Green synthesis of *Aconitum violaceum*-mediated Ag-ZnO nanocomposites and their potential biomedical applications

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Author's Declaration

I**, Fazal Rahman S/O Fazal Qayum,** Registration no 02272111025, MPhil Biotechnology scholar, Department of Biotechnology, Faculty of biological sciences, Quaid-I-Azam university Islamabad, Pakistan, hereby declare that the quoted data in thesis entitled "**Green synthesis of *Aconitum violaceum*-mediated Ag-ZnO nanocomposites and their potential biomedical applications"** is based on genuine work carried under the supervision of **Dr. Bilal Haider Abbasi** and has not been submitted or published somewhere else.

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DEDICATION

I DEDICATE MY WORK TO MY PARENTS WHO HAVE ALWAYS LOVED ME UNCONDITIONALLY AND WHOSE GOOD EXAMPLE HAS TAUGHT ME TO WORK HARD FOR THE THINGS THAT I ASPIRE TO ACHIEVE. I ALSO DEDICATE THIS THESIS TO MY ALL RESPECTABLE AND HONORABLE TEACHERS WHO HAVE SUPPORTED ME IN DEVELOPING MY PERSONALITY AS A COMPETENT PROFESSIONAL.

Fazal Rahman

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All praise is due to **Almighty Allah**, the omnipotent, the most compassionate and His Prophet **Muhammad (P.B.U.H),** the most perfect among all ever born on the surface of the earth, who is a forever source of guidance and knowledge for humanity.

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Fazal Rahman

Certificate of approval

This is to certify that the Department of Biotechnology, Faculty of Biological Sciences, Quaid-I-Azam university Islamabad, Pakistan accepts the dissertation entitled **"Green synthesis of *Aconitum violaceum*-mediated Ag-ZnO nanocomposites and their potential biomedical applications"** submitted by Fazal Rahman, in the present form as satisfying the dissertation requirement for the Degree of Master of Philosophy in Biotechnology.

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List of abbreviations

$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticles
ZnO	Zinc oxide
Ag-ZnO	Silver-zinc oxide
NMS	Nanomaterials
NCs	Nanocomposites
DPPH	2, 2-diphenylpicrylhydrazyl
HPLC	High performance liquid chromatography
KP	Khyber Pakhtunkhwa
PEG	Polyethylene glycol
PGA	Propylene Glycol Alginate
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium
NADPH	Nicotinamide adenine dinucleotide phosphate
AV	*Aconitum Violaceum*
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
XRD	X-ray Diffraction
FTIR	Fourier-Transform infrared spectroscopy
SEM	Scanning electron microscopy
EDX	Energy dispersive x-ray spectroscopy
DLS	Dynamic light scattering
CVD	Chemical vapor deposition process
SDR	Spinning disc reactor

Abstract

Nanotechnology is one of the most promising technologies of the current era which gained a dynamic interest in the field of life sciences. Conventional approaches have been employed for nanoparticles synthesis, but they have many adverse effects. The utilization of medicinal plants for the production of nanoparticles have many benefits over other methods. Numerous bioactive compounds found in plants have the potential for the reduction and capping of nanoparticles. The purpose of this study was to synthesize silver-zinc nanocomposites (Ag-ZnO NCs) from roots, leaves and stem of *Aconitum violaceum* with their relevant extracts followed by characterization. The biosynthesized NCs were subjected to characterization using XRD, FTIR, SEM, EDX, DLS and HPLC. XRD pattern confirmed crystalline structure of NCs with average size of 62-72 nm. FTIR showed capping of NCs with phytochemicals present in root, leaf and stem of *Aconitum violaceum*, which acts as stabilizing and reducing agents in the synthesis of NCs. DLS was used to measure the zeta potential and particle size distribution. SEM demonstrated spherical structures either less or more clustered with some agglomerated particles. HPLC analysis was performed for both extract and Ag-ZnO NCs which revealed the presence of bioactive compounds (Benzoylmesaconine, Benzoylaconine, Benzoylhypaconine, Mesaconitine, Hypaconitine, Indaconitine, Aconitine). These biosynthesized NCs and their relative extracts demonstrated a broad spectrum of usages. Highest hemolytic activity (6.6 %) and brine shrimp lethality assay of Ag-ZnO NCs LC50 value (64.5±4.78 ug/ml) shows the toxicity of these nanocomposites. Anti-cancerous activities were tested using ROS/RNS, Caspase 3 gene expression, mitochondrial membrane potential and MTT assay. All the activities confirmed that Ag-ZnO NCs have high anticancer applications. This could be used for cancer therapy and drug delivery agents. Whole outcomes showed that the bio-fabricated Ag-ZnO NCs have vast in vitro biological and biomedical applications and could be utilized as a wide range agent for a wide range of biomedical applications. However, more investigations are required to explore and evaluate the therapeutic effects of Ag-ZnO NCs on animal models.

1. Introduction

Nanotechnology has placed the basis for tremendous industrial applications and exponential growth. For example, in the medical field, nanotechnology has had a reflective impression on medical devices like diagnostic biosensors, drug delivery and imaging probes. The utilization of nano-materials in food as well as cosmetics industries has extremely increased to improve production, packaging, bioavailability and shelf life (Hulla, Sahu, & Hayes, 2015). The use of nanoparticles is surprisingly increased in various fields like chemistry (organic & inorganic), material science, molecular biology, and medicine (Jamkhande, Ghule, Bamer, & Kalaskar, 2019). The word "nano" states to a Greek word which mean very small or dwarf. Roman utilized nanomaterials in fourth century AD, which revealed one of the most remarkable examples of nanotechnology in prehistoric era (Leon, Chung, & Rinaldi, 2020).

