Bachelor's thesis

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Green synthesis of silver nanoparticles for medicinal purposes

Bachelor's thesis in Chemistry (BKJ)
Supervisor: Elisabeth Egholm Jacobsen
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Contents

Abstract

Silver nanoparticles (AgNPs), among other nanoparticles, are known because of their unique attributes. To contribute to the study of medicine and the treatment of diseases like cancer, they are synthesized, typically using extracts from medicinal plants. Three different plants are applied and evaluated as anticancer agents to understand their properties and mechanisms of action. With green synthesized AgNPs, one can get energy efficiency, low toxicity, high yields, cost-effectiveness, eco-friendliness, and more.

The challenges and benefits of the green-based synthesis are taken into consideration along with possible future research directions. These environmentally friendly methods' results are contrasted with those of chemical processes, and the data is then inspected using different analytical methods.

1 Introduction

Green chemistry helps chemists achieve the intentional goal of sustainability. Then, to minimize negative impacts, it is distinguished by meticulous planning of chemical synthesis and molecular design. All industrial areas, including aerospace, automobile, cosmetics, electronics, energy, household supplies, pharmaceuticals, and agriculture, have adopted green chemistry.[1] This field has proven that chemists can develop lucrative products and methods that also benefit the environment and human health. So, whether it is toxicity, physical hazards, or global hazards, intrinsic hazards of a chemical substance or a chemical process can be designed to be minimized at every stage of a process.[1] The nature of the feedstock and raw materials used in the chemical transformations as well as the finished products may give rise to risks based on these hazards. Intrinsic risks in chemicals and procedures are decreased and/or eliminated through careful design.

Nanoparticles are particles found in natural, incidental, or manmade materials where 50% or more of the particles fall within the size range of 1-100 nm.[2] Due to the unique characteristics of their size, distribution, and morphology, nanoparticles play a crucial role in the rapidly expanding area of nanotechnology.[3] Metal nanoparticles have been widely researched due to their catalytic, optical, electronic, antimicrobial, and magnetic properties. Among noble metals, silver has been known, for more than 2000 years, as a metal that exhibits good medical properties thus the metal of preference in the field of biological systems, living organisms and medicine.[3,4] AgNPs have a diverse spectrum of applications in numerous branches of science and technology, so different synthesis protocols have been developed. Chemical, physical, and biological procedures can all be used to synthesize AgNPs.

For green synthesis, biological agents such as bacteria, fungi, plants, plant-derived pure compounds, algae, carbohydrates, and microorganisms are used.[5] One important area of nanotechnology is the green synthesis of AgNPs, where the use of biological components may provide an environmentally favorable substitute for chemical and physical processes. In the case of biological synthesis of AgNPs, molecules produced by living organisms replace the functions of the reducing agent for reducing Ag^+ ions and the stabilizing agents for avoiding AgNP aggregation.[4] Since toxic chemicals are not used in the synthesis protocol or on the surface of the particle, using environmentally friendly materials like plant leaf extract for the synthesis of AgNPs has many advantages in terms of eco-friendliness and

compatibility for pharmaceutical and other biomedical applications. But how does this plant-mediated green synthesis of silver nanoparticles help in cancer treatment? And why is green synthesis preferable to other chemical procedures?

2 Theory

2.1 Green Chemistry

The term "chemical" is frequently associated with the word "toxic" in the public, leading to chemistry being seen as a dangerous science. Risk can be decreased by taking safety measures and wearing protective gear. The risk, which is determined as a function of the danger and exposure, rises when safety precautions are ineffective. Even under unfavorable conditions, the risk can be reduced by decreasing the hazards part of the equation rather than just concentrating on exposure controls.[1] Designing safer, more environmentally friendly chemicals and methods necessitates minimizing inherent risks to lower the possibility of accidents.

Paul Anastas and John Warner presented the 12 principles of green chemistry in 1998.[1] These principles serve as a framework for the design of novel chemical products and processes, and they apply to all facets of the process life-cycle, including the raw materials used, the effectiveness and safety of the transformation, the toxicity and biodegradability of the products and reagents used. The first principle is “prevention”, which states that it is preferable to stop waste before it is produced than to treat or clean up waste after it has already occurred. The following principle is “atom economy”, indicating that all materials used in the production process should be incorporated as much as possible into the finished product. The third principle deals with “less hazardous chemical synthesis”: whenever possible, synthetic methods should be developed to use and produce substances that have low to no toxicity for both human health and the environment.[1] The design of safer chemicals is the fourth principle, where chemical products should be designed to maintain function effectiveness while lowering toxicity. The use of auxiliary substances, such as solvents, separation agents, etc., should be minimized and, when required, made harmless, according to the fifth principle, “safer solvents and auxiliaries”. The sixth principle is the “design for energy efficiency”, which explains that the minimization of energy requirements is necessary while considering their effects on the economy and the environment. The seventh principle, "use of renewable feedstocks", states that whenever it is technically and monetarily feasible, a

