



Green synthesis of nanoparticles and their applications in cancer treatment

Sansa Basaiawmoit *, Phidashisha Kharmalki, Larishisha Swer, Matsramch Marak, Rebecca Marwein and Lakhon Kma

Department of Biochemistry, North Eastern Hill University, Shillong, Meghalaya-793022

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Abstract

Cancer, through the years has always been a topic of interest in research. Even with the advancement in technology, researchers are constantly on the lookout for novel ideas to prevent the progression of cancer. Green synthesis of nanoparticles has been a widely studied area owing to its immense potential applications in environmental and medical industry. Green synthesis employs an eco-friendly, a non-toxic and cost-effective approach; being the much more favored method of synthesis over physical and chemical synthesis. The use of biological entities, taking plants for example, in most cases is safe. Plants in nature have the ability to absorb inorganic metals from the surrounding environment and synthesize nanoparticles intracellularly as well as extracellularly. However, not all plants can self-synthesize nanoparticles due to their enzymatic properties or metabolic processes. Therefore, there is the need to synthesize nanoparticles, using biological entities or their extracts by green synthesis approach through reduction. Plant-based nanoparticles have been extensively researched for their therapeutic applications. Nanoparticles synthesized from plants and fruits have been proven to possess anti-cancer properties owing to their phytochemical compounds possessing strong antioxidant properties and inhibiting cancer cell progression and proliferation. In this review, we discuss green synthesis of nanoparticle and their various applications in cancer treatment.

Keywords: Green synthesis; Plant extracts; Nanoparticles; Cancer

1. Introduction

Nanotechnology is the branch of science that deals with particles at the molecular or atomic level, their characterisation, manipulation and application. Nanotechnology has brought forward a wide range of applications such as in the field of optic science, biomedical sciences, engineering, mechanicals, chemical industries, space industries, medicine and so forth (Singh T et al. 2019). Nanotechnology deals with the synthesis and manipulation of particles of sizes ranging from 1 to 100 nm. This field of technology makes use of all the fields of natural sciences together with chemistry, physics, biological sciences, engineering, materials science, and computational sciences for the formulation of nanostructures (Shenton et al. 1999; Medvedeva et al. 2007). The biological nanostructures offer different applications depending upon their size, distribution, and morphology (Thakkar et al. 2009).

Nanoparticles (NPs) have been highly studied due to their impressive properties of being small in size with a large surface area to volume ratio (Ray 2010). However, manipulation at the nanometric size produced particles that were toxic in nature. To overcome this problem, a new approach termed as 'Green Synthesis' was introduced that was environmental friendly, cost-effective and non-toxic (Duan et al. 2015). In general, NPs can be found as carbon nanotubes, quantum dots, nanorods, nanocapsules, nanoemulsions, fullerenes, metallic NPs, ceramic NPs, and polymer NPs (Sudha et al. 2018). Amongst these, the metallic nanoparticles are the most commonly studied in research owing to their impressive properties and mode of action for which they play important roles in many applications including treatment of cancer, which will be outlined in this review. This further led to extensive research in the synthesis of NPs,

*Corresponding author: Sansa Basaiawmoit

via physical, chemical and biological methods, out of which the biological method gave rise to a green synthesis approach. This review will outline the development in green synthesis of metallic nanoparticles and applications in the field of cancer.

2. Synthesis of nanoparticles

Generally there are two methods for synthesis of nanoparticles, top-down approach where larger materials are broken down to nanometric size using chemical, physical and biological processes, and bottom-up approach also known as “self-assembly approach” where atoms grow in clusters to form NPs again via physical, chemical and biological reactions. The chemical and biological processes employ the bottom up approach of synthesis. Chemical and physical processes often use toxic chemicals resulting in the synthesis of NPs that are toxic and may often show carcinogenicity and environmental hazard (Gupta and Xie 2018). The use of these toxic chemicals limits the potential applications of NPs in various fields (Hua et al. 2018). This led to the need for a clean, reliable, environmentally friendly, and a biologically safe method to synthesize NPs, which gave rise to the Green Synthesis method, a process involving both chemical as well as biological, following the bottom up approach which produces NPs with minimum defects and a homogenous chemical composition (Jamkhande et al. 2019).