Nanotechnology deals with the materials at molecular or atomic level, size between 0.1 to 100 nm (Nikolova, Slavchov, & Nikolova, 2020). The nano-objects including nanoparticles, nanocomposites, nano-pores, nano-capacitors, nanotubes and nano-channels are developed via nanotechnology which improved the world. These nano-objects have numerous analytical applications throughout the world (Rahman et al., 2019). Nanostructured materials have at least one dimension in nanometer in the form of thin film with nanoscale thickness, nanowires, nanorods, nanoparticles (NPs) and bulk materials with nanoscale building blocks (Sahani & Sharma, 2021). Nanotechnology includes the fabrication and use of physical, chemical and biological systems at scale range from single molecule or atoms to submicron dimensions. It also includes the incorporation of these resulting materials into larger systems (Nasrollahzadeh, Sajadi, Sajjadi, & Issaabadi, 2019). Numerous methods like chemical, physical and biological methods can be used for the synthesis of nanoparticles (Sajid & Płotka-Wasylka, 2020).

A variety of negative effects have been linked with chemical synthesis due to the presence of different surface absorbed toxic chemicals. Biological methods using microorganisms, fungus, enzymes, plants and plants extracts for nanoparticles synthesis, are the eco-friendly alternatives to chemical and physical methods (Hasan, 2015). Plants are among the natural products that have played a significant role to treat various diseases. Around 70,000 plant species, from lichens to tall trees, are thought to have been used for medicinal purposes (Kuruppu, Paranagama, & Goonasekara, 2019).

Green nanotechnology offers approaches to transform biological systems that could synthesize environmental friendly NMs, while avoiding any linked toxicity (Nasrollahzadeh, Sajjadi, Sajadi, & Issaabadi, 2019).

Top-down and bottom-up are the two approaches for nanoparticles synthesis. Various physical or chemical approaches are used in top-down synthesis to reduce the size of macroscopic systems to nanometers. Components of atomic or molecular dimensions can be gathered together to make nanoparticles via bottom-up approach. The bottom-up approach is used to synthesize nanoparticles, because it allows the control over their size (MODAN & PLĂIAȘU, 2020). Green synthesis approach involves the plant materials like fruits, leaves, flower, root, stem and bark etc. which serves as stabilizing and reducing agents, are mixed with chosen metal solution, like $AgNO_3$ (sliver nitrate), $Zn(CH3COO).2H_2O$ (zinc acetate), $CuSO_4.5H_2O$ (Copper (II) sulfate pentahydrate), ZnO (zinc oxide), TiO_2 (Titanium Oxide) and other metals (Chandra, Kumari, Bontempi, & Yadav, 2020).

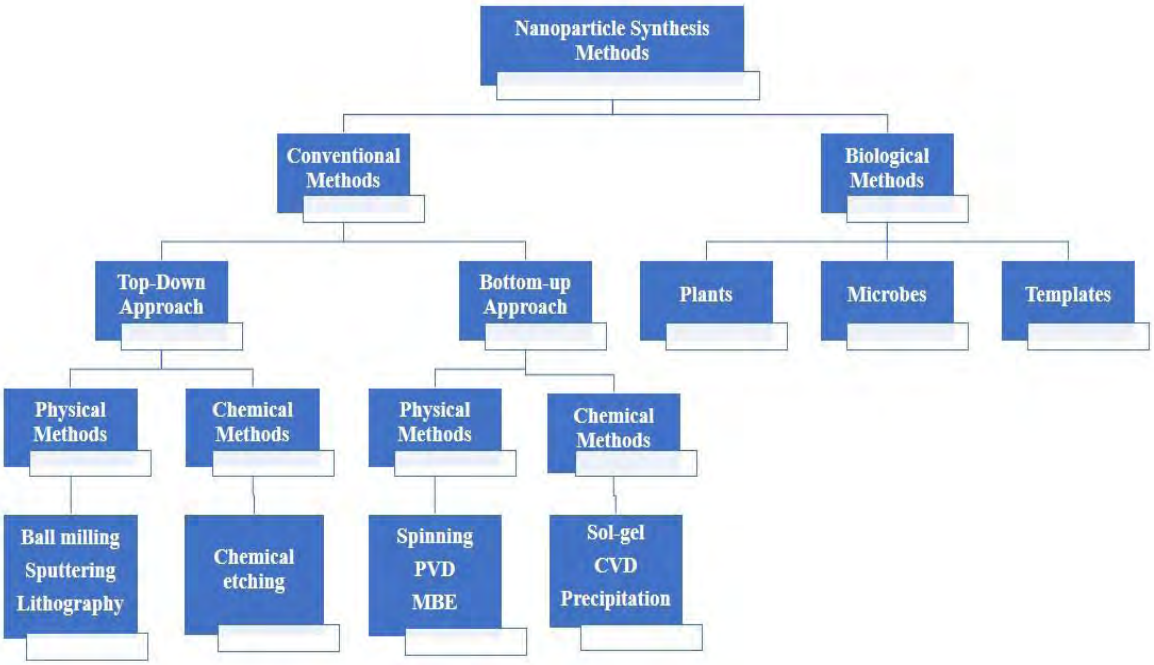


***Figure 1*: Various physical, chemical, and biological routes of NPs synthesis.**

It is well-known today that various metals including Zn, Ag, Te, Al, Sb, As, Pb, Cd, Ni, Co, Mn, Cr, Mo, Ga, and Cu etc. have a wide range of medical applications including antimicrobial activity (Gudkov et al., 2021). Metallic nanoparticles play a key role in various scientific fields like physics, mechanics, pharmaceutics, medicine and so many others (Shnoudeh et al., 2019). Ag (Silver), ZnO (zinc oxide) and TiO_2 (titanium oxide) are utilized in various fields to control microbial growth (da Silva et al., 2019). ZnO-NPs are a class of metal oxides having both photocatalytic and photo-oxidizing abilities against biological and chemical species. It also have a wide range of applications in pre-ecological, biomedicinal, and biomedical (drug delivery and bioimaging) fields as it is biodegradable, biocompatible and less toxic materials (Chikkanna, Neelagund, & Rajashekarappa, 2019) (Arya et al., 2021) (Muhammad, Ullah, Haroon, & Abbasi, 2019).