raw material should be renewable rather than depleting. The eighth principle relates to "reducing derivatives"; unnecessary derivatization (use of blocking groups, protection/deprotection, transient modification of physical/chemical processes) should be limited or avoided, as these processes call for extra reagents and have the potential to produce waste.[1] The ninth principle, "catalysis", describes how more selective catalytic chemicals perform better than stoichiometric reagents. The tenth principle, known as "design for degradation," asserts that chemical products should be made in such a way that, when they have served their purpose, they degrade into harmless byproducts and do not remain in the environment. "Real-time analysis for pollution prevention" is the eleventh principle: to enable real-time, in-process monitoring and control before the formation of hazardous substances, analytical methodologies need to be further developed. Lastly, the twelfth principle represents the “inherently safer chemistry for accident prevention”, where substances used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.[1]

2.2 Chemical approaches for synthesis of AgNPs

Chemical synthesis of AgNPs employs three main components: metal precursors, reducing agents and stabilizing/capping agents.[4] In general, different reducing agents, like sodium citrate, ascorbate, sodium borohydride, Tollens reagent, N,N-dimethylformamide (DMF), etc. are used for reduction of silver ions (Ag^{+}) in aqueous or non-aqueous solutions.[6] These reducing agents reduce silver ions into metallic silver (Ag^{0}), which then aggregates into oligomeric clusters. Metallic colloidal silver particles ultimately form from these clusters. To avoid the agglomeration of nanoparticles that can bond to or absorb onto nanoparticle surfaces, it is crucial to use protective agents to stabilize nanoparticles during the preparation of silver nanoparticles. Surfactants with functionalities for interacting with particle surfaces, such as thiols, amines, acids, and alcohols, can prevent sedimentation, agglomeration, and loss of surface properties while regulating particle growth.[6]

The issues with chemical methods include the extensive use of toxic chemicals, the process's lack of environmental friendliness, the need for expensive chemicals with high energy input, the need for sophisticated instrumentation, and additional factors that result in the presence of some toxic chemicals adsorbed on the surface that may be toxic to humans and have negative effects in biomedical applications.[4]

2.3 Properties of AgNPs

The physicochemical properties of AgNPs provides them the ease of entrance into the cells and gives them the ability to efficiently interact with biomolecules inside the cells or on the cell surfaces.[7] The hemocompatibility should be considered when picking a drug material for the treatment of a certain disease like cancer. AgNPs have great potential in anti-cancer activity since they show selective participation in the interruption of mitochondrial respiratory chain that leads to the production of reactive oxygen species (ROS) and disruption of adenosine triphosphate (ATP) synthesis, thus resulting in nucleic acid damage.[7]

2.3.1 Shape and Size of AgNPs

Various studies have established that temperature, pH of the solution, precursor concentration, the molar ratio of capping agent to that of precursor, the types of reducing agents, the strength of chemical interaction between the precursor and different crystallographic planes of AgNPs and the synthesis method determine the size and shape of the AgNPs.[7]

It is also proved that nanoparticles at smaller size distribution present higher rate of antibacterial activity against the tested pathogens compared with the larger size nanoparticles.[8] This happens because the small size nanoparticles will transport into cells much faster than larger ones. Smaller size AgNPs also show larger available and active surface area which is ideal for interaction with many kinds of bacteria. AgNPs can possibly not only interact with the surface of membrane, but also influx into the bacteria.[8]

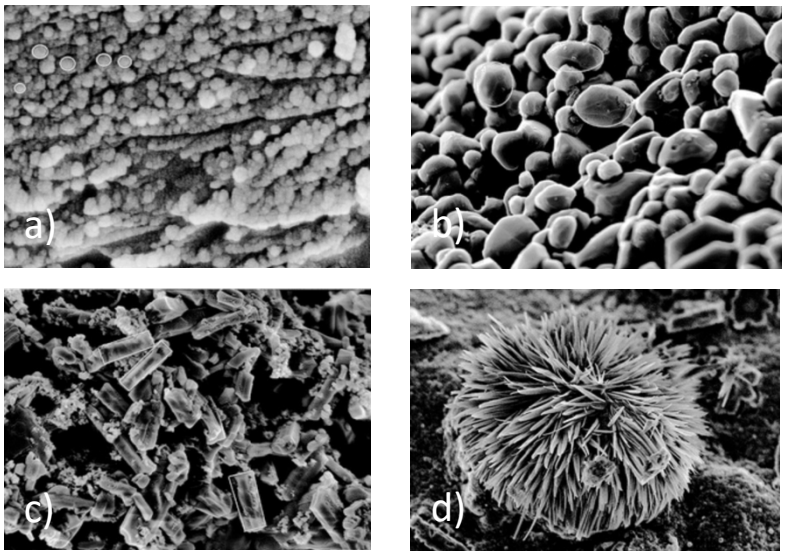

Figure 1.1: FE-SEM images of various shapes of silver nanoparticles (**a)** spherical, **b)** oval, **c)** rod and **d)** flower-shaped). Adapted from ref. [9] with permission from The Royal Society of Chemistry.