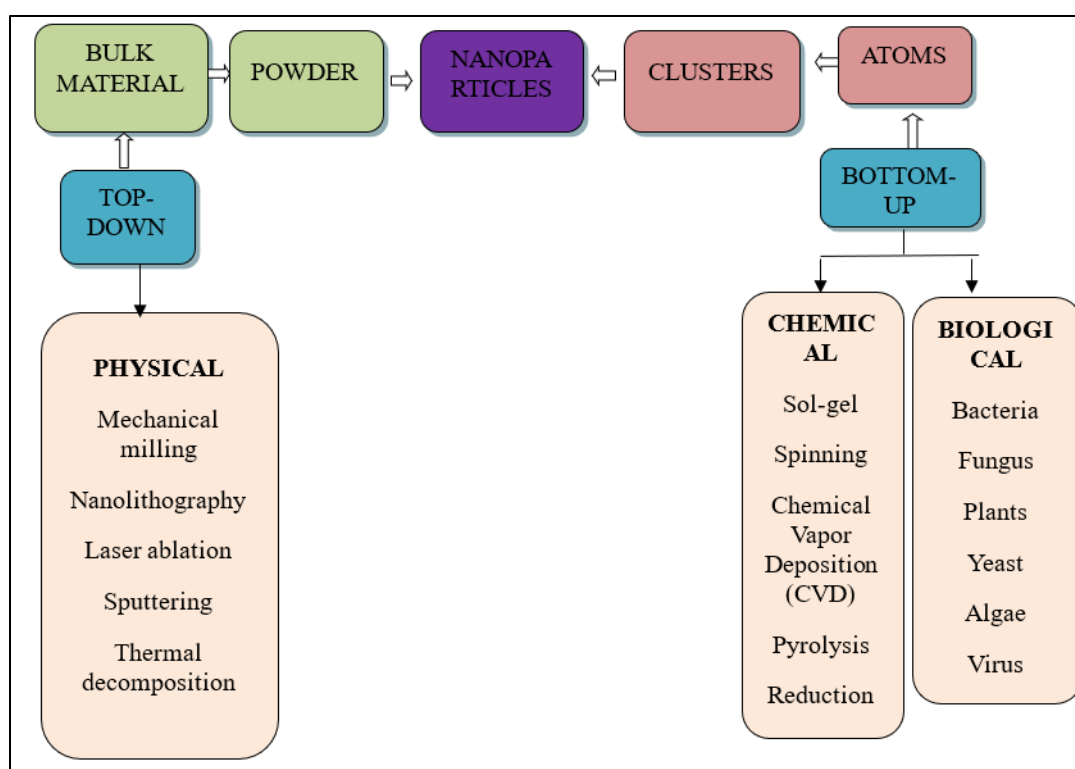


Figure 1 Different approaches to the synthesis of nanoparticles with their methods (Zhang et al. 2014)

3. Green synthesis

Plants and microorganisms have long developed a mechanism to adapt to the high concentration of metals in their surrounding niche converting them into non-toxic particles (Bisen et al. 1987). The formation of nanoparticles can be viewed as a defence and/or resistance mechanisms of the organisms against the toxic concentration of metals. This property of plants and microorganisms makes them the ideal choice for the synthesis of non-toxic nanoparticles, and studying their possible applications in drug delivery system.

