An overview of the green road to the synthesis of nanoparticles

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The world today is witness to the exponential rise of nanotechnology in our daily lives with a vast number of applications in energy, medicine, food, water, cosmetics and drugs. The attractive properties of materials in the nanoscale have brought to light many new discoveries and innovative applications of nanoparticles (NPs). The progress and implementation of this new and exciting technology requires caution especially during nanoparticle production and synthesis. The fabrication of these all important NPs should be done in a clean, cheap, sustainable and safe manner with minimum risk to health and environment. This article reviews and reports eco-friendly, sustainable and energy efficient green methods of synthesizing NPs, with low environmental pollution and health hazards. Our aim is to highlight and summarize the use of green synthesis methods that use biological units, namely, plants and microorganisms (bacteria, yeast and fungi) for the production of inorganic NPs, with a focus on research works during the last five years. The added advantage of disease inhibition of NPs produced using the green approach, due to their excellent antibacterial, antifungal and antitumor properties, has also been discussed and illustrated with specific examples from literature.

Keywords: ***nanoparticles, environmental safety, green synthesis, plants, microorganisms, antibiotic***

Introduction

All matter and life originates at the nanoscale with atoms and molecules and this provides us with a very useful set of tools and techniques called nanotechnology, which includes nanoparticles (NPs), nanofabrication, nanobiotechnology and other cognitive sciences. The unifying force is the manipulation of nanoscale matter for the diverse applications that we see today. Nanomaterials have dimensions of 1-100 nm and the main appeal is their large surface to volume ratio which gives them unique and enhanced properties. The size, shape and type of the NPs greatly influence its properties and the field of applications. Nanomaterials find applications in environment and toxic gas sensing [1-5], food and agriculture [6, 7], drugs and medicine [8-12] and in energy [13-15].

While nanotechnology makes many things in life easier and provides benefits to society, the large scale production of nanomaterials and the use of self replicating nano-machinery can pose incalculable risks. It is important to understand the role of nanoparticles in the uptake of pollutants and nutrients and also the toxicity of nanoparticles in food and agriculture. Safer design and manufacturing practices have to be adopted to ensure minimum risk to environment and human health. The concerns of toxicity and energy efficiency have lead to substantial research on green and sustainable production of NPs. This is a rapidly growing research field and the last two decades have witnessed the use of biological resources such as plants, microorganisms and natural biomolecules for reduction and stabilization of a large number of NPs [16-20]. This mini review focuses on the recent literature mainly last five years, to highlight and summarize the green synthesis routes, which use biological units from plant, bacteria, fungi and yeast with sustainable and energy saving protocols for NPs fabrication. The review emphasizes through published data that NPs of a wide range of chemical composition, size and shape, with the minimum of environmental contamination are possible using green synthesis and varying experimental parameters. The important added benefit of disease inhibition through the excellent antibacterial, antimicrobial and antitumor properties of green NPs is also explored and illustrated using specific examples from literature. The review is organized under the sections: importance of green synthesis, methods of green synthesis, disease inhibition using green NPs and conclusions.

I. GREEN SYNTHESIS AND WHY IT IS IMPORTANT

A. *What is green synthesis?*

Green synthesis is the production of NPs using natural resources like plant extracts, microorganisms and energy saving methods in a sustainable, non-toxic and economical way. Extracts from different plants can be used to produce NPs with special characteristics and functionality to suit a specific application [10, 21, 22]. The microorganisms can be bacteria, fungi, yeasts and the choice will be based on the type, size and functionality that are desired of the NPs [23-25].

B. *Benefits of Green Synthesis*

Why use green synthesis when conventional methods of production exist? The answer lies in the numerous benefits that come with the use of a green route for NPs synthesis. First of all, NPs produced in this manner are more stable and effective in comparison with those produced by physicochemical methods. Next, they are eco-friendly, sustainable, inexpensive and free of contaminants. In comparison physiochemical methods are costly and can be unhealthy (as a result of toxic chemicals used) with high chances of contamination. Purity of NPs is a major consideration for biological and medical applications and green NPs are mostly contaminant free. In addition, they are energy efficient and do not need high pressure, temperature, or toxic chemicals. Moreover, most NPs produced by the green method show excellent antifungal, antibacterial and anti-parasitic properties. Other advantages are the ease of large scale synthesis and the disposal of the non-toxic waste products.