2.3.2 Optical character of AgNPs

AgNPs efficiently interact with light through a phenomenon called surface plasmon resonance (SPR) more efficiently than any other organic or inorganic chromophores. This strong interaction is a result of both the restriction of a large density of conducting electrons to smaller dimensions compared to the mean free path and the unique frequency dependence of the dielectric function in metallic silver.[7,10] This gives rise to SPR related properties, as both the frequency and resonance strength can be determined by the size and shape of NPs as well as the dielectric function of the surrounding medium. The light-interaction cross-sections of AgNPs is dependent on the photon electric field which can extend up to 10 times larger than the geometric cross-sections of the AgNPs. These structures are then capable of interaction with light photons that are not physically incident upon them.[7]

Some optical characteristics of AgNPs can also be adjusted to obtain the necessary results. For example, the absorption spectrum of AgNPs is tunable through careful optimization of the synthesis conditions to the near-infrared region. This eliminates the interference from tissue auto-fluorescence, resulting in NPs which have great promise in tumor-targeting and deep tissue-imaging.[7]

2.4 Analytical methods

After synthesis, silver nanoparticles must be characterized for factors like size, form, size distribution, surface area, shape, solubility, aggregation, and more. This is because a particle's physicochemical characteristics should be considered before toxicity or biocompatibility can be identified.[11] The physiological interactions between nanomaterials and their intended biological targets may be substantially influenced by a range of physiochemical properties, including size distribution, electrostatics, surface area, general morphology, and aggregation.[11] Numerous analytical methods, such as ultraviolet visible spectroscopy (UV-vis spectroscopy), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), etc., can be used to analyze the synthesized silver nanoparticles.

2.4.1 Characterization of synthesized AgNPs by UV-vis spectroscopy

For the primary characterization of synthesized nanoparticles as well as for keeping track of the synthesis and stability of AgNPs, UV-vis spectroscopy is a very reliable and efficient technique. AgNPs have unique properties that make powerful interaction with light wavelengths possible.[12] Additionally, UV-vis spectroscopy is quick, easy, practical, delicate, specific for various types of NPs, requires only a brief measurement time, and does not require calibration for the characterization of nanoparticles. Particle size, the dielectric medium, and the chemical environment all have an impact on absorption.[7]

Conduction and valence bands in AgNPs are relatively near to one another, allowing for simple electron motion. Due to the collective oscillation of the electrons of the metallic nanoparticles in resonance with the light wave, these free electrons give rise to the SPR absorption band.[13] With the maximum reduction and formation of metallic silver nanoparticles being defined by the absorbance intensity, UV-vis spectra can be used to track the production of AgNPs. However, UV-vis spectroscopy is inadequate on its own to provide detailed descriptions about AgNPs.

2.4.2 Characterization of synthesized AgNPs by FTIR spectroscopy

FTIR spectroscopy has been predominantly used to analyze the proteins connected to the silver and surface atoms of the capping agents of the nanoparticles. Precision, repeatability, a high transmission ratio, and the ability to tell whether biomolecules are actively involved in the formation of nanoparticles are all provided by FTIR. The study of nano scaled materials has also been done using FTIR, including the detection of functional molecules covalently bound to silver, carbon nanotubes, graphene, and gold nanoparticles, as well as enzyme-substrate connections during catalytic reactions. The presence of peaks indicates that the secondary metabolites of plants, including aldehydes, ketones, carboxylic acid, phenols, glycosides, and terpenoids, are coated with NPs.[13] The decrease of metal salts in crude extracts is caused by the elevated levels of flavonoids and antioxidants.

The absorption band in a FTIR spectra of plant-mediated synthesis of AgNPs and plant extracts is due to the vibration effect of phytochemicals present in the plant extract, which is responsible for capping and stabilization of AgNPs.[14] FTIR can confirm that the amino acid residue and protein carbonyl group have a stronger ability to bind the metal and might prevent agglomeration.

