polyphenolic compounds; terpenoids, the major constituent of the volatiles present in the aromatic plants, especially in the essential oil, and nitrogen-containing alkaloids. Flavonoids and hydroxyl groups, containing natural moieties could play a vital role in the green synthesis of the NPs. These hydroxyl groups are suggested as being excellent for metal complexation [16, 24, 25]. As mentioned earlier, the use of *A. indica* plant extract is reported to produce NPs even less than 1 nm size (0.5nm). The role of flavonoids and hydroxyl groups, containing natural products in this is obvious by screening its phytochemical profile. Aqueous and ethanolic extracts of *A. indica* leaves confirms the occurrence of saponins, flavonoids, terpenoids, and cardiac glycosides in excess [26]. The plant-derived metabolites like flavonoids, quinones, carotenoids, sterols, phenolics, and anthocyanins make it best for the biosynthesis of NPs [27-29].

The process of the complex formation plays an essential role in the development of the NPs, by offering active sites for metal ions. The complexation process also assists in controlling the size of the metal ions. [30-34]. Flavonoids and carotenoids have previously been documented as potential reducing moieties and are found actively engaged in the electron transfer in order to quench various hazardous free radical species such as superoxide peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), and hydroxyl ($OH^{\bullet}$) moieties, formed during the process of the photosynthesis [35-36]. Along with this, there are strong shreds of evidence that these species are actively engaged in the chelation of metal ions in order to stabilize and reduce their catalytic activity [37]. Previous reports suggest that both the tendency of chelation and reducing capacity support the role of flavonoids in the formation of NPs. Tendency to chelate metal ion breaches cluster of metal ions into a smaller nanosized group of atoms or ions, depending upon the precursor used in the process of NPs synthesis (Fig. **1**). As the reduction potential of most of the flavonoids falls in the range of 0.2 to 0.8 V [38] they can efficiently reduce the number of metal ions from their radical or cationic state to neutral NPs. Among various flavonoids, quercetin has shown the best electron-donation capacity. The favourable electron-donating properties originate from the electron donating 0-3 hydroxyl groups in the C ring, which is conjugated to the catechol (B ring) ring through the 2,3-double bond [39]. Along with secondary metabolites, primary metabolites like starch could also act as a natural reducing agent.

For the very first time, pomegranate seeds molasses as a green capping fuel was used for a novel green/facile synthesis of spongy defective zinc oxide nanoparticles (ZnONPs) [40]. Natural carbohydrate polymeric reducing agents have frequently been investigated for the synthesis of many inorganic NPs [1, 41]. The polymeric chains of starch possess a large number of hydroxyl groups, which are excellent for metal complexation. These hydroxyl groups offer active sites for metal ions enabling good control of the size, shape, and dispersion of the formed metallic NPs [24, 25, 33, 34]. These metabolites may interact in the synthesis process without using them as an isolate. The concentration of reducing plant material used in green synthesis affects the morphology and size of the NPs [42]. The size of these NPs plays an important role in deciding the catalytic properties of these nanoparticles. To avoid environmentally hazardous reducing agents, currently the synthesis of silver nanoparticles *via* green synthesis works as a capping as well as reducing agents [43]. As the concentration of reducing plant material increases, the size of nanoparticles is expected to get reduced. Kim *et al.,* studied the effect of the concentration of reducing agent (caffeic acid) on the morphology of Au NPs and observed that as the concentration of caffeic acid increases, the sizes of AuNPs were decreased due to the adsorption and stabilizing effect of oxidized caffeic acids [42]. Some of the plants species have been reported with extra stabilizing effect of NPs obviating the use

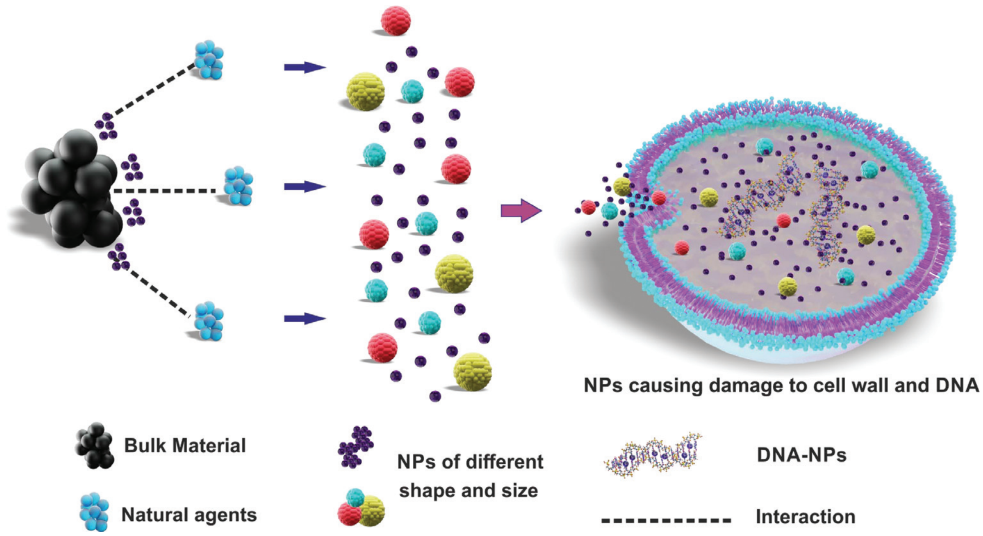


Fig. (1). Mechanism of nanoparticle synthesis and antimicrobial activity. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

of extra capping agents to maintain the stability of the synthesized NPs [43].

On the whole, the procedure of green synthesis methods can be summarised in two simple steps, the first step is to prepare the extract of the effective part of the plant. The selection is based on the phytochemical screening of the plant material. Parts with a high yield of flavonoids or hydroxyl terpenes are favoured owing to their tremendous capacity to reduce the metal ions and stabilize the NPs. In addition, the selection of plants is also based on cost-effectiveness, natural abundance, chemical profile, and medicinal properties of the plant material. The collection of plant material is followed by cleaning it. To extract essential phytochemical from the plant material, fine particles of plant material are left in double-distilled water for a few hours, and then the extract can be prepared by boiling it for a few hours. The time required to boil the plant material depends upon the solubility of the required phytochemicals in water. The extract is cooled down and filtered, Whatman filter paper no.1 is in general used for the filtration, and the extract can be stored for further use. The extract can now be used in the second step of synthesis where metal ions could be reduced and capped by it. In general, salts of metals are used as a precursor for the synthesis of nanomaterials. Small concentration solutions of these salts are prepared in the water such as 1, 2, 3, 4, and 5 mL. In order to optimize the process, mixing is done by two processes. The first is to keep the concentration of salt solution constant and to add different concentrations of the plant extract and *vice versa*. This procedure can be repeated for confirmation or to optimize the process further. The reduction of M^{n+} to M can be confirmed by a change in the colour of the solution. The preliminary evidence for the synthesis of NPs comes from the UV absorbance of the NPs. The particle size and surface morphology can later be analyzed by using Transmission Electron Microscopy (TEM), and other techniques.