Similarly silver nanoparticles have become a valuable low temperature bonding materials due to their unique properties (Yan, 2021). Silver nanoparticles undergone extensive research for various applications such as consumer products, optical sensors, therapeutics, water purification (Defnet & Zhang, 2021) (Deshmukh, Patil, Mullani, & Delekar, 2019) and for the removal of 4-nitrophenol due to their specific properties like photothermal effects and resistance to oxidation and corrosion (Rezaei & Dinari, 2021). Silver nanoparticles have the potential utilization in medicine for biosensing, bioimaging as well as anticancer (Xu et al., 2020) and antibacterial (released Ag^+ ion to destroy the bacterial membrane, interfere with DNA/RNA replication, and thus wipe out the bacteria) therapies (Xie et al., 2020). These NPs can reacts with various microorganisms like fungi and viruses thus they act like ant-parasitic agents (Fahmy et al., 2019). Ag NPs can be utilized as a better catalytic agent due to their high surface to volume ratio for the degradation of pollutants (Dawadi et al., 2021). These type of nanoparticles act as efficient anticancer drug carriers for the HeLa cell lines when combined with alendronate and doxorubicin (Liao, Li, & Tjong, 2019).

Ag NPs also possess an extensive effects against spoilage fungi (Simbine et al., 2019) and can be utilized as a material medium for the transportation of antibiotics (Ahmad et al., 2020). Novel investigations showed that Ag NPs have the potential of high antimicrobial in both bacterial cultures and within the macrophages (Tăbăran et al., 2020), also plays a key role to control tumor through their cytotoxic effects (Ratan et al., 2020), as well as to kill SARS-CoV (Jeremiah, Miyakawa, Morita, Yamaoka, & Ryo, 2020).

Ag NPs inserted in wound dressing polymers which stimulate wound healing and controlled the growth of microorganisms (Kalantari et al., 2020).

Nanocomposites are the materials composed of a combination of one or more components where at least one of which have nanometer size (Omanović-Mikličanin, Badnjević, Kazlagić, & Hajlovac, 2020). Nanocomposites can be used for a wide range of applications such as wastewater treatment, energy storage, electrolysis, biomedical, food, automotive, oil and gas pipeline construction etc. (Ates, Koytepe, Ulu, Gurses, & Thakur, 2020) (Hassan et al., 2021). Various types of matrix materials and nanoparticles are used to form nanocomposites (Shameem, Sasikanth, Annamalai, & Raman, 2021). Nanocomposites shows new electrochemical and mechanical characteristics and can be classified depends upon nano-structured filler type. Significant attention have been given to the utilization of nanocomposite coatings to protect the surface of metal from corrosion (Deyab, 2020). Graphene NCs revealed a favorable candidate for the purification of water and disinfection (P. Singh et al., 2020).

Silver-cellulose NCs remarkably showed toxic effects against *S.aureus* and *E.coli* (Kwiczak-Yiğitbaşı et al., 2020). Ag@Fe_2O_3 NCs from the leaf extract *Adathoda vasica* showed significant properties against cancer (Alavi & Varma, 2021). Ag/MgO NCs was studied for their multifunctional effectiveness towards catalytic degradation of MB (methylene blue), O-nip (O-nitro phenol), MO (methyl orange), antibacterial and the cytotoxicity of these NCs towards lung cancer cell lines (A549) (Jayapriya, Premkumar, Arulmozhi, & Karthikeyan, 2020). WO3/ZnO NCs showed visible light induced photo-catalytic anticancer effects against Hela cells (T. A. Singh, Das, & Sil, 2020). Few investigations demonstrated that the combination of ZnO NCs with vaccine agents revealed as a promising immune modulator. Similarly different types of ZnO NCs have also been displayed to boost anticancer immunity when used as tumor agent carriers (Sharma et al., 2019). ZnO\Fe_2O_3 was investigated to catalyze maxilon blue GRL dye, and the skin infection which caused by resistant *Staphylococcus aureus* could be controlled and managed with the use of ZnO/CdS NCs (Nemiwal, Zhang, & Kumar, 2021) (Liu, Wang, Ma, Peng, & Wang, 2019).

Ag-ZnO NCs were obtained for photocatalytic purposes (Hamrayev, Shameli, & Korpayev, 2021), and demonstrated as effective antimicrobial agents against various pathogenic bacteria like *B. subtilis and S. aureus* (gram positive) , *Pseudomonas aeruginosa* (gram negative), as well as Green fluorescent protein (GFP, gram negative recombinant) expresses antibiotic resistant

E.coli (Pradeep, Bindu, Suresh, Thadathil, & Periyat, 2022) (S. Wang et al., 2019) (Shreema, Mathammal, Kalaiselvi, & Gopi, 2022). Ag-ZnO NCs showed beneficial results for both catalytic and photocatalytic degradation of water, organic substances including para-Nitro phenol, Methylene blue and Paracetamol drug (Basnet, Samanta, Chanu, Mukherjee, & Chatterjee, 2019). Ag-ZnO NCs have greater antioxidant activity in term of scavenging DPPH free radicals (Zare et al., 2019b).