2.4.3 Characterization of synthesized AgNPs by SEM & TEM

SEM and TEM are used to characterize AgNPs shape and morphology.[15] It has been demonstrated that mostly spherically shaped NPs are detected by electron microscopy in case of plant mediated AgNPs.[7] Although SEM images have better depth of field than TEM images, these have a thousand times greater resolution. TEM is a significant, extensively used, and essential technology for measuring particle size, particle distribution, and shape quantitatively. The magnification of a TEM is determined by the distance between the objective lens and the sample as well as the distance between the objective lens and its image plane.[13] The drawbacks of TEM include the time-consuming sample preparation process, the small sample size, and the high-pressure requirement. To accelerate the electrons to the desired velocity and frequency before they interact with the sample that is to be analyzed, electron backscatter diffraction examinations are frequently conducted in TEM.[13]

2.5 Anticancer effect of AgNPs

Cancer is a fatal disease that leads to deaths all over the world. It is a complex illness brought on by an intricate web of hereditary and environmental influences. Even so, knowledge of the genetic, biochemical, and cellular causes of cancer can offer novel therapeutic aims and tactics. Many anticancer drugs reach their target site at insufficient concentrations and exert the pharmacological effect without causing irreversible damage to healthy tissues and cells.[13] Nowadays, a variety of chemotherapy medicines serve to treat different kinds of tumors, yet they carry serious side effects and require lengthy intravenous infusion procedures.[16] A variety of cell lines have been used by various research facilities to examine the prospects for developing a brand-new cancer treatment.

The finest and most effective alternative therapeutic approach for treating cancer is nanoparticle-mediated therapy. Because of their capacity to target diseased cells or tumor tissues by encasing the therapeutic substance in the nanoparticles, NPs have been used as medication delivery systems. The variation of the tumor and its surroundings present a significant challenge for medical professionals and nanotechnologists in creating specialized compositions to specifically target individual cancers, despite the development of several nanoparticle-mediated techniques. To obtain greater specificity, less toxicity, biocompatibility, and better efficiency while overcoming the drawbacks of conventional chemotherapy, new nanoparticles are used in single platform-based techniques.[13] It is

important to address the challenges and limitations of using nanoparticles to treat cancer, such as their limited carrying capacity and physiological restrictions.

2.5.1 Mechanism of action of AgNPs

AgNPs in mitochondria may offer the perfect surface for Ag^+ to bind with DNA and proteins and interfere with their functions. Additionally, oxidative stress and ROS production emerge as the initial process that triggers toxicity brought on by nanoparticles.[17] Due to substantially improved mitochondrial function, cytotoxicity is induced. AgNPs alter the physiological functioning of cells as well as their shape, stability, and oxidative stress levels, which leads to mitochondrial dysfunction. AgNPs produced biologically are intended to alter the structure of cancer cells, which is a step toward apoptosis.[16] Apoptosis could be identified using transmitted light microscopy by observing morphological changes in cells. Besides regulating the expression of genes and pro-inflammatory cytokines, AgNPs are shown to be efficient at absorbing cytosolic proteins from their surface. Autophagy inhibitors enhance the apoptosis that AgNPs causes in cancer cells. Autophagy may aid in cell survival at low concentrations, but at large concentrations, it may also cause cell death.[13] The production of ROS is accelerated by the elevated intracellular ion concentration. AgNPs that have been endocytosed are destroyed by lysosomes, and cells become injured when Ag^+ ions escape into the cytoplasm. AgNPs' cytotoxicity and genotoxicity are affected by the amount of time spent synthesizing them.[16] AgNPs could then trigger apoptosis by several different pathways, including elevated lactate dehydrogenase release, increased apoptosis and autophagy gene expression, mitochondrial dysfunction, caspase stimulation, and DNA damage.

2.5.2 Plant examples with synthesized AgNPs

2.5.2.1 Taraxacum officinale

Furthermore, various examples of medicinal plant mediated synthesized AgNPs are tested for their anticancer activity, such as AgNPs with Taraxacum officinale leaf extracts, which are active against human liver cancer cells (HepG2). The anti-tumor potential of Taraxacum officinale-mediated AgNPs is comparable to commercial anticancer medicines, which is significant.[13] The increase in SPR centered at 435 nm indicates synthesis of AgNPs, and no change in position over time is observed. The NPs were stored up to 4 months at room temperature and there was no difference in the SPR peak or aggregation of NPs. A wide peak at 3360 to 3400 cm^{-1} can also be seen in the FT-IR spectrum of the TOL extract, which may

be caused by the stretching vibration of both the -NH_2 and -OH groups in primary aromatic amines and alcohols.[18] Strong absorption bands at 1610 and 1063 cm^{-1} are attributed to the existence of carbonyl group stretching and C-OH stretching, respectively, in the extract. The stretching at 3360 to 3400 cm^{-1} of the stabilized TOL-AgNPs' FTIR spectrum decreased and slightly shifted, which may be a sign that the intermolecular H-bonding has become weaker. A 50-nm scale HR-TEM image of TOL-AgNPs revealed nearly spherical shape and size in the 5 to 30 nm region.[18]