II. METHODS OF GREEN SYNTHESIS

The production of nanoparticles (NP) using natural substances is an important and emerging area in nanotechnology. The conventional methods of synthesis of NPs using chemicals as precursors or reducing agents have potential risks of toxicity and in general are not environmentally friendly or quick processes. To overcome these disadvantages the use of renewable resource natural biological systems to produce NPs is finding widespread acceptance. The different methods of green synthesis will be expanded based on the type of resource used and classified under: a) use of plants and plant extracts b) use of microorganisms and c) use of energy saving methods.

A. *Use of plants and plant extracts*

Green synthesis using biological molecules from plant extracts has proved to be far superior to chemical means. Plants are readily available, diverse and safe making them very appealing as sustainable bio resources. The huge plant diversity provides a natural bank of resources that can be utilized to rapidly synthesize in a one step protocol different types of NPs having a range of antimicrobial activity and applications. The reaction rate of plant mediated NPs synthesis is rather fast (few minutes to hours) and occurs mainly at room temperature leading to easy scalability. The use of plant extracts to produce NPs of high quality, specific morphology and function is wide spread due to the simple steps involved in nanoparticle recovery. Fig 1 shows a schematic diagram of the steps involved from [26]. The steps involved are extraction of plant by hand grinding/blender, filtration of the extract, addition of metal NP salt, stirring the resultant solution and finally recovery of NPs from precipitate.

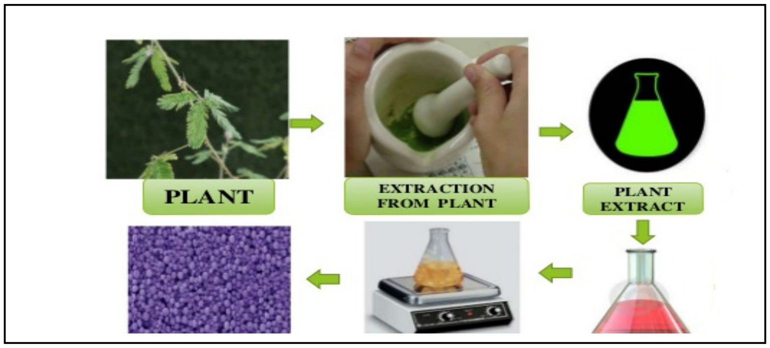

Fig. 1 The various steps involved in the extraction of nanoparticles using plants [26]

Different parts of the plant like stem, fruit, fruit peels, bark, root and leaves can be used to produce the NPs. Taking silver (Ag) NPs as an example, the specific parts of the various plants used for synthesis is presented in Table 1 adapted from Ahmed et al [10].

TABLE I. SYNTHESIS OF SILVER NANOPARTICLES USING EXTRACTS FROM DIFFERENT PARTS OF VARIOUS PLANTS

Plants	**Size (nm)**	**Plant's part**	**Shape**	**References**
Alternanthera dentate	50–100	Leaves	Spherical	[27]
Acorus calamus	31.83	Rhizome	Spherical	[28]
Boerhaavia di□usa	25	whole plant	Spherical	[29]
Tea extract	20-90	Leaves	Spherical	[30]
Tribulus terrestris	16-28	Fruit	Spherical	[31]
Abutilon indicum	7-17	Leaves	Spherical	[32]
Ziziphora tenuior	8–40	Leaves	Spherical	[33]
Cocous nucifera	22	Inflorecence	Spherical	[34]
Pistacia atlantica	10–50	Seeds	Spherical	[35]

The advantage of using plant extracts over other biological methods is that there is no need for elaborate culturing or cell maintenance. Another attractive feature is its easy scalability in comparison with microorganisms [10, 36]. The success of producing metal, metal oxide and chalcogenide NPs with excellent antimicrobial properties by this method for various applications has been reported in several research articles and reports [10, 21, 22, 26-39].