The family of Ranunculaceae contain various medicinal plant species including *Aconitum violaceum* (*AV*) (Phumthum, Balslev, & Barfod, 2019). *Aconitum violaceum* has remarkable medicinal properties due to the presence of various flavonoids and alkaloids (Safdar & Bibi, 2020). The ethnomedicinal uses of this plant are well-known throughout Gilgit and Baltistan as it is common medicinal plant species of Deosai meadows (Abbas et al., 2021). It is a vulnerable medicinal plant species with limited worldwide distribution (Hadi et al., 2022). Phytochemically, this species is enriched in alkaloids, anthraquinones and saponins which exhibits higher potential of strong antioxidants, cytotoxic, antifungal, antibacterial, and powerful inhibitor of acetylolinesterase. Generally, the plant is very toxic but the toxicity could be reduced via extracting these metabolites using polarity-based method (F. A. Khan et al., 2021a).

It is suitable to treat various disorders like skin allergy, boils, wound healing, blood disorders, malaria, inflammation, infectious fever, respiratory disorders (such as cough and catarrh), intestinal disorders and digestive disorders (upset digestion, dyspepsia, dysentery, abdominal colic and diarrhea). Dried roots of *A. violaceum* in limited amount could be utilized to treat cough. The roots are dissolved in mother's milk and given to newborns with allied gastric complaints, severe diarrhea and stomatitis (Semwal et al., 2019) (Shah et al., 2020).

The current study emphasizes the synthesis of Ag-ZnO nanocomposites via biological route using the extracts of different parts of *Aconitum violaceum*. The active secondary metabolites produced by the medicinal plants are responsible their biological characteristics. The plant (*Av*) has a broad range of Alkaloids, diterpenoid, anthraquinones and saponins, and is generally used to treat urinary tract infections, intestinal disorders, malaria, insect bites, blood disorders, fever, joint pains allergy, stomach, liver disorders and wound healing. Various studies confirmed that this plant have multiple activities like anticancer activity, antibacterial, antifungal, strong antioxidant and strong inhibitor of acetylcholinesterase (Ali, Chouhan, Sultan, Hassan, & Gandhi, 2021).

The leaves, stem and roots extracts of *Aconitum violaceum* acted as a stabilizing and reducing agent. The synthesized NCs were subjected to characterization via XRD, FTIR, and SEM. In order to study biomedical applications various assays were performed to analyze the anticancer potency of the synthesized Ag-ZnO NCs. Moreover, cell viability tests were also performed to check the suitability of Ag-ZnO NCs for broad range of applications.

2. Literature review

Nanotechnology is the science and art of producing, manipulating, controlling, characterizing, and utilizing smaller, stronger, faster and smart structures, materials, products or devices having between 1 and 100 nm size range (Bayda, Adeel, Tuccinardi, Cordani, & Rizzolio, 2019). The properties at the nanoscale level vary from those at the micro or macro level which can be used for various environmental or industrial applications. The composition, surface chemistry, size, shape and porosity of nanomaterials (NMs) can be controlled at nanoscale, and this offers unique features to NMs when compared to bulk materials. Nanomaterials can be categorized in different groups according to their origin (natural, engineered, bioinspired, incidental), composition (organic, inorganic, carbon and hybrids), dispersion state (aggregated or dispersed), phase (multi or single phase), morphology (low to high aspect ratio) porosity (mesoporous, macro, nano) and dimensionality (0-3D). Numerous approaches including chemical, physical and biological methods can be utilized for the synthesis of NPs (Harish et al., 2022). Top-down and bottom-up are two methods to approach nanoscale materials. By using top-down strategy, the size of the structure is reduced towards nanoscale. On the other hand, bottom-up approach is used to synthesize the large nanostructures from smaller atoms and molecules (Abid et al., 2021).

2.1 Bottom-up approach

The bottom-up approach consisted of building NMs from atoms into clusters and finally into nanoparticles. This process is unique because it just using self-supported one step (annealing) for nanoparticles synthesis (Belusso et al., 2019). Common methods used in bottom-up approaches including Sol-gel, CVD (chemical vapor deposition) and spinning methods. The benefit of using this method is to obtain a well homogeneous material with small defects.

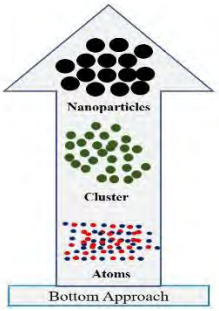


***Figure 2*: Bottom-up approach for nanoparticles synthesis.**

2.1.1 Sol-gel processes

Sol-gel is one of the well-known synthetic processes to synthesize high-quality NPs and NCs. The surface and texture characteristics of the materials can be controlled significantly with this method. Generally this approach could be defined with five key steps; hydrolysis, poly-condensation, aging, drying and thermal decomposition (Parashar, Shukla, & Singh, 2020a).

Due to affordability, reproducibility, uniformity, simplicity, low processing temperature in comparison to other methods, this method is being considered beneficial for the synthesis of NMs. The synthesized nanoparticles via sol-gel method offers good morphological control, physical and optical characteristics (Arya et al., 2021). It is simple, cost effective and less time required method (Habte et al., 2019) (Moein, Abdel-Rehim, & Abdel-Rehim, 2019). The recovery of nanoparticles is done by many methods like filtration, sedimentation, and centrifugation while drying is used to remove moisture content.