2.5.2.2 Vitex negundo

By using AgNPs synthesized with Vitex negundo (leaf), activity against the HCT15 line of human colon cancer growth inhibition is shown.[19] Cytomorphology observation reveals the compacted, constrained, treated, and healthy regulated cells. Propidium iodide staining and DNA fragmentation by single-cell gel electrophoresis are used to analyze apoptotic shifts and nuclear condensation. The biosynthesized AgNPs at IC50 of 20 µg/ml inhibits the multiplication of colon cell line HCTL5 (human), stops the G0/G1 process, observes decreased DNA synthesis, and induces apoptosis.[13] The UV-Visible spectrum shows a peak around 410 nm corresponding to the Plasmon absorbance of the AgNPs. In the FTIR spectrum, the combination of nanoparticles with V. negundo biomolecules resulted in intense peaks at 3435, 3331, 2214, 1557, 1416, 1124, and 1051 cm^{-1}.[20] The bonds pertinent to the hydroxyl single bond -OH (3331 cm^{-1}) and carboxyl group (3435 cm^{-1}) were identified by these FTIR peaks. According to a study of V. negundo's bioactive substances, the plant has a high concentration of total phenolic compounds and flavonoids, which are both recognized as powerful natural antioxidants.[20] This demonstrates how polyphenolics, such as tannic acid, supply the free energy necessary to convert silver ions into AgNPs. With a size range of 5 to 47 nm, TEM reveals that natural particles are spherical and evenly distributed.

2.5.2.3 Solanum trilobatum

Since they also exhibit promising activity, AgNPs synthesized by Solanum trilobatum unripe fruit extract are useful when evaluating the cytotoxicity against breast cancer MCF7 cell line.[13] The bands at around 400–420 nm correspond to the AgNPs and UV–vis absorption spectra shows broad surface plasmon resonance at 432 nm.[21] To determine the potential biomolecules responsible for the reduction of Ag^+ ions and capping of bio-reduced silver nanoparticles synthesized by the fruit extract, FTIR analyses of both the aqueous fruit extract

and synthesized dried silver nanoparticles were performed. The fruit aqueous extract shows strong bands at 3446 cm^{-1} (O–H stretch), 2853 cm^{-1} (C–H stretch), 1623 cm^{-1} (–C=O stretch), 1385 cm^{-1} (C–C stretch) and 1020 cm^{-1} (C–N stretch). AgNPs synthesized using S. trilobatum then show similar results with strong bands at 3424 cm^{-1} (O–H stretch), 2853 cm^{-1} (C–H stretch), 1639 cm^{-1} (–C=O stretch) and 1020 cm^{-1} (C–N stretch).[21] SEM results demonstrate the synthesis of higher density polydispersed spherical AgNPs of different sizes. Only a small number of the nanoparticles are dispersed since most aggregate. To characterize Solanum trilobatum, the Western blot analysis and RT-PCR were also used to better understand its mechanism. The RT-PCR results show that MCF 7 cells treated with AgNPs, exhibit reduced levels of Bcl-2 expression, while Bax is increased.[21]

2.6 Advantages & disadvantages of green synthesis

Green synthesis possesses some advantages over traditional chemical methods. It is simple, cost-effective, and usually involves a one-pot reaction. The toxicity-associated hazardous chemicals are eliminated, increasing the biocompatibility of the resulting product with normal tissues for in vivo applications. Green biological entities can also be used as reducing agents and capping agents, providing AgNPs with enhanced colloidal stability, which is an important factor when making claims regarding the biological activity of AgNPs.[5] In fact, biosynthesis results in low-energy use and environmental impact, with respect to conventional chemical synthesis methods. The high specificity of biomolecules involved in the biosynthesis process is also important since it enables an efficient control of AgNPs size and shape, whose tight control is critical to optimize applications.[11]

AgNPs have numerous features that make them an ideal candidate for potential medicinal applications, although their toxicity is not one of them. AgNPs are commonly advertised as very efficient antibacterial agents that are safe for healthy mammalian cells but the toxicity of AgNPs relates to their conversion to biological and environmental systems.[22] Since they are used in electronic technologies, cleaning products, scientific equipment, the food processing industry, etc. the widespread usage of AgNPs as antimicrobials and disinfectants may increase bacterial resistance.[13] AgNPs can cause hazardous risk to aquatic creatures, making this toxicity in aquatic species larger than that in land animals and humans. This ecological toxicity is primarily linked to the continuous release of nano-sized silver, and the chemicals' impact on marine life is due to their larger dispersion and discharge. The physicochemical

characteristics of AgNPs such as dispersion rate, concentration, surface characteristics, size and shape impact the genotoxicity and cytotoxicity.

The use of plants as sustainable and renewable resources in the synthesis of AgNPs is more advantageous over microorganisms, which need expensive methodologies for maintaining microbial cultures and more time for synthesis.[11] Using plant extracts is then considered the most economical, energy efficient and cost-effective out of all the applications that can be used in green synthesis of AgNPs. It protects human health and environment leading to lesser waste and safer products. Also, previous studies reported that the bio reduction potential of the plant extracts is comparatively higher than the microbial culture.[11] Plant-mediated synthesis brings less contamination and so reducing the impact on the environment.