B. *Use of microorganisms*

Many microorganisms can be considered as nano factories that produce metal nanoparticles with different efficiency, size and shape. The use of physical and chemical means for NP synthesis may result in contamination due to toxic solvents, precursors and generate harmful byproducts with generally low yields. In contrast, the use of micro-organisms like fungus, yeast and bacteria for NPs synthesis is relatively nontoxic, safe, reliable, clean and environmentally friendly with high yields [17-20, 40-42]. Due to the ease of control of yeast under laboratory conditions, availability of numerous enzymes and the rapid growth with the use of simple nutrients, the yeast strains are preferred over bacteria [20]. Fungi have the ability to digest extracellular food and excrete enzymes that hydrolyze complex molecules to simpler ones. Thus, they act as reducing agents of metal salts through secretion of enzymes and proteins to yield NPs. The mechanism may be extracellular that is outside the cell or between group of cells or intracellular meaning inside the cell or cytoplasm. This form of biosynthesis has great potential as large scale production of NPs from various strains of fungi is possible and they can even be grown *in vitro*. Table 2 lists some of the noble metal, metal oxide, chloride and sulphide NPs obtained from diverse fungi and yeast species [43-55]. The extraction of NPs synthesized through intercellular mechanism requires extra processing steps, so extracellular is favored in most microbial approaches, as seen in Table 2.

The extracellular biosynthesis of Ag NPs using filamentous fungi like '*Aspergillus fumigatus*' has been reported by Kuber et al [23]. Well dispersed Ag NPs of 5-25 nm were formed within minutes of silver ion coming in contact with the cell filtrate showing that the extracellular reduction process is a very fast and feasible biosynthesis method for Ag NPs. These filamentous fungi are excellent candidates for extracellular process applications, since, they secret a variety of enzymes and are easy to grow and handle.

NANOPARTICLES OF VARYING SIZE AND SHAPE FABRICATED FROM FUNGAL AND YEAST SPECIES

Fungi and Yeast	NPs	Size (nm)	Shapes	Location	Ref
Alternaria alternate	Au	12 ± 5	Spherical triangular hexagonal	Extracellular	[43]
Aspergillus clavatus	Au	24.4 ± 1 1	Triangular spherical hexagonal	Extracellular	[44]
A. fumigatus	ZnO	1.2–6.8	Spherical hexagonal	Extracellular	[45]
A. oryzae TFR9	$FeCl_3$	10–24.6	Spherical	–	[46]
A. sydowii	Au	8.7–15.6	Spherical	Extracellular	[47]
A. terreus	Ag	1–20	Spherical	Extracellular	[48]
A. tubingensis	$Ca_3P_2O_8$	28.2	Spherical	Extracellular	[49]
Aureobasidium pullulans	Au	29 ± 6	Spherical	Intracellular	[50]
Candida albicans	Au	5	Mono dispersed spherical	Cell-free extract	[51]
C. glabrata	CdS	–	–	Intracellular	[52]
Coriolus versicolor	Au	20–100, 100–300	Spherical ellipsoidal	Intra- and extracellular	[53]
Cylindrocladium floridanum	Au	19.05	Spherical	Extracellular	[54]
Fusarium oxysporum	Pt	70–180	Rectangular triangular spherical aggregates	Extracellular	[55]

'*Fusarium oxysporum'* is one of the most utilized filamentous fungal species for production of NPs. The work of Govender et al [55] shows the successful reduction of octahedral H_2PtCl_2 and $PtCl_2$ by the hydrogenase enzyme released from *Fusarium oxysporum.* This is a two step process; first at a pH of 9 and 65°C, the large H_2PtCl_2 molecule undergoes a two electron reduction to a smaller $PtCl_2$ molecule that can fit the enzyme surface, then at an optimal hydrogenase enzyme activity of pH of 7.5 and 35°C a second two electron reduction of $PtCl_2$ occurs to give Pt NPs.