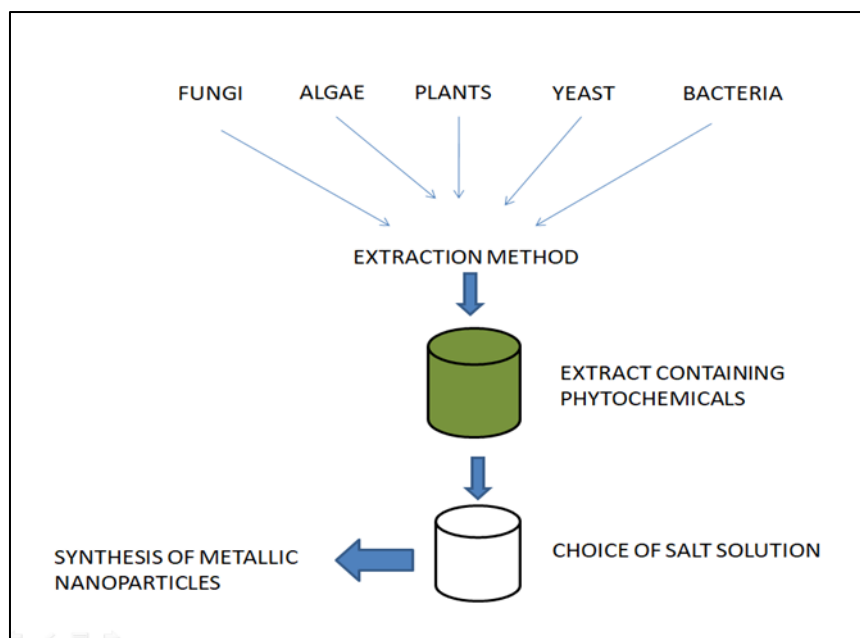


Figure 2 Schematic green synthesis method to obtain metallic nanoparticles

The synthesis of metallic nanoparticle is split into two categories:

- **Bioreduction:** Chemical reduction using biological means leads to the synthesis of more stable form of metal ions. The metal ion is reduced while the enzyme gets oxidized (Deplanche et al. 2010).
- **Biosorption:** In this category, the metal ions directly attached themselves to the organism either from their aqueous or soil sample. The metal ions are either bonded to the cell wall or in other cases there is an assembly of peptides, produced by plants and microorganisms, into nanoparticles (Yong et al. 2002).

The most important feature in the synthesis of metallic nanoparticles is to adopt a non-toxic, environment-friendly and cost-effective method. The biosynthetic route offers the best method to synthesize metallic nanoparticles from plants and microorganisms for various applications (Razavi et al. 2015). One of the first reported green synthesis method produced silver nanoparticles (AgNPs) by utilizing starch solution (Raveendran et al 2003). Following this, there had been numerous review papers highlighting the synthesis of NPs from plant extracts including fruits and microorganisms (Mittal et al. 2013; Iravani et al. 2014). Till date, green synthesis of NPs from plant extracts has become a topic of interest to many researchers, as it provides an effective role in many other applications, biological and otherwise. Numerous plant and fruit extracts have been used due to the presence of phytochemical which acts as stabilizing and reducing agents (Narayanan and Sakthivel 2011). Different parts of plant and fruit extracts have been used to elucidate their potential application.

4. Green synthesis using microorganisms

There is a huge interest in using bacteria, fungi and yeast for exploring nanoparticle synthesis, however the process is being held back by the possibility of contamination of culture, extraction methods being inadequate and lengthy, and inability to control size of nanoparticles. Despite the drawbacks, numerous reports have come up over the years establishing microorganisms as ideal subjects of nanoparticles synthesis with distinctive applications.

Bacteria mediated green synthesis: Bacteria are common biological factories for the synthesis of nanoparticles (Iravani 2014). Depending on the localization of the nanoparticles, the synthesis of NPs can be intracellularly or extracellularly (Rafique et al. 2017).

The most common metal nanoparticles synthesized in bacteria are gold (Au), silver (Ag) and palladium (Pd). Au and Ag nanoparticles have been extensively studied due to their anti-microbial property. Copper nanoparticles (CuNPs) are highly unstable and can be easily oxidized to copper oxide, hence require an extra step of stabilization (Baco-Carles et al. 2011).