2.7 Future of AgNPs

The pharmaceutical industry has recently paid a lot of attention to the green synthesis of AgNPs. AgNPs' high biodegradability and clearing rate are crucial in preventing the possibility of long-term toxicity.[7] Although clinical trials of AgNPs-based nanomedicine are required for directing the future direction of their application, the AgNPs demonstrated excellent potential in cases of nanomedicine-based treatment. Investigations into biodegradability, dose, and administration route are currently the main challenges that need to be overcome in clinical trials. AgNPs can also be used as a crucial instrument for cancer cell visualization and detection when making an early diagnosis of the disease. AgNPs' green synthesis has already been demonstrated to be beneficial for in vivo fluorescent tumor imaging.[7]

Business experts have extrapolated that the future of the global nanotechnology industry could be promising. A substantial amount of commercially produced AgNPs are being manufactured, and more and more NPs are being used as therapeutic agents. Based on the positive outcomes of prior research studies as well as their cost, AgNPs are potential drug leads.[23] However, there are some fundamental toxicity issues that prevent the use of AgNPs as therapeutic drugs. The AgNPs must be biocompatible, non-toxic, and symptom-free to get past these obstacles and be used in preclinical experiments on humans or any other living organisms.[7] Finally, in-depth datasets that reveal possible toxicity and pharmacological problems like side effects are needed before using biogenic AgNPs as a cancer nanomedicine.

3 Discussion

The goal of the anticancer mechanism of plant mediated AgNPs is to achieve apoptosis. Although the main parts and purposes of this mechanism were already mentioned in section 2.5.1, a more detailed step-by-step explanation of the mechanism is necessary to fully understand how these AgNPs help in cancer treatment. The anticancer mechanism starts with the AgNPs upregulating caspase-8, which leads to the activation of pro-apoptotic proteins (Bid and tBid). This further boosts the mitochondria's release of cytochrome C, which triggers Apaf-1 and results in the formation of an apoptosome.[24] Following the activation of caspases, apoptosis occurs. To accelerate apoptosis, AgNPs also directly upregulate cleaved caspase-3. Secondly, AgNPs cause mitochondrial apoptosis by making the mitochondrial membrane more permeable and decreasing ATP synthesis.[16,24] Apoptosis is induced by AgNPs by upregulating the apoptotic proteins Bak and Bax, which also causes a rise in cytochrome C release. Furthermore, AgNPs damage genetic material DNA by increasing the generation of ROS, which produces oxidative stress. These nanoparticles stop the cell cycle at the G2/M region and inhibit protein kinases. The P-23 Protein, which activates other pro-apoptotic proteins to start the apoptosis process, is upregulated by AgNPs. Intracellular pro-apoptotic proteins are produced after DNA damage, which triggers caspases and results in cell death. Finally, the VEGF-2 receptor is how vascular endothelial growth factor (VEGF) promotes angiogenesis (tyrosine kinases).[24] The proliferation of tumor cells resulted from VEGF's subsequent induction of angiogenesis—the creation of new blood vessels from already existing ones—can turn a benign tumor into one that is malignant. It is therefore clear that AgNPs activate apoptosis through cellular damages, the anti-angiogenic pathway, and the caspase cascade pathway.

In section 2.5.2.1, there was an increase in SPR at 435 nm and no change in position over time, which might indicate formation of uniform particle shape. Even after 4 months, there was no difference in the SPR peak which shows the significant stability of AgNPs. The TOL leaf extract was then used in the FTIR to determine the potential biomolecules implicated in the capping and reduction of AgNPs. The hydroxyl groups may form intermolecular hydrogen bonds with one another, which would explain the peak's broadness. The carbonyl groups and aromatic rings were discovered to be involved in the nanoparticle formation in the FTIR spectrum of the stabilized TOL-AgNPs.[18] Many pharmacologically active compounds, including flavonoids and phenolic acids, may have contributed to the reduction of silver ions

and formation of the matching AgNPs in the leaf extract. In the absence of other powerful ligating agents, these flavonoid and phenolic acids may interact with metal nanoparticle surfaces through π-electrons and become adsorbed there.[18] Therefore, it appears that the biomolecules in leaf extract were effectively reducing, capping, and stabilizing the manufactured AgNPs.

Thus, there are many benefits to using TOL extract over other biological resources, including: a green synthesis route that combines the medicinal effects of the plant preparation with those of AgNPs; a short incubation time for the synthesis of AgNPs with long-term stability; a higher antibacterial potential against two important phytopathogens; and the possibility of using a synergistic antibacterial effect with conventional antibiotics to form nanomaterials.[18]