Another typical example using '*Fusarium oxysporum*' is the size controlled synthesis of silver NPs to obtain well-dispersed nanoparticles with size between 5 and 13 nm by Husseiny et al [9]. The NP type, size and morphology are controlled by the environmental and nutritional parameters via substrate concentration, temperature, pH and weight of biomass.

The biosynthesized NPs showed excellent antibacterial and antitumor activities which will be discussed in more detail in section IV.

C. *Use of energy saving methods*

The synthesis of molybdenum disulfide nanostructures (MSNs) for host of applications by the 'green' microwave-assisted (MW) solvothermal synthesis method [56] is a clear example of energy saving techniques for green NP synthesis. In this process, ammonium molybdate and elemental sulphur are mixed in 1:1 molar ratio in 10 ml of hydrazine monohydrate and 30 ml deionized water under magnetic stirring for 5 minutes. The resultant solvent mixture is transferred to a Teflon vessel and subjected to microwave radiation of 270 Watt in a microwave oven for 10 minutes, and is allowed to cool down naturally to room temperature. Black precipitate settled at the bottom of the solution is filtered, washed with distilled water, diluted hydrochloric acid and ethanol successively and centrifuged to remove any unreacted precursors. The final product is dried in vacuum oven at 50 degrees Celsius for 4 hours to obtain MoS_2 nanostructures. This technique qualifies for the energy efficient 'greener' approach by drastically reducing the reaction time (~300 times faster than the conventional method). Microwave radiation penetrates through Teflon vessel and interacts with the solvents directly causing localized heating and thus generates supercritical conditions favorable for nucleation and growth of nanoparticles. The schematic illustration of the strategy for MW green synthesis of MSNs via generation of supercritical conditions is shown in Fig. 2 taken from the work of Qureshi et al [57].

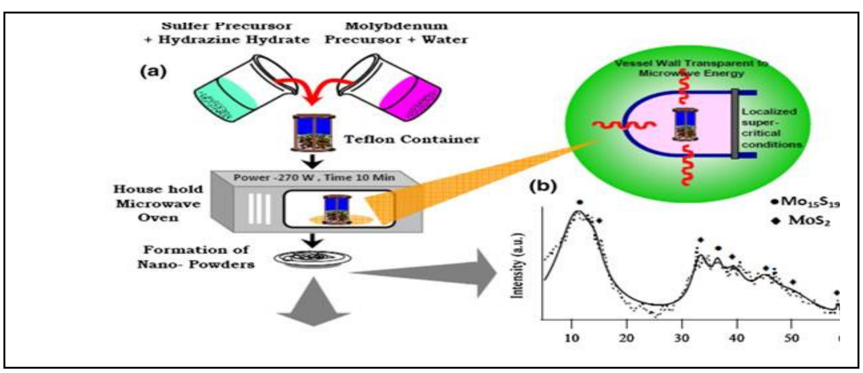

Fig. 2. The Schematics for microwave assisted green synthesis of Molybdenum disulphide nanoparticles [57]

In their work they demonstrated interestingly, biofilm inhibition and antimicrobial behavior of MSNs produced using this method. This is a significant discovery since the biofilm protects pathogenic organisms from drugs and immune system by resisting entry and recognition, respectively. They also showed the MSNs to be non-cytotoxic which gives them biocompatibility and increases their antimicrobial potential.

The green synthesis of Ag NPs with demonstrated antibacterial activity by Fatimah [21] using MW is another example of energy efficient green process for NP synthesis. The use of MW shows the rapid formation of Ag NPs with similar properties to those obtained through the time consuming aging method. In addition, the use of microwave irradiation yielded larger particles. The profound effect of irradiance on the reduction rate of $AgNO_3$ to Ag NPs using fig leaves extracts shown by Ulug et al [58] is another instance of energy efficient green synthesis.