2.1.2 Chemical vapor deposition process (CVD)

CVD is a typical bottom-up vapor phase growth process which deposits materials as a thin layer from vapor species onto substrates via chemical reactions (Saeed, Alshammari, Majeed, & Al-Nasrallah, 2020). Solid and gas are common precursors in this process. The reaction included the deposition of materials via heat and gas. The temperature of the substrate is the most significant parameter in the CVD method. The gaseous precursor may be controlled more accurately in vapor deposition process as compared to solid precursor. The structure and morphology of the final product can be controlled in this process via temperature and at the level of super-saturation (Bhutto et al., 2019). Free of contamination and high crystalline quality samples and the ability of scaling up are the greatest benefits of this method (Qin et al., 2020). Due to less processing cost and higher production, CVD is considered as the most promising and acceptable method (Pant, Singh, Negi, Tiwari, & Singh, 2021).

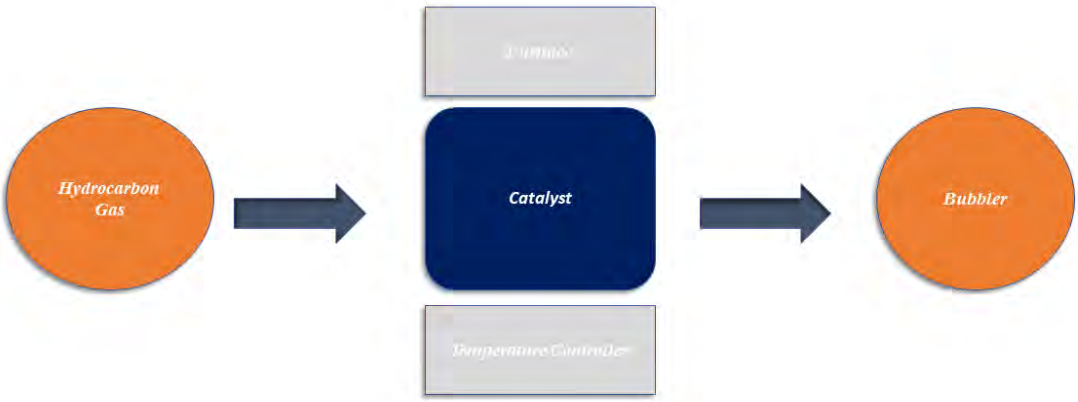


Figure 3: **Schematic diagram of a catalytic CVD setup**

Although, the drawbacks of this methods may include chances of chemicals hazards because of corrosive, toxic, and explosive precursor and multicomponent deposition is challenging (Jamkhande et al., 2019).

2.1.3 Spinning

The NPS can synthesized via SDR (spinning disc reactor), which comprise of revolving disc where physical parameters like temperature could be controlled (Ijaz, Gilani, Nazir, & Bukhari, 2020). The vessel is typically filled with some inert gases or specifically nitrogen to stop chemical reactions and remove oxygen (Mushtaq, Hassan, & Mughal, 2022b). The physical parameters like temperature are controllable inside the chamber. The disc of the chamber is continuously rotated and water along with precursor is introduced in it. The atoms or molecules fused inside the chamber due to spinning. The obtained pallet is collected and dried (Gu et al., 2019). The characteristics features of nanoparticles obtained from SDR depend upon the disc speed, precursor/water ratio, location of feed, liquid flow rate, etc.

2.2 Top-down approach

Top-down methods entails to minimize the size to nanoscale. These methods comprise in the transformation of a bulk materials of the desired parameter into nanomaterial (Dang-Bao, Favier, & Gómez, 2021) (Krishnia, Thakur, & Thakur, 2022). The size and shape of nanoparticles via these methods can easily be controllable. Although, some difficulties like stability of nanoparticles, scaling-up of fabrication, and effective delivery are still the issues to be solved (Garemark et al., 2020).

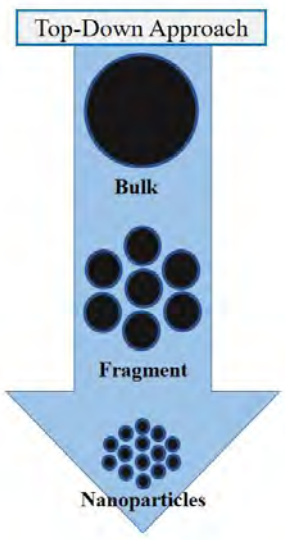


Figure 4: **Top down approach for nanoparticles synthesis**

This method is appropriate for laboratory experiments but not for large scale production as well as relatively costly and not appropriate for soft samples (Patil et al., 2021). Mechanical milling, laser ablation and nanolithography are the most extensively used methods in top-down methods for nanoparticles synthesis (Jaji et al., 2020).

2.2.1 Mechanical milling

Mechanical milling is a top-down method for nanoparticles synthesis of different sizes (Mushtaq, Hassan, & Mughal, 2022a). It is most widely used for nanoparticles synthesis in comparison to other top-down approaches. Mechanical milling process mainly linked with powder processing with the application of medium or high speed agitators (Mughal & Hassan, 2022). This method is used for NPs synthesis due to low chemical waste, comparably less expensive equipment and simplicity (Yarahmadi et al., 2021) (Ismail, Saputri, Dwiatmoko, Susanto, & Nasikin, 2021).