In recent times, various nanoparticles systems have been synthesized by using plant materials. Natural precursor tea leaves and ground coffee were also used for the successful synthesis of Silver deposited titanate nanotube array composite (Ag/TNA-c). The synthesis method from natural herbs was effective, eco-friendly for the synthesis of nano-composite [44].

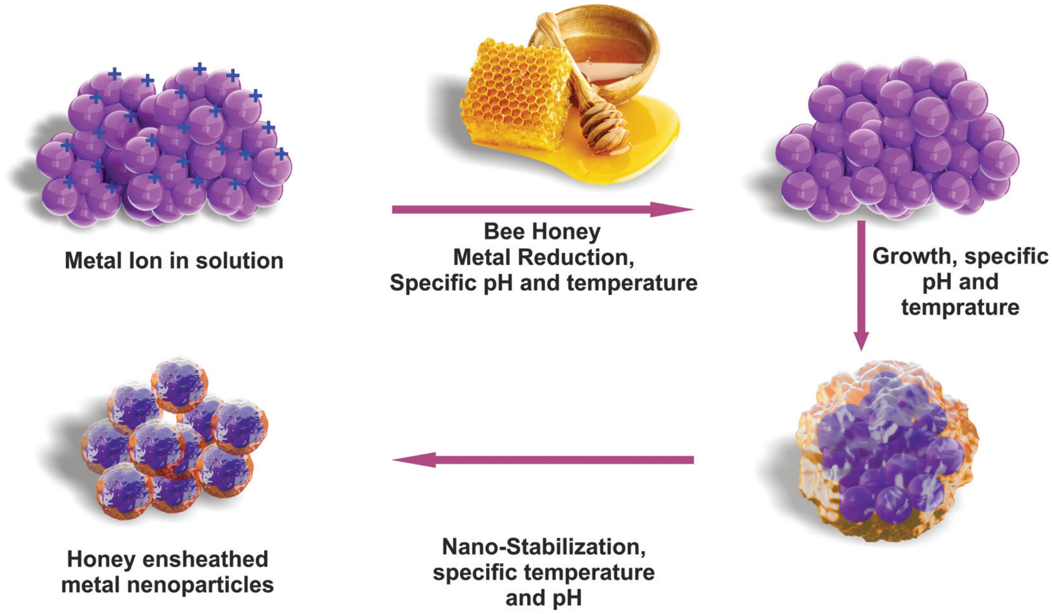
The most common among them are Ag NPs. Along with Ag nanoparticles, other synthesized NPs are AuNPs, ZnO NPs, Bimetallic Ag/Au NPs, AgClNPs, MnO_2NPs, Lanthanum NPs, CuNPs, PtNPs, *etc*. Shaikh *et al.,* synthesized Copper nanoparticles (CuNPs) using *Vitis vinifera* leaf extract. The X-Ray diffraction analysis reveal the synthesis of highly crystalline CuNPs with an average of 3-6 nm [45]. Synthesis of NPs is not only restricted to d- block elements. Chatterjee *et al.* [46] prepared lanthanum NPs using the extract of *Vigna radiata* beans [46]. Afshar *et al*. [47], synthesized silver NPs at ambient temperature using water extract of *Satureja hortensis* L. with an average size of 15 ± 7.402 nm and spherical in shape [47]. Rao *et al.* [48] synthesized various structural analogues (nanoflowers, nanorods) of ZnO by using algae *Chlamydomonas reinhardtii.* The nanorods measured 330 nm in length and while nanoflowers were measured approximately 4 µm [48]. Krishnaraj *et al.,* [49] developed a method of Silver NPs (AgNPs), manganese dioxide NPs (MnO_2NPs), and silver-doped manganese dioxide NPs (Ag-doped MnO_2NPs) simultaneously by using plant extract of *Cucurbita pepo*. The simultaneous synthesis turned out to be highly successful as the size reported of NPs was in the range of 15-70 nm. Bimetallic Ag/Au NPs (BNPs) were prepared successfully by using *Antigonon leptopus* a flavonoids rich plant species. The size of BNPs was found to be in the range of 10 to 60 nm. (13) Sushma *et al.,* reported the synthesis of zinc oxide NPs [ZnO NPs] by using *Ocimum tenuiflorum* leave extract [50]. Altaf *et al.,* reported the synthesis of AuNPs by using leaf extract of *Aloe arborescens*. The process of AuNP synthesis turns out to be cost-effective with no extra requirement of any of the capping agents [51]. Recently, the demand of herbs has been increased significantly in the agriculture and food industry. There are new greener biological methods for the synthesis of different varieties of nanoparticles. The development of magnesium oxide (MgO) nano-flowers from plant extract is the best example of it [52]. Recent literature on the NPs synthesis has been tabulated in Table **1**.

Table`1. Various plant species used for the synthesis of NPs.

S. No.	NPs	Plant Species	Applications	Reference
1	AuNPs	*Aloe arborescens* leaf extract	-	[29]
2	ZnO NPs	*Ocimum tenuiflorum*	Antioxidant activity	[35]
3	Bimetallic Ag/Au NPs	*Antigonon leptopus*	-	[13]
4	AgNPs	*Melon aqueous* extract	Insecticidal activity	[36]
5	AgNPs	Bacterial culture media	-	[69]
6	AgNPs	Using root extract of *Chelidonium majus*	Antibacterial	[42]
7	AgNPs	*Ficus carica*	-	[70]
8	AgNPs, AgClNPs	*Solidago altissima*	-	[58]
9	AgNPs	*Vigna radiata*	-	[64]
10	AgNPs, MnO_2NPs, Ag-doped MnO_2NPs	*Cucurbita pepo*	Antibacterial activity	[34]
11	AgNPs	*Bauhinia variegata*	Insecticidal activity	[71]
12	Ag NPs	*Satureja hortensis* L	Antibacterial properties	[32]
13	AgNPs	*Bacillus thuringiensis*	Antibacterial activity	[72]
14	Lanthanum NPs	*Vigna radiata*	Antioxidant	[31]
15	CuNPs	*Vitis vinifera*	Antimicrobial	[30]
16	AgNPs	*Dioscorea alata*	Antibacterial activities and optical limiting behavior	[73]
17	AgNPs	*Erigeron bonariensis*	Catalytic activity	[63]
18	AgNPs	*Salvinia molesta*	Antimicrobial efficacy	[40]
19	AuNPs, AgNPs	*Parkia roxburghii*	Antimicrobial efficacy	[8]
20	AuNPs	*Nepenthes khasiana*	Biocompatibility studies	[74]
21	AgNPs	*Rubus glaucus*	Cytotoxicity on Hepatic cancer (Hep-G2) cell line and their antioxidant activity:	[75]
22	AgNPs	*Terminalia cuneata*	Catalytic action	[62]
23	Ag NPs	*Diplazium esculentum*	Photocatalytic and anticoagulative activities	[68]
24	AgNPs	*Azadirachta indica*	Antibacterial activities	[76]
25	ZnONPs	*Cassia fistula*	Photodegradative, antioxidant and antibacterial activities	[59]
26	Pt NPs	*Azadirachta indica*	-	[77]
27	AuNPs	*Genipa americana*	Antioxidant efficacy	[78]