III. DISEASE INHIBITION USING GREEN NPS

The excellent antibacterial, anti-parasitic and antitumor properties of green NPs allows them to play significant roles in medicine, clinical and *in vitro* diagnostic applications [8-12, 18-25, 51, 57]; and also in agriculture, water treatment, food packaging and textiles [6, 7, 59-65]. Antibiotic resistance poses a major problem in healthcare due to the inherent tendency of microbial cells to alter their genes. In this context, the exploitation of inorganic NPs to develop antiseptics that are deadly to microbes and which demonstrate wide-range activity with lower prospects of microbial resistance is the most needed solution.

The use of Ag NPs in the biomedical sector has seen increasing number of applications with large number products such as ointments, dressing materials and body hygiene already in the market. Ag NPs have been reported to possess antimicrobial property against myriad array of pathogenic microorganisms. An excellent example is the work of Husseiny et al [9] who showed the inhibitory capabilities of green Ag NPs towards bacteria and tumor. The NPs biosynthesized with '*Fusarium oxysporum*' showed excellent antibacterial activities when studied using agar well diffusion and zone of inhibition method against pathogenic strains of *E. coli* and *S. aureus*. Also, their work reveals promising antitumor capability against human breast carcinoma cell line (MCF-7). Fig. 3 shows the antibacterial and antitumor activities taken from their work.

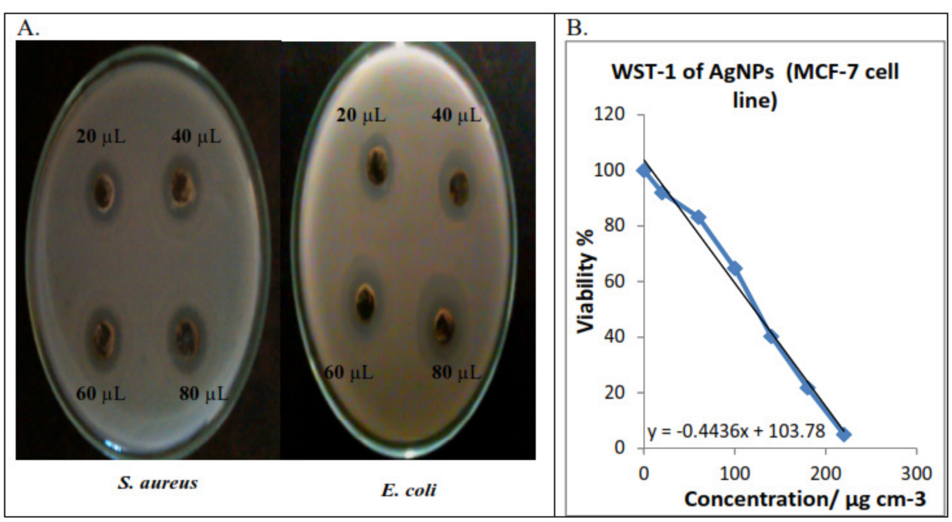

Fig. 3. The Antibacterial activity of different concentrations of biosynthesized AgNPs (A) against *E. coli* and *S. aureus* strains (B) viability chart of biosynthesized AgNPs against MCF-7 cell line. [9].

A serious concern is bacterial resistance to conventional antibiotics based on organic molecules. The prevalence of multidrug-resistant bacteria requires an effective solution and green NPs have come to the rescue. An example is the interesting microbial resistance mechanism using non-cytotoxic MSNs by reactive oxygen species (ROS) via disruption of cellular functions, as demonstrated in the work of Qureshi et al [57]. Fig. 4 taken from their work compares and illustrates the effectiveness of MSNs with the reports of works for other inorganic NPs like Ag, Pd, Fe–Pt, Fe_2O_3, TiO_2, ZnO, MgO, CuO [66-77] against various forms of bacteria.