Delftia acidovorans a bacterium that possesses secondary metabolites with the ability to protect itself against toxic gold and causes biomineralization of inert gold nanoparticles within the cytoplasm (Johnston et al. 2013). The bacteria *Rhodospseudomonascapsulata* was investigated for its ability to synthesize gold nanoparticles and demonstrated that controlling the pH generated nanoparticles of different shapes and sizes. At neutral pH, spherical nanoparticles of 10-20 nm size range whereas at pH 4 nanoplates were observed (He et al. 2007). *Bacillus licheniformis* was also screened for its ability to biosynthesize silver nanoparticles and also speculated that the synthesis of silver nanoparticles could involve the enzyme nitrate reductase (Kalimuthu et al. 2008). Silver nanoparticles were biosynthesized using *Bacillus spp.* producing crystalline shaped of 5-15 nm sizes. The metallic nanoparticles were deposited within the periplasmic space making the recovery of biosynthesized nanoparticles easier (Pugazhenthiran et al. 2009).

Fungi mediated green synthesis: Fungi as compared to bacteria are more easily available providing a cost effective and ease of laboratory setup thereby is used for large scale production of NPs (Molnár et al. 2018). The most common metal nanoparticles synthesized by fungi are silver, gold and platinum (Pt). Out of the three, the most commonly synthesized is silver for their antimicrobial property (Rai et al. 2008) while most fungi are unable to synthesize gold nanoparticles (AuNPs). AgNPs synthesized from *Verticillium sp.* reported nanoparticles of size 25 ± 12 nm, formed below the cell due to the action of enzymes present in the cell wall membrane. It was also reported that fungal cells continue to grow even after the biosynthesis of nanoparticles, hence proving that the silver nanoparticles are non-toxic to the cells (Mukherjee et al. 2001).

One particular work investigated the extracellular biosynthesis of silver nanoparticles using *Aspergillus fumigates* generating spherical and triangular nanoparticles with the size range of 5-25 nm in diameter. Nanoparticles were generated as soon as the salt solution came into contact with the extract, thus providing a fast and efficient method of nanoparticle biosynthesis (Bhainsa and D'Souza 2006). A fungal mediated biosynthesis of silver nanoparticles using *Coriolus versicolor* reported the formation of spherical silver nanoparticles of sizes 25-75 nm. It was also demonstrated that fungal proteins and glucose were responsible for the reduction process generating nanoparticles intracellularly as well as extracellularly (Sanghi and Verma 2008).

Yeast mediated green synthesis: Yeasts are also a better counterpart to bacteria for the synthesis of NPs as their growth can easily be maintained in laboratories using available required nutrients (Moghaddam et al. 2015). Yeasts have the property to absorb lethal metals in their surrounding due to their large surface area and convert them into non-toxic counterparts (Bhattacharya and Gupta 2005). Silver nanoparticles biosynthesized using *Aspergillus sydowii* displayed antifungal property against pathogenic fungi; and antiproliferative activity against cancer cells HeLa and MCF-7 proposing the following points as possible mechanisms; inducing oxidative stress, damaging DNA, damaging mitochondria, activating immune system, inducing cell cycle arrest and activating apoptosis (Wang et al. 2021). The endophytic fungus *Talaromyces purpureogenus* has been reported for the synthesis of silver nanoparticles which showed antibacterial activity and effective antioxidant activity (Sharma A et al. 2021). An anti-microbial cellulose based fabric was designed employing green synthesis of zinc oxide nanoparticles using *Phanerochaete chrysosporium* for diverse applications (Sharma J et al. 2021).

Algae mediated green synthesis: The use of algae to synthesize NPs has also been explored due to the presence of bioactive compounds such as macromolecules, vitamins and secondary metabolites with a wide range of applications (Arya et al. 2019). Algae also display the same property of accumulating surrounding heavy metals and synthesizing metallic nanoparticles. *Chondrus crispus* and *Spyrogira insignis* have been reported for the biosynthesis of nanoparticles to aid in the recovery of gold from dilute solutions using algae. *C. crispus* gold nanoparticles were synthesized whose diameters were observed in the range of 30-50 nm and spherical in shape, whereas *S. Insignis* reported silver nanoparticles of exact 30 nm diameter with no defined shape but formation of aggregates were observed (Castro et al. 2013). Green micro algae *Chlorella vulgaris* was also reported to intracellularly synthesize gold nanoparticles of 40-60 nm in diameter. Other salt solutions were also used which synthesized their respective nanoparticles however they were at a lower concentration as compared to gold. Thus *Chlorella vulgaris* provides a larger platform for large scale synthesis of gold nanoparticles (Luangpipat et al. 2011). Silver nanoparticles of variable shapes with 8-27 nm in diameter were synthesized from marine alga *Sargassum wightii* and screened for their antibacterial potential against bacterial strains *Staphylococcus aureus*, *Bacillus rhizoids*, *E.coli* and *Pseudomonas aeruginosa*, proving to be effective (Govindaraju et al. 2009).