3. GREEN SYNTHESIS OF NANOPARTICLES USING HONEY

In recent years, green synthesis mediated nanoparticles have been taking place using local honey. The honey, although varies from source to source, is reported to have fructose and glucose as major sugars (maltose, sucrose, isomaltose, turanose, nigerose, melibiose, panose, maltotriose, and melezitose as other sugars); 0.1%-0.5% proteins; vitamins such as C, B1 (thiamine) and B2 complex involving riboflavin and nicotinic acid; flavonoids; phenolics; and various bioactive compounds such as ascorbic acid, tocopherols, catalase (CAT), superoxide dismutase (SOD) together with reduced glutathione (GSH) as antioxidant enzymes. Also minerals such as copper, manganese, iron, zinc, calcium, and sulphur



Scheme (1). Fabrication of silver nanoparticles from metallic silver with honey. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

are found in honey, whereas enzymes such as invertase, diastase, and glucose oxidase are major bioactive constituents of honey [53-59].

Several metal nanoparticles have been formed by using honey. For example, gold (Au) nanoparticles have been formed by reducing $HAuCl_4$ with honey where fructose has been proposed as a possible reducing agent and protein as a stabilizer [60]. Nanoparticles in the size range of 6.2 nm to 13.6 nm have been successfully manufactured by this method [61] and showed antibacterial activity against *S. aureus* infection.

Silver (Ag) nanoparticle has been synthesized successfully using honey varying the concentration and pH of the solution (Scheme **1**). NPs as small as 4 nm have been synthesized using a metallic solution of silver at pH 8.5 [62]. Instrumental analysis has revealed that crystallographically different silver nanoparticles have been synthesized with face-centred cubes such as {111}, {200}, {220}, {311} and {222} cubes [63]. The antimicrobial potential is also one key advantage of these silver nanoparticles [64].

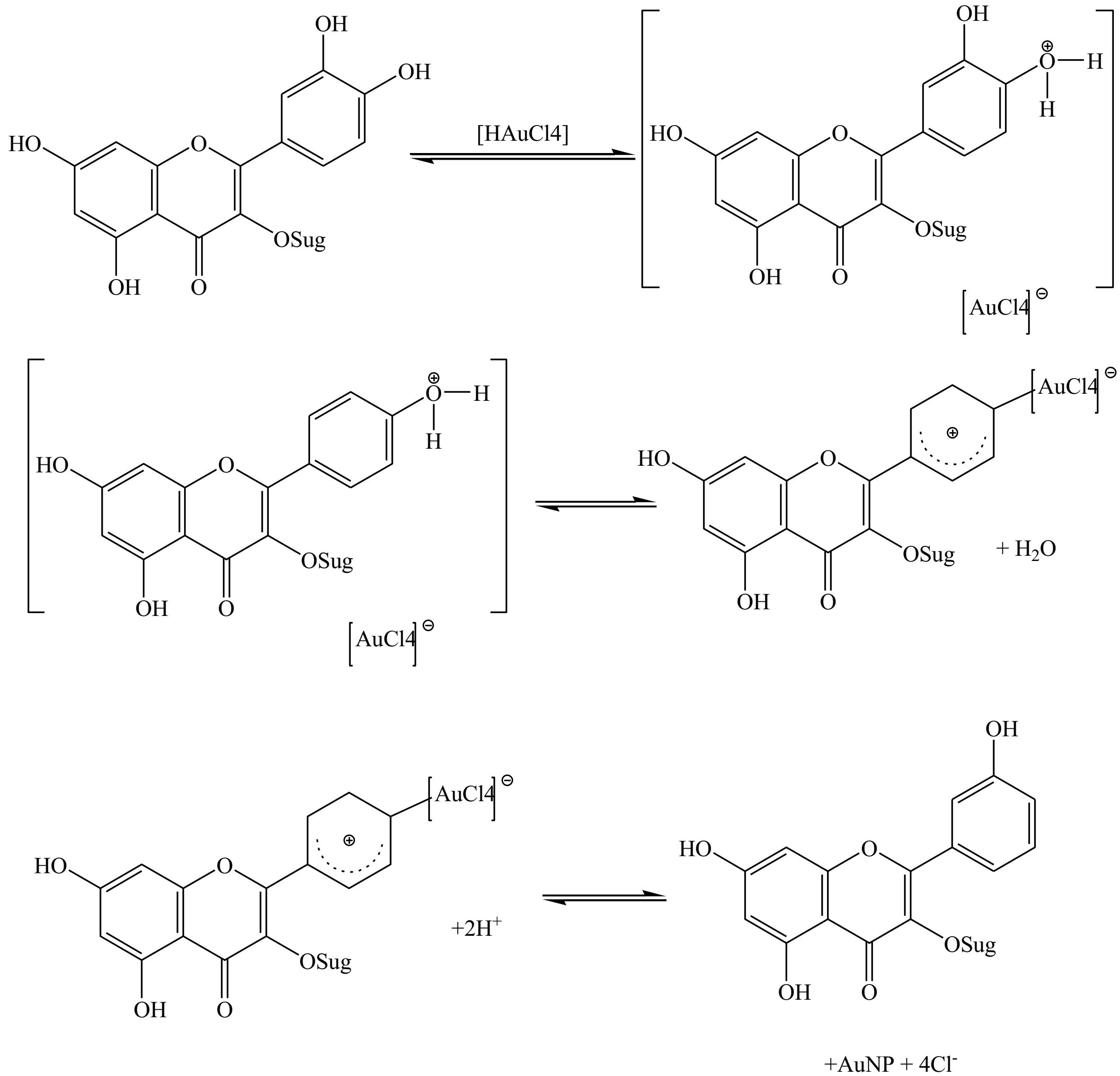
Palladium (Pd) and carbon (C) nanoparticles have also been manufactured with the help of honey. Especially Pd nanoparticles, which have been of a highest stability, from 5-40 nm have been synthesized using honey as a catalyst [65]. Interestingly, honey was used as a precursor for synthesizing carbon nanoparticles. For example, surface coated polysorbate and polyethylene glycol C nanoparticles have been synthesized from honey and were of smaller sizes than the previous genre of carbon nanoparticles [66]. In very precisely con trolled honey-mediated C nanoparticles, nano-systems as small as 2 nm have been fabricated. The C-nanoparticles have been used for sensor application, fluorescence emission, and bioimaging probes in various fields of biomedical instrumentation [67]. These green synthesize nanoparticles have great importance in various fields such as magnetic devices, photocatalysts, microe-lec-tronic devices, anticorrosive coatings and biomedical [68-72].