2.2.2 Laser ablation

Laser ablation is an approach of top-down methods for various nanoparticles synthesis. In solution, laser ablation synthesis arisen a flexible method for NPs synthesis (Crivellaro, Guadagnini, Arboleda, Schinca, & Amendola, 2019). Laser wavelength, liquid medium, laser fluency and pulse repetition are the most significant parameters that effects the optical absorption, shape and size (Dheyab et al., 2021).

Laser ablation can synthesize the nanoparticles with high purity because the particle's purity mostly determined by the purity of the targeted and ambient media (liquid or gas) without impurity from the reactor (Kim, Osone, Kim, Higashi, & Seto, 2017). This met hod demonstrated to be most appropriate as it provides a replacement for conventional synthesis methods like chemical methods. Stable nanoparticles fabrication can be achieved in organic solvents and water and thus no stabilizing agents are required in this process.

2.2.3 Nanolithography method

Nanolithography is a very powerful and adventitious method. It is a multi-step method and can be utilized to make precise 2D metal arrays on the surfaces with a precisely controlled size, shape and spacing. The main advantage of this method is that it can fabricate the NPs of uniform size and shape (Y. Khan et al., 2022). Nanolithography is one of the top-down approaches that can synthesize smaller nanomaterials (Jaji et al., 2020). Another advantage of this method is it can transform a single nanoparticle into a cluster of the desired size and shape (Upadhayay, Khan, Singh, & Singh, 2019). Requirements of complex and expensive equipment are the drawbacks of this method (V. Singh, Yadav, & Mishra, 2020).

2.3 Nanoparticles classification

The nanoparticles classification depends on their size, shape, types of material, and activity. However, the major classificational distribution relies on the type of material used for their synthesis. Nanoparticles can be inorganic, organic, composite-based or carbon-based.

2.3.1 Carbon based nanoparticles (CNPs)

Carbon-based nanomaterials are the most promising products of nanotechnology due to their unique characteristics which make them suitable for a broad range of uses including electronics and drug delivery (Yuan, Zhang, Sun, Wei, & Wei, 2019). Carbon-base NMs including CNPs (carbon nanoparticles), CNTs (carbon nanotubes), and graphene have been fabricated and widely studied because of their higher electrical, magnetic, optical and physical properties.

CNPs are extensively utilized in numerous areas of life. The characteristics such as higher surface area, good compatibility, low toxicity and low cost make these NPs most flexible in various industrial and scientific applications such as photocatalysis, biological imaging, optical, chemical, and biomedical sensing and biological imaging (Asadian, Ghalkhani, & Shahrokhian, 2019) due to their tremendous mechanical, thermal, physical and chemical characteristics (Narayanan, Sakthivel, & Han, 2021).

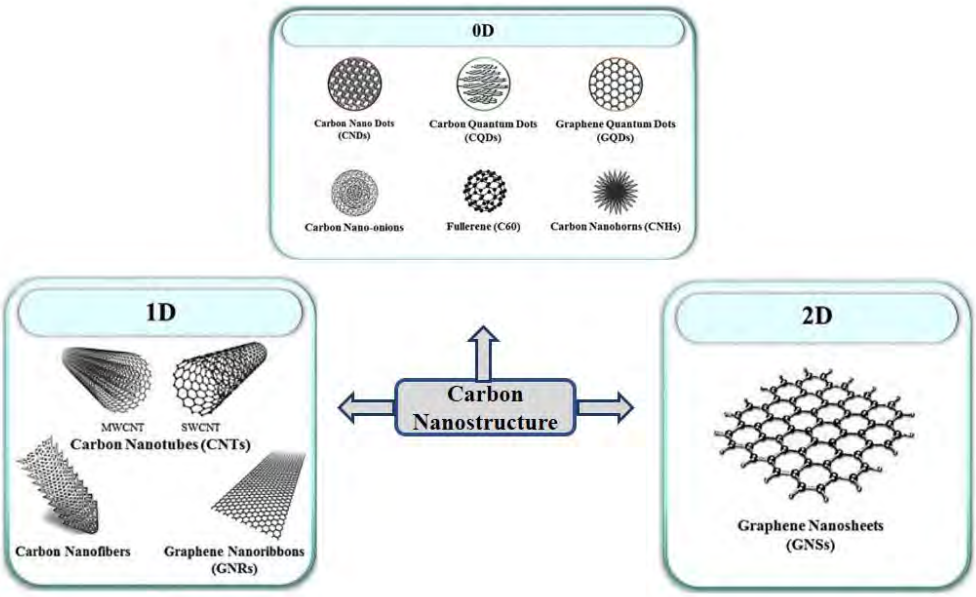


Figure 5: **Various types of carbon nanostructures**

2.3.2 Metal and metal-oxide nanoparticles

Metal and metal-oxide nanoparticles possess greater thermal, mechanical, optical, chemical and optical characteristics. These types of nanoparticles have a wide range of importance due to their large surface area, controlled size and morphology. The applications include biomedical, molecular sensing, energy harvesting, catalysis and environmental remediation etc.

The fabrication method involved top-down and bottom-up approaches. The most synthesized metal and metal oxide nanoparticles are nickel, gold, silver, selenium, iron oxide, zinc oxide, titanium oxide, magnesium oxide, zinc peroxide and cerium oxide (Agarwal, Nakara, & Shanmugam, 2019).

2.3.3 Biomolecules derived nanoparticles

Nanoparticles can be fabricated via biomolecules like polysaccharides, lipids, proteins, fungi and nucleic acids, and they have remarkable characteristics (Guilger-Casagrande & Lima, 2019; J. Wang, Li, & Nie, 2021). Due to their potential of non-immunogenicity, biocompatibility, biodegradability and broadly availability of biomolecule-derived NPs are a new topic of investigation and utilized in many applications.