5. Nanoparticles synthesis using viruses

Virus contains an outer capsid protein that facilitates the synthesis of nanoparticles (Makarov et al. 2014). Tobacco mosaic virus has been reported to synthesize Au and Ag nanoparticles (Kobayashi et al. 2012).

6. Green synthesis in plants

Green synthesis of nanoparticles in plants have a wider range of application than the above mentioned microbes as they are non-pathogenic and much research has been done. Most methods employed in the synthesis of nanoparticles from plants involve taking any part of a plant and collecting its extract either in aqueous form or non-aqueous. Different parts of a plant may show differences in effectiveness of the NPs. The addition of plant extracts on silver nitrate (AgNO_3) leads to the reduction of Ag^+ (Beach et al. 2009). There is no known literature that can elucidate the exact mechanism of reduction and stabilization of nanoparticles. Plant extracts contain polyphenols and active biomolecules that are responsible for NPs formation (Parandhaman et al. 2019). Polyphenols and flavonoids are the most common constituents found to be involved in the plant-mediated biosynthesis of nanoparticles. The proposed mechanism of nanoparticle biosynthesis involves the reduction of ions to metals, followed by accumulation of the atoms forming clusters and finally further amplification of the clusters leading to the formation of nanoparticles and their protection by capping agents to prevent aggregation. The reduction process and stabilization of nanoparticles are not entirely performed by the same one or set of compounds (Alahmad et al. 2021).

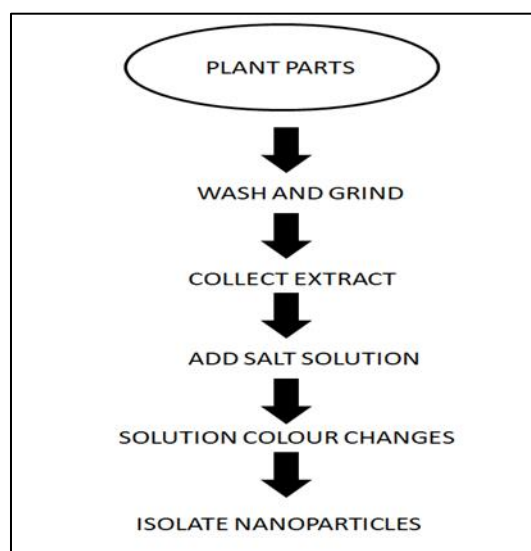


Figure 3 A general representation of synthesis of nanoparticles using plant extracts

Syzygium cumini mediated biosynthesis of zinc oxide nanoparticles has been investigated for their role in wastewater treatment. The leave extract of the plant acts as reducing, capping and stabilizing agent of nanoparticles, generating spherical nanoparticles of 71 nm average size. The zinc oxide nanoparticles also showed an enhancement in seed germination of *Pennisetum glaucum* (Rafique et al. 2022).

Plant mediated green synthesis of metallic nanoparticles have also been proven to possess anti-bacterial, anti-fungal and anti-cancer properties. Silver nanoparticles biosynthesized from aqueous fruit extract of *Conocarpus lancifolius* have been reported to show effectiveness against fungal pathogens such as *Rhizopus stolonifera* and *Aspergillus flavus* and against bacterial strains *Streptococcus pneumonia* and *Staphylococcus aureus*. They also show activity against breast cancer cell lines MDA MB-231 by inducing apoptosis (Oves et al. 2021). The use of leaves part of *Capsicum chinense* showed that the groups of molecules involving in reduction/stabilisation of gold and silver nanoparticles were polyphenols, reducing sugars and amino acids. The silver nanoparticles also inhibited the growth of bacterial strains which have been known to be highly resistant to antibiotics (Lomelai-Rosales et al. 2022). *Glycosmis pentaphylla* biosynthesized silver nanoparticles displayed remarkable synergistic effect with fungicides and antibiotics proving to be much more effective than conventional fungicides and antibiotics thus opening up a new approach to agriculture and hospital management (Dutta et al. 2021).