4. MECHANISM OF GREEN SYNTHESIS OF NANOPARTICLES

Taking into account, nowadays researchers showed great interest in the synthesis of metal and metal oxides NPs employing bio-genic route that utilized aqueous plant extract and microbes as they are environment-friendly, stable, clinically adaptable, bio-compatible, and cost-effective [73]. The mechanism of nanoparticle synthesis by the green method, especially by plant and plant compounds has been discussed by various authors. For example, Borodina and Mirgorod, 2014 [74], proposed a mechanism for Au-nanoparticle (Au-NP) synthesis. They proposed that Au-nanoparticles are mainly formed due to the reduction of Au^{+2} ions from $AuCl_4$ by flavonoids such as Rutin (Scheme **2**). Herein, rutin acts as a base that forms salt with acidic $HAuCl_4$. In addition, the acid forms a charge-transfer complex with rutin which on decomposition forms Au-NPs [74], (Scheme **2**).

In the case of Ag-nanoparticles, Ag is reduced by -OH groups on 3' and 4' carbon atoms of the flavonoid, where the flavonoid acts as an acid. The -OH groups subsequently are converted to keto groups and forms initially a charge-transfer complex of < 1nm. Later it gets stabilized and forms stable Ag-nanoparticles of size ~1 nm [75].

For C-nanoparticles (CNPs), the precursor carbohydrate hydrolyses to yield glucose which on isomerization forms fructose. The fructose on dehydration converts to hydroxyl methylfurfural, which further undergoes polymerization to form condensed or polyfuronic polymer (Scheme **3**). The condensation finally leads to a supersaturation point, initiates the nuclear de-aggregation, finally resulting in the formation of CNPs [76].

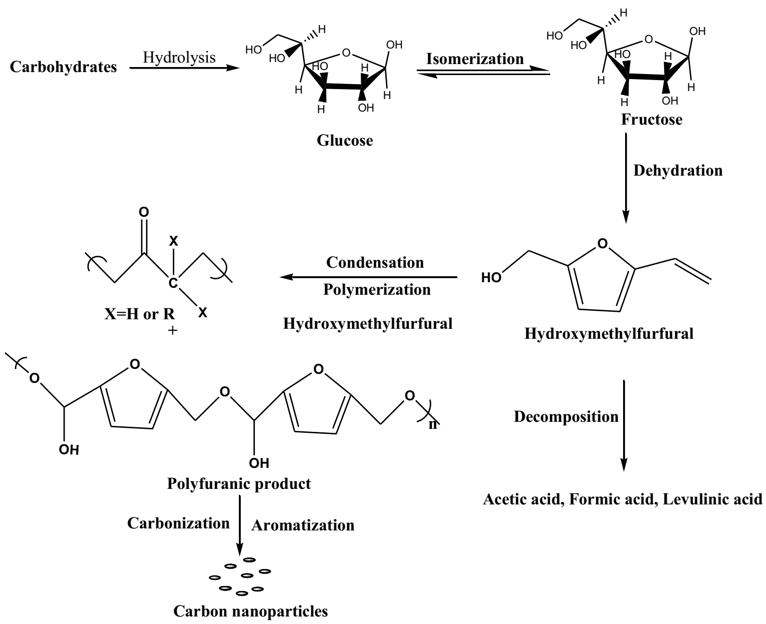


Scheme (2). Mechanism of synthesis of Gold nanoparticles from $HAuCl_4$ [58].

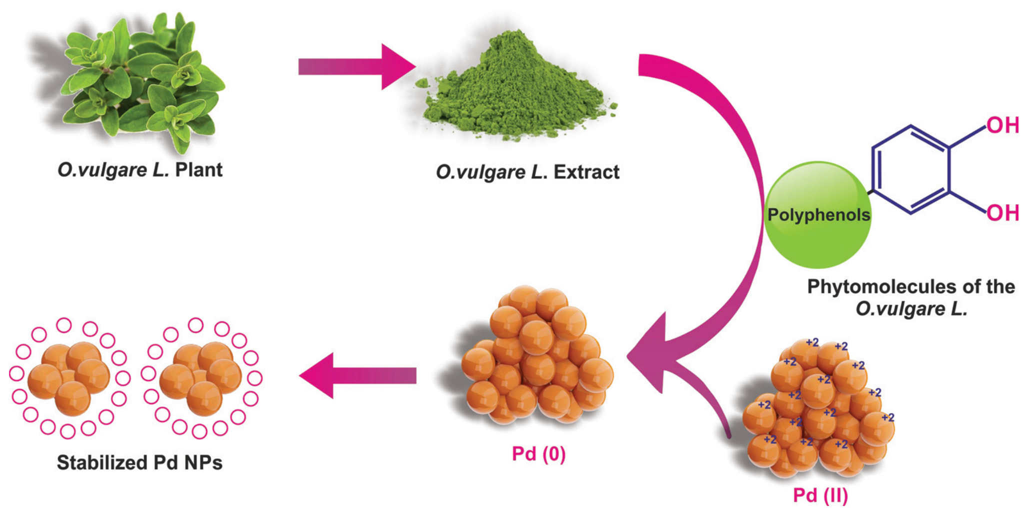
For Pd-NPs, the reaction mechanism for synthesis has been proposed by two possible pathways. First, for the synthesis of single Pd^{+2} nanoparticles, plants are used having polyphenol compounds that reduce bivalent Pd^{+2} into Pd (0) NP (Scheme **4**), [77]. In the second pathway, Pd-nanocomposite is synthesized over a graphene oxide-Fe_3O_4 system. In this mechanism, first Graphene Oxide (GO) is used as a nano-carrier where -COOH group is chlorinated to yield -COCl. The resulting -COCl is then condensed with a 1, 4-phenylenediamine to produce an amide derivative with a free -NH_2 terminal. The resulting complex was further condensed with pyridine-2-aldehyde to yield a corresponding imine derivative. The resulting complex when exposed with $PdCl_2$ solution, the Pd^{+2} forms a coordination complex with two N-donor ligands on the imine complex [78]. Subsequent treatment with Fe_3O_4 converts it to a nanoscale resulting in the formation of Pd-Fe_3O_4-GO nanocomposite (Scheme **5**). The Zinc Oxide NPs are also formed by first reduction and subsequent oxidation of $Zn(NO_3)_2$ solution by plant flavonols such as L-ascorbic acid, chlorogenic acid, and quercetin [79], (Scheme **6**).

5. APPLICATIONS OF NPS SYNTHESIZED BY GREENER ROUTES

As quantum confinement of particles imparts marked differences in the properties of a material at the nano-level in comparison to that of properties exhibited by the same material in bulk. These altered properties of the nanomaterials could well be exploited in various applications. Most of the nanoparticles are found to have antibacterial activity and have been used most widely in the health industry, food storage, textile coatings, and several environmental applications. In addition, the unique band gap in some of the nanoparticles could further be exploited in the development of cost-effective and sufficient energy storing and conversion devices. Applications of NPs in the field of biotechnology enlarged because of their promising properties like biocompatibility, antimicrobial and anti-inflammatory activities, and targeted drug delivery [15]. The next segment of the review paper is oriented toward the interaction of NPs with the microorganisms in order to understand the basic mechanism involved in the superior activity of the NPs against them.