In an aqueous medium, the plant extract from Vitex negundo reduces and stabilizes the synthesis of AgNPs. $AgNO_3$ is reduced by vitex negundo's leaf extract through the oxidation of the amino group which involves the transfer of electrons from the functional group to the Ag+ ions.[20] The biosynthesis of AgNPs from the leaves of Vitex negundo prevents the development of the HCT15 colon cancer cell line and causes apoptosis, as mentioned in section 2.5.2.2. The AgNPs are produced in vitro and have cytotoxic effects against human colon cancer cells because of a weak chemical interaction with components of the leaves extract. In a concentration-dependent way, the leaves extract-capped nanoparticle solution was toxic to HCT15 cells. The findings imply that G0/G1 cell cycle arrest is a mechanism by which AgNPs promote apoptosis.[20] This resulted from DNA damage and increased levels of reactive stress in cells exposed to AgNPs. Therefore, by conjugating with metabolites, biologically produced silver nanoparticles can be used to treat cancer cells in addition to being used to deliver drugs.[20]

By comparing the two FTIR spectra of Solanum trilobatum (section 2.5.2.3), changes can be seen in the peak at 3446 cm^{-1}, where stretching vibrations of the hydroxyl/amine groups of unripe S. trilobatum fruits change noticeably to a lower wavelength of 3424 cm^{-1}, indicating the attachment of silver nanoparticles to the surface. Ag^+ ion reduction results in the peak at 1623 cm^{-1} shifting to a higher wavelength (1639 cm^{-1}), which indicates the presence of Ag nanoparticles.[21] The AgNPs contribute to the absorption band at around 400–420 nm in UV–vis spectra which means spherical shape. The RT-PCR results together with the Western blot results imply that AgNPs induce cell line death and that the mitochondrial pathway may be

implicated. Mitochondria are crucial signaling centers during apoptosis, and diverse apoptosis regulators can either promote or inhibit mitochondrial integrity damage.[21] The discharge of cytochrome C from the mitochondrial intermembrane space into the cytosol is another way that oxidative stress is thought to trigger caspase activation. The findings indicate that AgNPs activates caspases 3 and 9 to cause cell death. By activating the intrinsic apoptotic pathway, which is characterized by modulation of Bax, mitochondria may be a significant site for AgNPs-induced ROS generation.[21] Bcl-2 expression in the mitochondria subsequently causes cell death by the caspase dependent pathway.

It is important to note that AgNPs have the potential to cause considerable oxidative stress to the cellular membrane and organelles including the nucleus, mitochondria, and lysosomes resulting in necrotic or lethal responses.[13] Hence, AgNP-induced oxidative stress could trigger inflammatory reaction, including the stimulation of innate immunity and an increase in endothelial leakage. AgNPs can therefore cause chromosomal abnormalities, DNA damage, and potential carcinogenicity when inoculated at non-cytotoxic dosages.[13] However, in comparison to conventional physical and chemical techniques, plant-mediated synthesis of AgNPs is simpler and easier to carry out without requiring any operating conditions. Higher reproducibility of the procedure and higher stability of the synthesized nanoparticles can be obtained using this bio-based nanoparticle synthesis protocol. As a result, green-based nanoparticle fabrication is appropriate for large-scale production by being very eco-friendly and secure for use in human therapeutics.[25]
The biosynthetic method using plant extracts has a practical technique and is a good option to traditional chemical and physical nanoparticle preparation methods due to the various benefits, stated in section 2.6, and outstanding features over other methods. By reducing the use of toxicity-related hazardous chemicals, the green synthetic approach of AgNPs based on plant extracts helps to safeguard the ecosystem and our health. Once more, to promote the safe use of AgNPs, the associated toxicity and safety concerns should be thoroughly investigated due to the growing number of applications for AgNPs.

AgNPs show great promises as effective anti-tumor drug-delivery systems. Conventional cancer treatments as chemotherapy, radiotherapy or surgery have their limitations associated with drug toxicity, unpredictable side effects, drug resistance problems and lack of specificity.[7] However, AgNPs overcome these issues by reducing the side effects and

increasing the efficiency of cancer therapy. One of their main features is the ability to cross various biologic barriers and to provide targeted delivery of drugs.

Green synthesis of AgNPs with specific delivery of anticancer drugs to tumor tissues offers an innovative approach in improving cancer treatment. The theranostics approach (combination of therapy and diagnosis) is one of the most attractive but also challenging approaches.[26] It was proven to be effective in cancer treatment since it helps getting a personalized treatment according to each patient. AgNPs are also plasmonic structures, thus capable of scattering and absorbing the lights impinging certain areas, as mentioned in section 2.3.2. After their selective uptake into cancerous cells, AgNPs-derived scattered lights can be used for imaging purposes.

Future ambit for silver nanoparticles in medicine include fluorescence imaging and cancer therapeutics, where the nanoparticles enable targeted drug administration, improved bioavailability, and sustained drug release in the target tissues, as well as improved nanoparticle drug stability. However, more research is necessary to clarify the mechanism of plant mediated AgNPs used in biomedical applications.[13] A clearer understanding of the mechanism and utility of silver nanoparticles may result from such research.