7. Factors affecting green synthesis of nanoparticles

Nanoparticles of interest can be synthesized by manipulating the crucial parameters such as temperature, pH, reaction time, reaction concentration and exposure to environmental conditions.

- **pH:** Size, shape and stability of nanoparticles are highly depended in the medium pH, wherein large sized NPs have been shown to be synthesized in acidic conditions and small sized NPs in the alkaline conditions as confirmed by zeta potential values and SPR absorbance peaks (Dubey et al. 2010).
- **Reactant concentration:** Depending on the concentration of the extract and salt solution, the shape and size of the nanoparticles can be manipulated. Higher extract concentration leads to early capping thus producing smaller shaped nanoparticles (Alahmad et al. 2021).
- **Reaction Time:** Biosynthesis of silver nanoparticles using *Dunaliella salina* extract increases with passage of time recording a maximum absorbance (430 nm) at 30 minutes however there was no increase in absorbance upto 35 minutes (Singh et al. 2017).
- **Exposure to sunlight:** Exposure to sunlight is another parameter that is required by most plant extracts for the synthesis of nanoparticles. *Dunaliella salina* reaction mixture showed a change in colour with exposure to sunlight whereas no such change was observed when kept in the dark (Singh et al. 2017).
- **Temperature:** Shape, size and yield of nanoparticles are some characteristics that are temperature dependent. The average size of AgNPs synthesized from *Citrus sinensis* extract decreased with increase in temperature (Kaviya et al. 2011). It was also observed that on higher temperature, nanoparticle synthesis occur at a faster rate than lower temperatures.