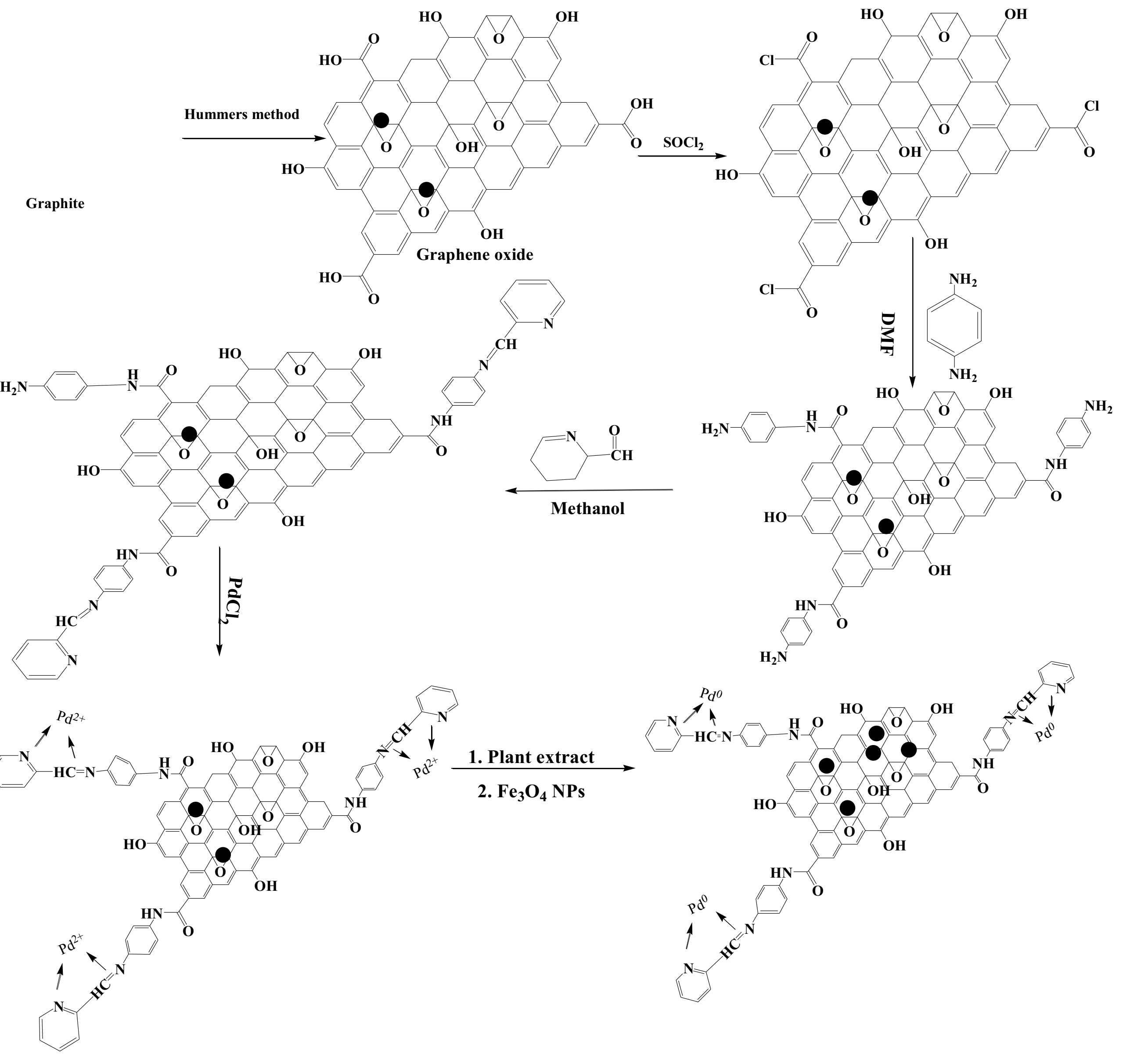
Scheme (3). Mechanism of carbon nanoparticle synthesis by green route. Reprinted with permission from [58].



Scheme (4). Synthesis of Palladium nanoparticles from plant. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