2.3.4 Ceramic nanoparticles

Ceramic nanoparticles are inorganic nonmetallic solids, fabricated via heat and successive cooling. They can be found in dense, amorphous, porous, polycrystalline or hollow forms. Due to their utilization in many applications including catalysis, imaging applications, photo-degradation of dyes, ceramic nanoparticles receiving much attention for research (I. Khan, Saeed, & Khan, 2019). These NPs are extremely advantageous for photo-degradation of dyes, imaging and drug delivery due to heat resistant, chemical inertness, stability, high loading capacity and simple conjugation to either hydrophobic or hydrophilic drugs (Montaseri, Kruger, & Abrahamse, 2021).

2.4 Methods for nanoparticles synthesis

2.4.1 Chemical methods

Chemical reducing agents, basically organic and inorganic solvents, are needed to synthesize chemical based nanoparticles. The main benefits of this approach are it allows particles with define structure, dimension, size and composition that can be broadly utilized in numerous research areas including sensing, imaging, catalysis and drug delivery. Furthermore, the working principles of chemical based NPs fabrication can be easily predicted (Deepak et al., 2019). This method is typically simple, needed ambient temperature processing conditions, as a result the materials produced with narrow size distribution and strong uniformity (Matussin, Harunsani, Tan, & Khan, 2020). The most common chemical methods includes polyol, chemical vapor deposition, sol-gel, co-precipitation, hydrothermal and microemulsion method etc. (Kolahalam et al., 2019).

2.4.2 Physical methods

Physical methods uses high electric and thermal energy, energy radiation and high mechanical pressure which comes under the top-down approach (Parashar, Shukla, & Singh, 2020b). It has various advantages and disadvantages. The advantages include the synthesis of uniform and stable NPs with no chances of contamination. Although the major disadvantages include the need of wide space for instrumentation and required high energy to run a reaction. Various physical methods are available including arc discharge, laser ablation, melt mixing, sputter deposition and flame pyrolysis (Jeyaraj, Gurunathan, Qasim, Kang, & Kim, 2019).

2.4.3 Biological methods

Biological methods are also known as green synthesis that are basically significant to reduce toxic by-products and harmful chemicals (Lee et al., 2020). These methods utilizing reducing agents like fungus, bacteria, polysaccharides, biological microorganisms and plants extracts (Yaqoob, Umar, & Ibrahim, 2020).

Biological sources comprise various biomolecules such as alkaloids, flavonoids, tanins, saponins, steroids, proteins, carbohydrates, enzymes, cell wall components, amino acids, etc. that helps in the bio-reduction, stabilization and capping of the NPs (Kaur & Sidhu, 2021). Biological methods have different benefits over physical and chemical methods as it is non-expensive, eco-friendly and easily scaled up for large scale production (Xu et al., 2020).

2.5 Ag-ZnO nanocomposites

Nanocomposites (NCs) can be synthesized from a wide range of materials. NCs are materials made of different components and mixed at nanoscale (Krasno & Swathi, 2018). Nanocomposites are composed of metallic, non-metallic and polymeric material through specific process (Omanović-Mikličanin et al., 2020). On the basis of their matrix type, NCs are categorized into three main classes: metal matrix, polymer matrix and ceramic matrix (Yadav, Gadi, & Kalra, 2019). Various metal oxides have been fabricated including Ag-ZnO, ZnO-Nio, ZnO-Mg, Co_3O_4-ZnO, CuO/ZnO and CeO_2-ZnO (Sakib, Masum, Hoinkis, Islam, & Molla, 2019). Due to their beneficial characteristics and ease of industrialization, the chitosan/Ag NCs sponges would have high potential as wound dressings (Zhou et al., 2021). The Cu_2O-Ag NCs demonstrated excellent long-lasting antibacterial ability against *P. aeruginosa* and *S. aureus* (Yang et al., 2019). ZnO NPs are physical fused with activated carbon to produce NCs (Veeman et al., 2021).

Ag-based NCs basically contain bimetallic Ag-based NCs and supported Ag NCs (G. Liao et al., 2019). ZnO-Ag NCs have higher oxidation capabilities due to their synergic effects. This demonstrated that the fabricated ZnO-Ag NCs have higher antioxidant activity in term of scavenging DPPH free radicals (Zare et al., 2019b). Ag-ZnO NCs can increase ROS production and offer a cumulative effects on photo-catalysis, anticancer and antibacterial activities (Sharwani, Narayanan, Khan, & Han, 2022).

Some studies showed that Ag-ZnO NCs revealed higher antimicrobial effectiveness against *S. mutans* and *E.coli* (Mtavangu, Machunda, van der Bruggen, & Njau, 2022a; Verma, Pathak, Srivastava, Prawer, & Tomljenovic-Hanic, 2021).

World Health Organization (WHO) and other investigations demonstrated that, one of the leading causes of death is hepatic cancer in recent years. Ag-ZnO NCs revealed high toxicity against HepG2 due to their higher cellular uptake and retention time; this was observably influenced by the concentration and time of Ag-ZnO NCs. The cytotoxic characteristics of these NCs were also tested against the normal NIH-3T3 cells, where the results exhibited lower toxicity of NCs than the HepG2 cells (Mousavi-Kouhi, Beyk-Khormizi, Amiri, Mashreghi, & Yazdi, 2021).