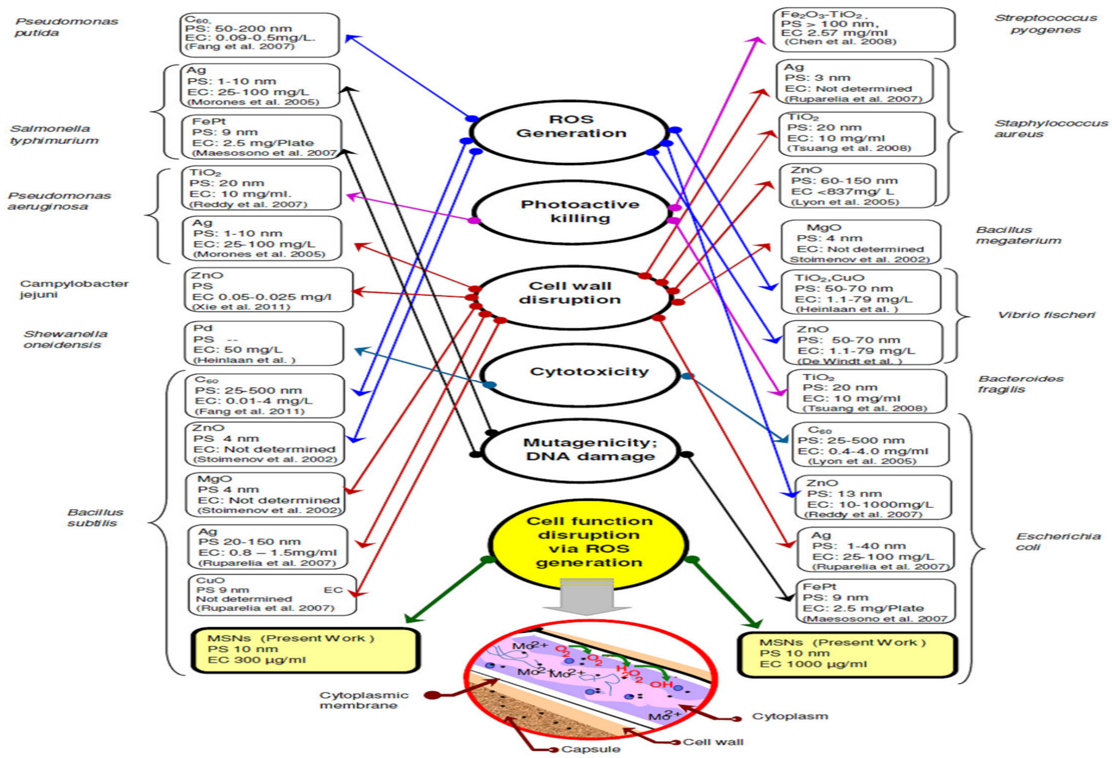

Fig. 4. Comparative illustration showing the antimicrobial effect of different NPs with MSNs on various Gram-positive and Gram-negative bacteria [57]

In comparison to the noble metal Ag, which is one of the most investigated and well known NP for its antimicrobial properties, the cytoxicity of MSNs is negligible and makes it more biocompatible and a comprehensive choice for all sorts of applications; Ag is limited in application by its higher cytotoxicity. Also, in comparison with the other NPs shown in Fig. 4 the MSNs have the lowest MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values making it a more superior multidrug antibacterial solution.

IV. CONCLUSIONS

The use of natural and biogenic resources for the production of NPs is an eco-friendly, sustainable, inexpensive and contaminant free process that satisfies the requirements of energy saving, green and healthy environment protocols. The NPs synthesized in this manner from plants and microorganisms are less toxic, more stable, controlled, and beneficial to humans and environment as compared with those produced by physical/chemical methods. Moreover, such green NPs are very effective in treatment against pathogens and multi drug resistant bacteria due to their excellent antibiotic activities.

Microbes develop resistant strains in a short span of time to conventional organic based antibiotics which is a major health concern that needs an urgent and innovative solution. The wide range of antibiotic activities available from green inorganic NPs makes them ideal to develop antiseptics that are deadly to microbes and which have a low probability of promoting microbial resistance. The diversity of biogenic resources and the multitude of NPs that can be synthesized with varying antimicrobial properties show the vast and rich possibilities in the field of green synthesis. An increased awareness to the health benefits of the green route of synthesizing NPs is desired along with renewed research to bring to light new and exciting discoveries in this direction.

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