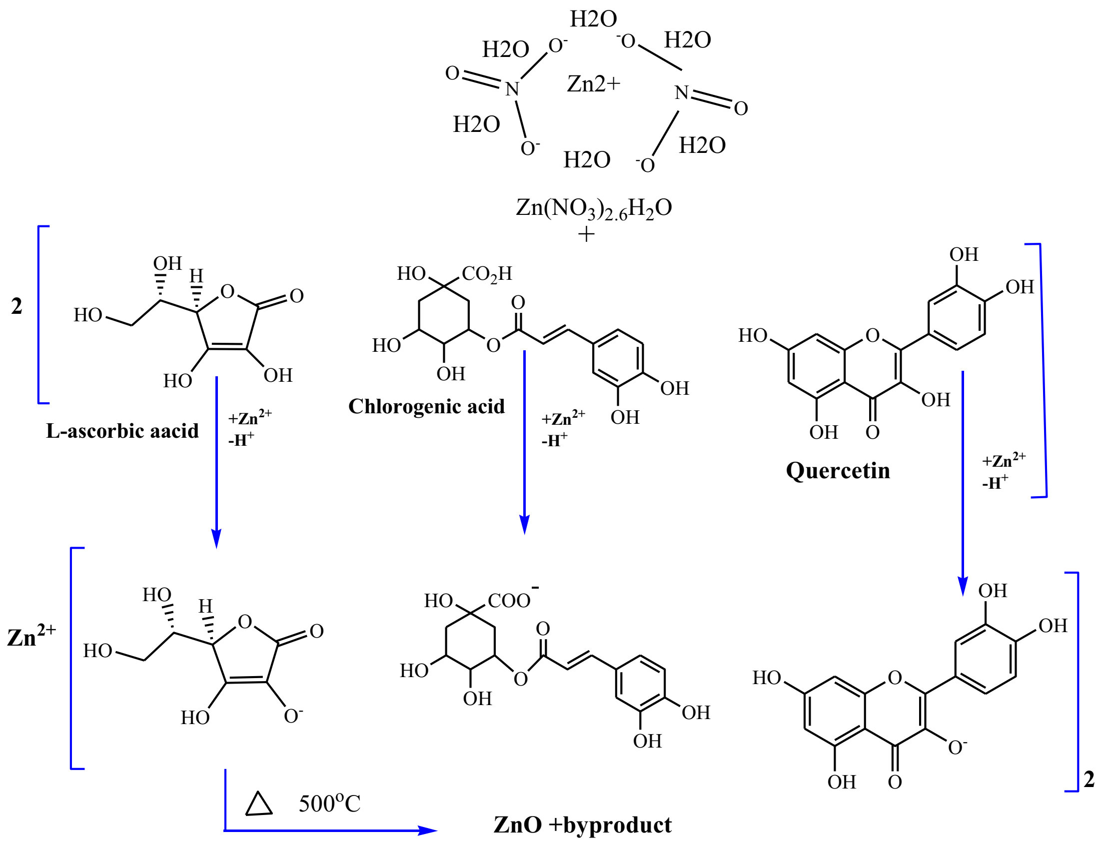
5.1. Antibacterial Activity

Antibacterial agents are of immense importance in various industries such as textile, food, medicine, *etc*. Various antimicrobial agents have been investigated in recent times. The main objective is to discover or invent a cost-effective and target-specific antibacterial agent. In the past few years, various metal and metal oxide NPs have been investigated in order to evaluate their antibacterial potential. This section of the review has been dedicated to summarising recent advancements in the search of antibacterial NPs and their future aspects. Various mechanisms have been proposed to resolve the mechanism and details of the action of NPs toward the bacterial cell owing to their great potential against the bacterial cultures. Several gram-positive and gram-negative cultures have been taken to evaluate the potential of NPs against them such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and, Enterococcus faecalis, etc*. The action of NPs on the bacteria is difficult to generalize as different types of bacterial cultures behave differently to the NPs. Tolerance of *E. coli, S. aureus,* and *B. subtilis* when evaluated against CuO NPs; *E. coli* was found to be more susceptible than the other two, but the effect of AgNPs against the same bacterial culture was more on the *E. coli* in comparison to the other two [80]. Different metal NPs have been proposed with different mechanisms for their action toward the bacterial cultures. These mechanisms are proposed considering parameters such as shape, size, charge density, nature of interaction with the bacterial cell, *etc*. A universally accepted mechanism is yet to be proposed. Fotou *et al.* proposed that interaction of AgNPs with the Gram-negative Bacterium *Escherichia coli* cell wall, and proposed that the interaction depends on the shape of the nanoparticles and the biocidal property of the NPs increases by the presence of a {111} plane when compared to spherical and rod-shaped NPs and with Ag^+ [81].



Scheme (5). Synthesis of Palladium-Fe_3O_4-Graphene Oxide nanocomposite. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Lignin particles (LPs) have importance due to their well-known biodegradability and bioactive potential. Nowadays, the green synthesis process is used for lignin nanoparticles synthesis. These LPs showed effective antimicrobial activity against *E. coli* and *Salmonella enterica* [82]. Interaction of NPs with the membrane seems to be the most relevant description for the activity of NPs. As the cell walls of Gram-positive bacteria contain a thick layer of peptidoglycan compared to the cell wall of Gram-negative bacteria. The electrostatic properties of the NPs govern the interaction between biofilm and the positively charged particles. The peptidoglycan is a unique and essential structural element in the cell wall of most bacteria consisting of glycan strands cross-linked by short peptides, the sacculus forms a closed, bag-shaped structure surrounding the cytoplasmic membrane. There is a high diversity in the composition and sequence of the peptides in the peptidoglycan from different species. It can be concluded that it is easier for NPs to destroy thinner cell walls; the evidence for such a conclusion come from various studies conducted on both the cell walls [83-84]. When ZnONPs were evaluated against *L. monocytogenes*, *S. aureus* and *E. Coli,* it was observed that the inhibition of the microbes was directly proportional to that of the concentration of the ZnO-NPs R. Yanping Xie *et al.* [85] analyzed the effect of ZnO- NPs in more detail. He studied the effect of NP accumulation on two oxidative stress genes (*KatA* and *ahpC*) and a general stress response gene (*dnaK*). He concluded that with increased concentration of the ZnO NPs levels of these genes increased 52, 7, and 17fold, respectively. In his study, he also reported a drastic change in the shape of the bacterial cell wall which turned in coccoid form to spiral form, when kept in ZnO NPs for 16 h. These coccoid forms were reported with some kind of memb-



Scheme (6). Synthesis of ZnO nanoparticles from $Zn(NO_3)_2$ solution by plant flavonoids. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

ers damages when analyzed by ethidium monoazide-quantitative PCR (EMA-qPCR), and hint at the role of damage to the cell membrane and oxidative stress for the activity of the NPs against the bacteria [85]. AgNPs were found to be an efficient antibacterial agents against both thick and thin layers of the polypeptidoglycan, resulting in antimicrobial activity against both of them [86]. The effect of the NPs is concentration, shape, and size-dependent. More inhibition was observed against tested bacteria when the concentration of ZnO-NPs increased [87]. The antibacterial activity of the ZnO NPs was inversely proportional to the size of the NPs in *S. aureus* (AAA). One school of thought also explains the activity of the NPs on the basis of interaction of NPs with genetic materials such as DNA. The affinity of Ag^+ ions for nucleosides in comparison to that of the other moieties, which is a selective interaction with the nucleic acid dilapidates the basic framework of the DNA, **(**Fig. **2)** results in the death of the cell. It is assumed that Ag^+ ion intercalates between base pairs in the DNA which, consequently, prevents effective hydrogen bonding between two ant-parallel strands [88]. The AgNPs also act as bactericidal embroideries due to their multifunctional cytotoxic payloads over the cells such as adherence on cell membrane thus damaging it and altering its transport efficiency; penetration into the cell membrane followed by dysfunctioning of the cellular ribosome, mitochondrion, and intracellular proteins; generation of reactive oxygen species (ROS) inside cell thereby oxidizing cellular proteins and lipids and lastly altering phosphotyrosine profile thereby altering cell signalling pathway [88], (Fig. **3**). Metallic and ionic forms of copper produce hydroxyl radicals that damage essential proteins and DNA [89]. The toxicity of CuNPs was reported to be dependent on the degree of dispersion of NPs. It has been observed that low temperature and high pH conditions lead to agglomeration of the NPs. In order to provide better dispersion and more surface area to interact with the bacterial cell walls, high temperature and acidic conditions are required [90].

Metallic nanoparticles like gold, zinc, copper possess antimicrobial activity. Due to their small size, these nanoparticles provide a large surface area for microbes interaction [91]. The synergistic effect of the NPs and other agents has also been observed against the bacteria. In a tremendous development, it is observed that chitosan gels and AgNPs could markedly prevent infections in chronic wounds. The literature states that *Staphylococcus aureus*, methicillin-resistant *S. aureus* and, *Pseudomonas aeruginosa* are mainly responsible for infections in burns [92-93]. Comparative antimicrobial testing of AgNP gel with available formulations in the market has shown that the zone of inhibition of AgNP gel was equivalent to 0.2% silver nitrate marketed gel. Studies suggest that patients receiving nano-silver had a significantly lower incidence of infections than those treated with existing regimes [94]. Ag NPs when used along with polyethylene glycol (PEG) and glutathione-stabilized (GSH) have shown marked effects on the microbial processes in the winemaking. The microbes involved in the winemaking process such as *Oenococcus oeni, Lactobacillus casei, Lactobacillus plantarum, Pediococcus pentosaceus*, and acetic acid bacteria (AAB) (*Acetobacter aceti* and *Gluconobacter oxydans*) can be controlled by using these PEG-Ag-NPs and GSH-Ag-NPs [95].