8. Role of nanoparticles in cancer treatment

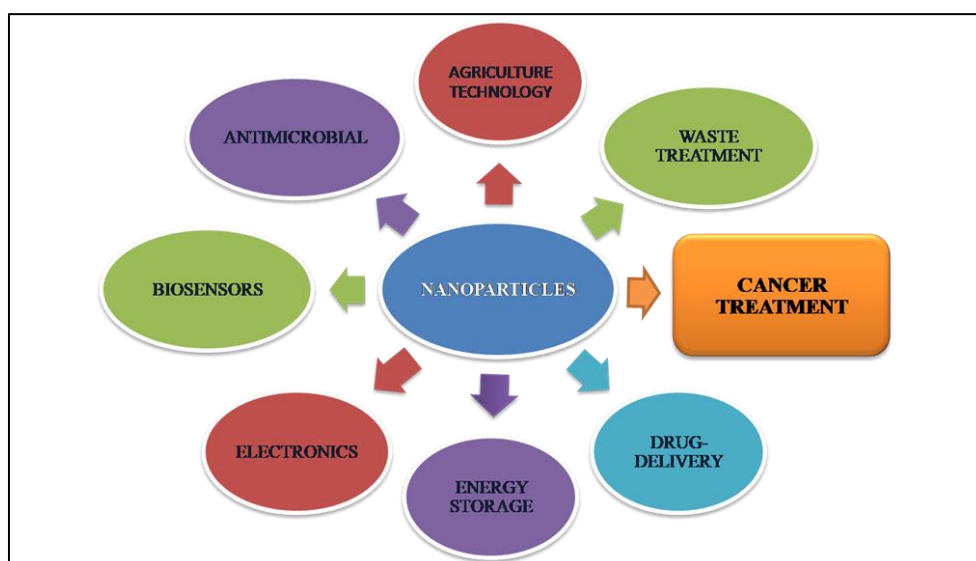


Figure 4 Applications of metallic nanoparticles

Cancer was first described by Hippocrates which has over the years been made several attempts to define the term cancer. According to the American Cancer Society, which provides the simplest definition of cancer, "Cancer is a group of diseases that possesses an uncontrolled growth rate and the property of abnormal cells proliferation". If the spread of cancer cells is not controlled, it will ultimately lead to cell death. Several causes and factors have been reported contributing to carcinogenesis. Cancer cells have been known to possess the property of evading apoptosis and proliferation, which is one of the hallmarks of cancer cells and has gained interest in the field of research (Jain et al. 2021). Other reported hallmarks of cancer are evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, and sustaining proliferative signalling (Hanahan and Weinberg 2011).

According to WHO, cancer is a leading cause of worldwide deaths. In 2020 alone, reported deaths due to cancer complications accounted for nearly 10 million. Cancer can be prevented by implementing day to day existing evidence-based prevention strategies. Available treatment regiments for cancer include invasive surgery, chemotherapy and radiotherapy. These existing strategies not only target cancer cells but are also toxic to healthy cells which overall brings about more side effects on the patient. In order to overcome this, the use of NPs has gain attention in the scientific research to come up with new therapeutic methods. The unique optical and electrochemical properties of AuNPs make them ideal candidates for potential in medical and biological fields (Shukla et al. 2005). Gold nanoparticles have been extensively used in medicine for the detection of cancer cells. They can also induce apoptosis in B-cell chronic lymphocytic leukemia. AgNPs and AuNPs synthesized from *Dunaliella salina* extract displayed no activity against normal cell line MCF-10A whereas they displayed anti-cancer property in cancer cell line MCF-7 by inducing apoptosis

(Singh A et al. 2019). Platinum nanoparticles (PtNPs) from Ajwa extract were also screened for anti-cancer activities and showed promising results (Al-Radadi 2019). According to published literature, AgNPs and AuNPs synthesized from leaf extracts of *Butea monosperma* were used as drug delivery system using doxorubicin against cancer cells and showed better therapeutic efficacy than free drug (Patra et al. 2015).

AuNPs have been reported to reduce cell viability and proliferation; and also showed that NPs restrict cancer cells in a time and dose dependent manner (Hoshyar et al. 2016). AgNPs synthesized with the extract of *Hypericum perforatum* displayed anti-cancer properties by decreasing cell viabilities of the cancer cell lines HeLa, HepG2 and A549 within 2 hours (Alahmad et al. 2021). Copper being an important biological trace element, finds application as an anticancer agent. *Wrightia tinctoria* copper nanoparticles have been found effective against breast cancer cell line MCF-7 causing 50% cell death in a dose dependent manner while displaying no toxicity against normal cell lines. It was proposed that copper ions are released from nanoparticles binding to DNA, causing damage and ultimately leading to cell apoptosis (Rajagopal et al. 2021).

9. Conclusion and future prospective

Since the beginning, history has been shown to be using plants in traditional medicine. Before the invention of drugs, man turned to nature to relieve of his ailments. It is our ancestors that paved the way to discovering the potential remedies using plants. Edible plants and fruits are taken orally or cooked knowing their useful benefits for overall human health. Even in modern times, plants continue to play an important role in treatment. Much research has been done on plants, elucidating their phytochemical compounds and their potential applications in medical treatment and cure. With the advancement in nanotechnology, there is an interest for synthesizing nanoparticles from plant extracts. Plant extracts NPs are more eco-friendly, cost effective and non-toxic. They are also more stable than NPs produced from other organisms. Metallic NPs have been shown to play a huge role in the field of medicine for their antimicrobial, antibacterial, antifungal as well as anti-cancer properties. Exploiting these properties, metallic NPs can be synthesized from exotic local plants which have been used for generations as treatment for various ailments. Plants have been used for centuries as components in ayurvedic medicine without elucidating the exact method of action. Metallic nanoparticles could be the key to exploring the potential of traditional medicine.

Compliance with ethical standards

Disclosure of conflict of interest

The authors report that there is no conflict of interest involved in this work.

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