4 Conclusion

AgNPs can be used as effective cancer therapeutics because of their capacity to disrupt the mitochondrial respiratory chain, which allows them to be developed to trigger the creation of reactive oxygen species, ATP synthesis, and ultimately DNA damage. AgNPs therefore conclude that ROS's oxidative stress is the main cause of toxicity against cells. Due to their large surface area, AgNPs may also effortlessly enter cells and interact with cell components, disrupting the cellular signaling pathway.

Green methods have great advantage compared to chemical methods. They are inexpensive, eco-friendly, non-toxic and no complex setup is required to conduct the synthesis process. It is also not necessary to use stabilizing agents to prevent agglomeration of the NPs and since these processes are carried out in ambient conditions, they are not energy intensive. These methods offer finer tuned control of the size and shape of the NPs compared to chemical and physical methods. There is still, however, some disadvantages that must be considered with green methods like not being as fast as synthesis by chemical methods. Besides all the disadvantages it is understood that green synthesis is the better method since it improves

human and environmental health safety by modifying how chemicals are manufactured, designed, and employed.

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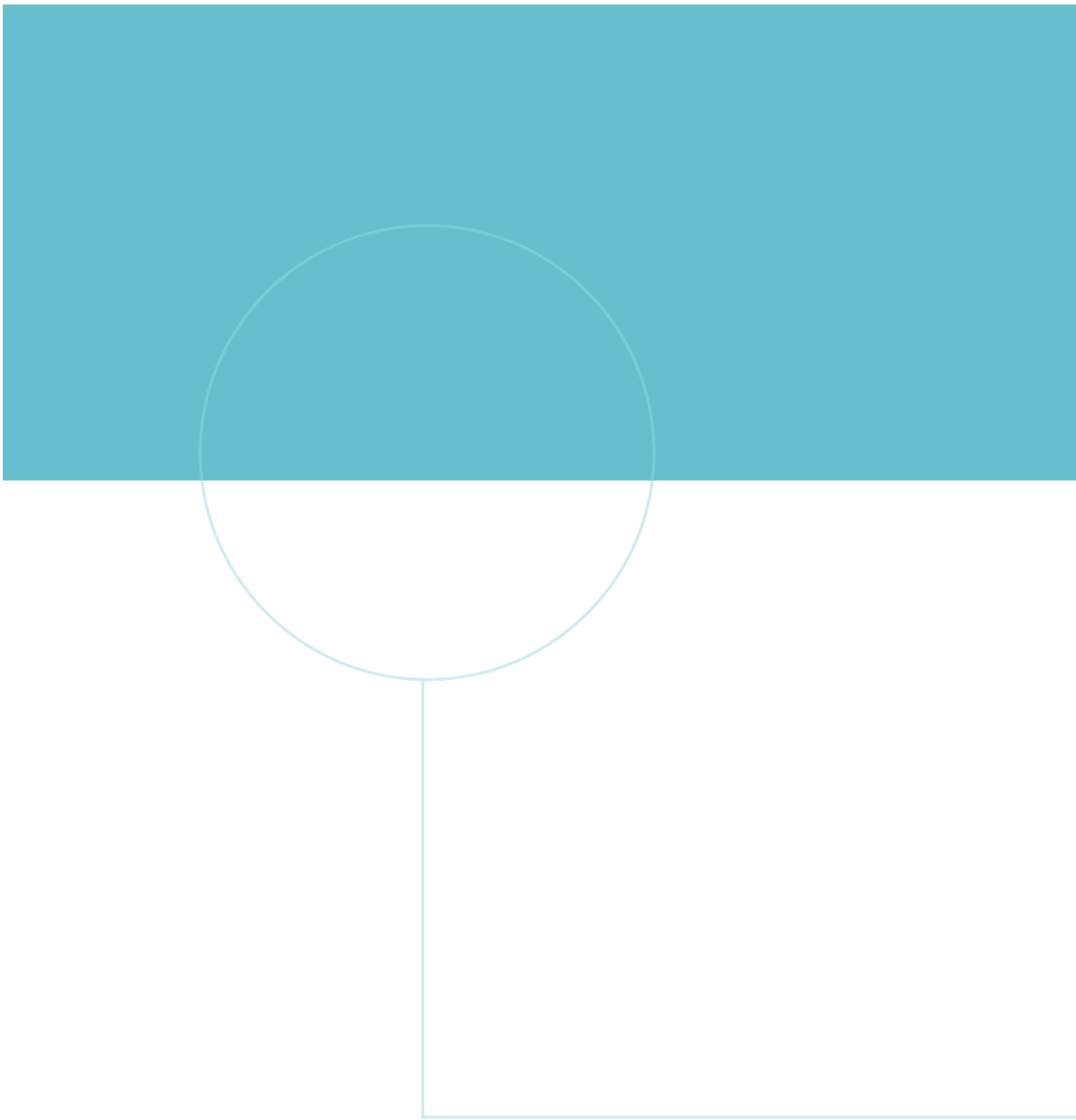

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