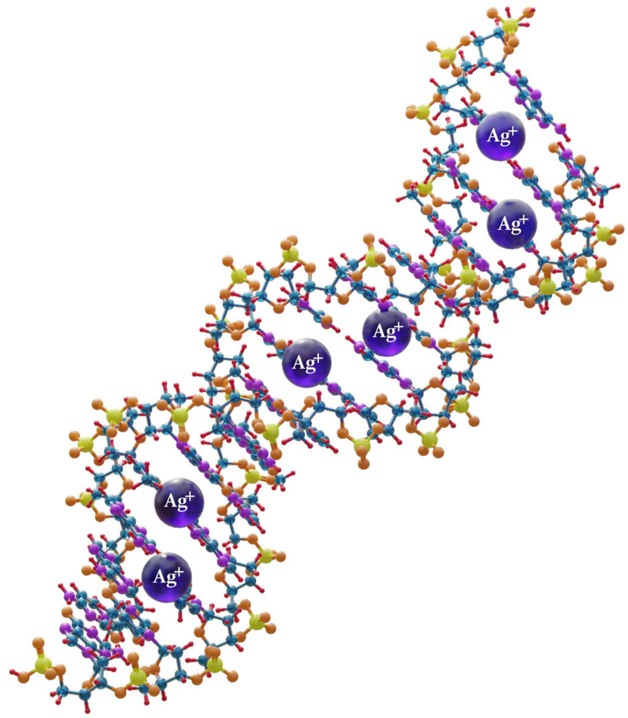


Fig. (2). NPs interacting with the DNAs. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

5.2. Catalytic Activity against Dyes

Organic dyes have frequently been used by various industries. The inherent problem of dyes is their toxic nature and difficulty in the degradation of these materials. Due to this, cost-effective and environment-friendly methods for the degradation of these dyes have always been a centre of attention. Due to the chemical and biological stability of dyes, it is very difficult for natural agents to degrade them [95-96]. Therefore, the degradation of organic dyes acquires great importance both environmentally and industrially. In recent years, various NPs have been investigated under solar light irradiation for the degradation of organic dyes. The photocatalytic functioning of NPs lies in the nano-scale size of these particles. Due to the nanostructure, the bandgap of these materials is greatly affected and excitation of electrons becomes much easier. Nowadays need for modified nanoparticles is increasing in the textile industry because they help in the protection of the catalytic degradation of various dyes [97]. The simplest mechanism of the photocatalytic reaction is the generation of electron-hole pairs on the surface of the semiconductor and its endpoint is as follows. When a photocatalyst is exposed to the light which possesses stronger energy than its bandgap energy, then electron-hole pairs diffuse out to the surface of the photocatalyst and partake in the chemical reaction with the electron acceptor and donor [89]. These free holes and electrons convert to the neighbouring oxygen and water molecules into OH free radicals and superoxides and these act as strong oxidizing agents for the degradation of dyes (Scheme **7**) **[**81, 98-100]. Various NPs synthesized with the help of the natural products or green techniques have been evaluated for their action against the degradation of the Dyes. The effect of the NPs on the degradation is in general monitored by UV-visible spectroscopy. The degradation of the dyes is very simple and cheaper than established hazardous methods. These NPs have been employed for the degradation of organic dyes such as methylene blue, rhodamine B, Congo red, yellow-12 Acridine Orange, *etc*. Kumar *et al*. synthesized AgNPs by the greener technique and evaluated the potential of the NPs in the degradation of methylene blue dye and reported successful degradation of the dye under direct solar light [101-102]. Maekawa *et al.* [103] synthesized Ag and AgCl NPs by using *Solidago altissima* plant material. These Ag and AgCl NPs have been utilized for the degradation of the rhodamine B (RhB). It is documented that degradation of RhB is effectively achieved by using Ag and AgCl NPs. He observed the major role in the degradation is being played by surface plasma on resonance and semiconductor properties of Ag and AgCl NPs [103]. Chitosan-silica sulphate nanohybrid (CSSNH) is a green and highly efficient heterogeneous new biopolymeric nanocatalyst. The catalytic activity of this catalyst was investigated in the green synthesis of thiiranes [104]. Methylene blue (MB) dye was effectively degraded under UV and Sunlight illumination in the presence of ZnO NPs [105]. Bimetallic combinations such as ZnO-Cu NPs have been reported with efficient catalytic activity against the degradation of harmful and toxic dyes such as Methylene blue (MB) and Congo red (CR). The degradation of these dyes has been achieved in the presence of $NaBH_4$at room temperature [106]. The ZnO-Cu combination is reported to be the best catalyst among all the other reported catalysts used for the degradation of these dyes with the shortest required time for the degradation of the dyes [107]. The catalytic activity of AgNPs also screened against dyes such as yellow-12 [108] and Acridine Orange (AO) [109]. Recent works show the potential of NPs synthesized with the help of green methods in the prevention of contamination caused by the use of dyes, especially industries such as fabric, printing, dye colours, paints *etc*.

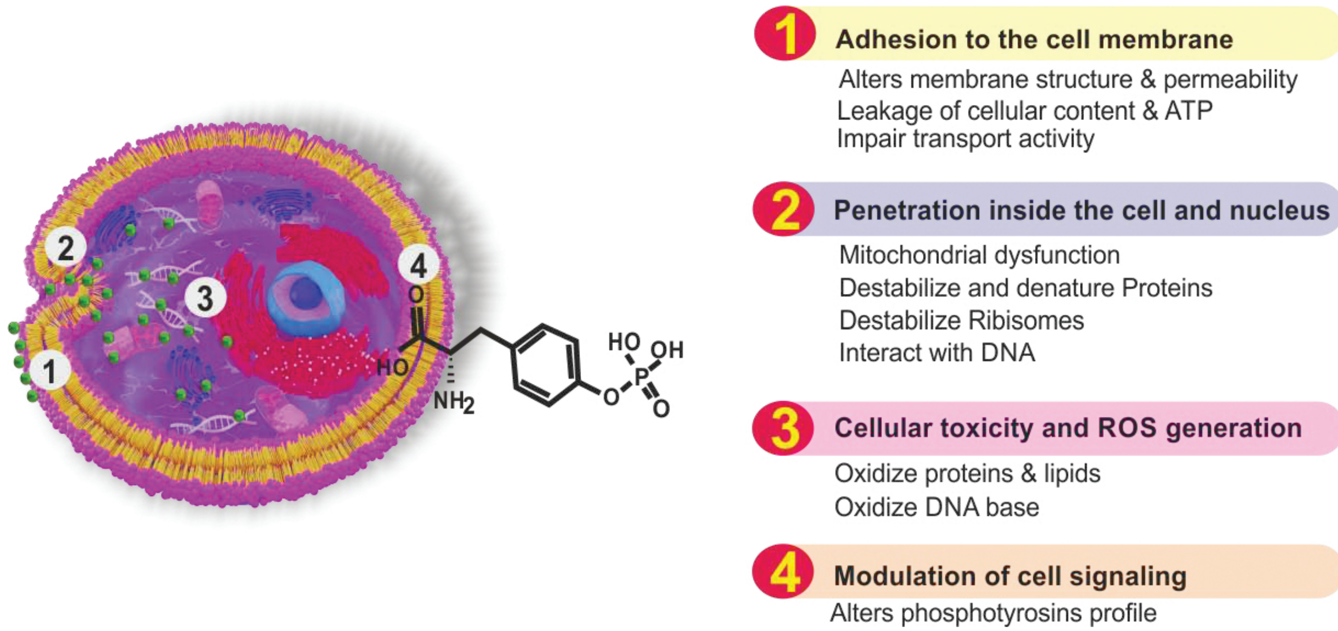


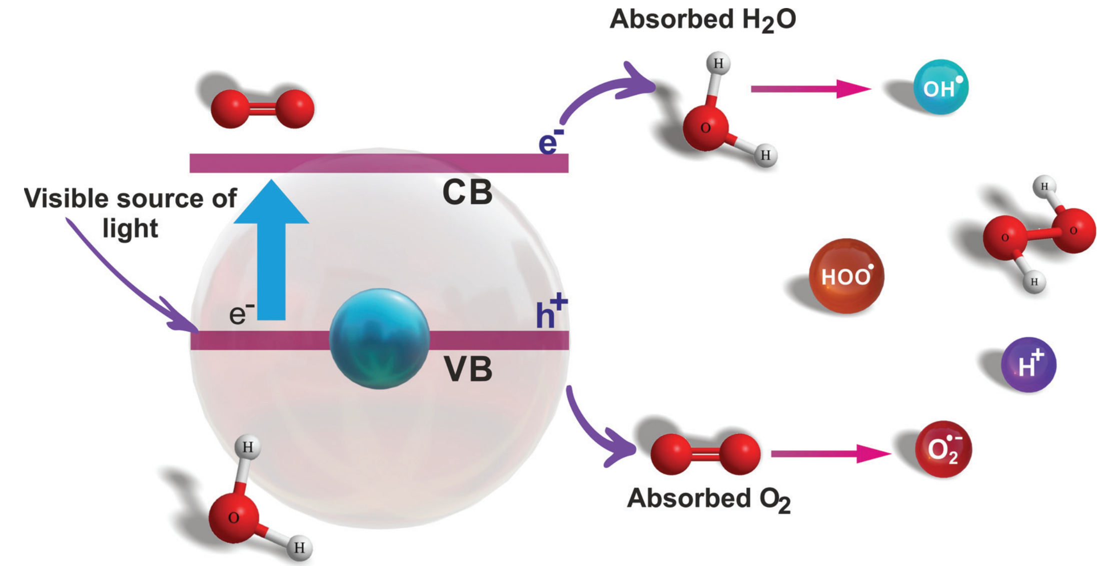
Fig. (3). Mechanism of Ag-nanoparticle toxicity inside the cell. 1, 2, 3, 4 are modes of action of Ag-nanoparticles on and inside the cell. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

5.3. Other Applications

NPs have opened a wide range of applications beyond their antimicrobial and catalytic activity. In the last decade, these materials have frequently been investigated for their potential use as anticancer agents, the development of cost-effective and efficient energy storage and conversion devices, and antioxidant agents. Anticancer and antioxidant activities of lanthanum NPs were done by Abraham *et al.* These NPs were synthesized with the help of extract of *Vigna radiate.* The activity of synthesized NPs was observed against osteosarcoma sixty-three cell lines and confirmed that the NPs can be used as potential members for the treatment of cancer [110]. Green synthesizes magnetite nanoparticles, using plant extract, which work as a reducing agent. These magnetite nanoparticles were used to test adsorption properties in the treatment of rosewater distillation wastewater [111]. Gutierrez *et al.* evaluated the potential of Ag NPs against human fibroblasts and observed that an increase in AgNP concentration affects the cell morphology of fibroblasts and suggested the potential application of NPs in chronic wound healing [112]. Chung *et al.,* have reviewed a large number of cases where NPs were evaluated for the anticancer activity of the NPs and referred to these materials as a potential source for the treatment of cancer [113]. Kamboh *et al.,* synthesized AgNPs by melon extract an eco-friendly, greener technique, and evaluate the potential of NPs against housefly *Musca domestica* [114]. Kuila *et al.,* used mango bark to prepare RGO/ Cu_2O composite. Used plant material simultaneously reduced the GO along with the formation of Cu_2O NPs. The cyclic voltammetry and amperometric analysis of the NPs resulted in the development of sensors toward the detection of H_2O_2 [115]. Currently, novel green adsorbents are in use for the removal of cephalexin (CEX) antibiotics from aqueous solutions without using any poisonous chemicals [116]. Poul *et al.,* discovered the effect of NPs on the Ag NPs on the human blood plasma and reported it to be coagulation inhibiting material [117]. Several other methods for green synthesis of nanoparticles involving callus culture, microbial tethering, fruit imbibed reduction and traditional plant intervention such as *Azadirachta indica,* together with their applications have been discussed in detail in various publications [118-127]. Especially, Pugazhendhi *et al*., reported that the silver NPs display a strange optical event called Surface Plasmon Resonance (SPR), due to the collective oscillation of the conducting metal surface electrons in resonance with the nonparticulate radiation at the nanoscale range. This property depends upon the type, shape, and size of the particle and this third-order optical nonlinearity of the AgNPs could be exploited in the development of the can be used as optical limiters [122]. The anticoagulative activity of the NPs remains the same even after a day. This study could turn out to be marked in the treatment of copious thrombotic disorders. Recently, a new modified zinc-aminolevulinic acid nano complex (n[Zn(ALA)2]) was prepared by using green conditions in water by sonochemical method. This foliar Zn amendment using synthetic Zn-ALA nano complex is useful as Zn-fertilizer in the agriculture industry [128].

Honey-mediated nanoparticles also have many potential applications. For example, since Au and Ag bear antimicrobial activities of their own, Au and Ag nanoparticles have been successfully used as potential antimicrobials as mentioned above. Pd nanoparticles have been used for organic catalysis, biosensor application, whereas carbon nanoparticles have been used for biosensing, bioimaging, and as quantum dots [129]. Real-time photoacoustic imaging is also another key application of honey-mediated C-nanoparticles [65].

There are many other applications where NPs have clearly shown their potential which is beyond the scope of this review.



Scheme (7). Pathway for catalytic activity of NPs and generation of reactive oxygen species (ROS). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

CONCLUSION

This review summarizes the potential synthetic routes and application of NPs through the green chemistry approach. Various routes of synthesis of NPs have been discussed such as using plants containing secondary metabolites like flavonoids. The electron capturing capacity of flavonoids for the formation of the NPs has been demonstrated. Not only the formation of the NPs, but the controlling of the size and shape of NPs either with plant extract or isolated molecule has also been described. It can also be noted that NPs having a size range of less than 10 nm could also be synthesized using plant extracts. Furthermore, relevant activities of greenly synthesized NPs have also been highlighted. For example, antimicrobial activity, anticancer activity, wound healing activity have been reported. Even, non-pharmaceutical activities such as reduction of chemical dyes, using NPs in voltammetric sensors for H_2O_2 detection have been explained. Thus, we propose that the green synthesis of nanoparticles has been an effective method for the synthesis of NPs as well as potential applications of these against various challenges.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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