The cytotoxic characteristics of these NCs were also studied for cervical (Hela) and ovarian (SKOV-3) carcinoma cells (Nagajyothi, Muthuraman, Tettey, Yoo, & Shim, 2021). Ag-ZnO NCs were also found to be efficient against various harmful fungi like *candida albicans, Fusarium spp.* And *Rosellinia necatrix*) (A. Chauhan et al., 2020; Noohpisheh, Amiri, Farhadi, & Mohammadi-Gholami, 2020).

2.5.1 Methods for synthesis of Ag-ZnO NCs

The location and shape of Ag-ZnO patterns are resembles to pure ZnO, showing that Ag-ZnO NCs are also hexagonal wurtzite structures (Panchal et al., 2020; Zhu et al., 2021). The properties of Ag-ZnO NCs are strongly associated with particle morphology, surface chemistry, size distribution, and particle reactivity. The activities of Ag-ZnO NCs are also strongly corresponded to its synthesis method, reactant concentration, reaction temperature, and the nature of used capping agents. Various methods have been documented for the synthesis of Ag-ZnO NCs such as sol-gel, chemical deposition and biological method (Taha, Ben Aissa, & Da'na, 2020).

a) Sol-gel method

Sol-gel method is one of the cheap, quick and easy methods and also has the advantages in the term of uniformity of the synthesized materials, low processing temperature, and has the ability to synthesize complex structures or composites materials (X. Wang & Li, 2021). Silver nitrate and zinc acetate were utilized as precursors for the synthesis of Ag-ZnO via this method. 1 M solution of zinc acetate was prepared in distilled water (DW) and dissolved by using magnetic

stirring at 60 ºC for 30 minutes. 0.2 M solution of oxalic acid was also prepared via the same procedure (magnetic stirring). Under the continuously stirring oxalic acid solution was added to warm zinc acetate solution. Milli-molar solution of silver nitrate (3mM and 5mM) were added to the solution for various silver quantities in samples. The resulting mixture was continuously magnetically stirred for 4 hours to make a thick xerogel that was further dried overnight at 80 ºC. The synthesized gel was then shifted into China dish and kept in the oven and heated for 1.5 h at 550 ºC to remove byproducts and other impurities that transmitted to the materials from the utilized apparatus. After the calcination process, the materials were cooled to room temperature which has white colored ZnO NPs and silver colored Ag NPs. The resulting materials were grinded into fine powder through glass rod. Finally, Ag-ZnO NCs with grey color were produced. The production of Ag-ZnO NCs via sol-gel method is quite good because there is no need for vacuum apparatus and costly chemical precursors as well as it offers good characterization results. Moreover, synthesis methods expressively affects the morphology and size of the particles (Bohan, Salman, & Ahmed, 2019; T. Iqbal et al., 2021).

b) Chemical deposition method

Chemical reduction method can be used to synthesize Ag-ZnO nanocomposites (Chen et al., 2021). Silver nitrate salt was first dissolved in distilled water to prepare different amounts of Ag^+ ions, after that the solution was added to ZnO suspension with different Ag^+ concentrations (1, 2.5 and 5%) in relation to ZnO. After that freshly prepared $NaBH_4$ (sodium borohydride) was added drop-wise while being stirred vigorously. The darkening of the solution confirmed that Ag has been reduced from Ag^+ to Ag^0. The suspension were then filtered, washed and dried to give Ag-ZnO NCs (Ebadi & Mozaffari, 2020).

2.6 Characterization techniques for nanocomposites

There are various procedures commonly utilized for the characterization of NPs of various sizes, shapes, surface area and dispersity nature. The widely used techniques includes UV-vis (UV-visible spectroscopy), FTIR (Fourier transform infrared spectroscopy), XRD (X-ray diffraction), SEM (Scanning electron microscopy), TEM (Transmission electron microscopy), DLS (Dynamic light scattering), Zeta potential and XPS (X-ray photoelectron spectroscopy) (Molla, Furukawa, Tateishi, Katsumata, & Kaneco, 2019; Muñoz-Fernandez, Alkan, Milošević, Rabanal, & Friedrich, 2019).

XRD is used to determine crystalline purity, size and shape. UV-vis spectrum range in between 300-800 nm shows the existence of nanoparticles, Ag-ZnO mainly depicted in arrange of 430,536 nm (Berhanu, Habtamu, Tadesse, Gonfa, & Tadesse, 2022; Majumder, Basnet, Mukherjee, & Chatterjee, 2020). FTIR identifies the active functional groups (Stanley et al., 2021) and surface residues i.e. phenols, flavonoids, hydroxyl groups etc. attached with the surface of NPs as capping and stabilizing agents. SEM offers information on the size, morphology and topology. TEM is basically used to find size, shape and morphology of nanoparticles at higher resolution (Ma et al., 2021; Peng et al., 2019). Size distribution and surface charges of NPs can be analyzed through DLS (Chitradevi, Lenus, & Jaya, 2019).

2.7 Nanotoxicology

Nanotoxicology is the branch of toxicology that deals with certain limitations of knowledge or field that have adverse health effects. It's an area of nanoscience that deals with the studies of negative effects of nanoparticles or engineered nanomaterials on living organisms (Egbuna et al., 2021). It was worried that how ENMs (engineered nanomaterials) influenced the environment and living organisms (humans, animals) and what are the safety concerns with ENMs. The unique features of ENMs are highly associated with crystallinity, aggregation, surface reactivity, surface groups, shape solubility, size and purity. NMs are known to prompt toxicity via reactive oxygen species (ROS) production (Umapathi, Kumawat, & Daima, 2022). According to reports, NPs with smaller size demonstrated greater toxic effect due to their higher surface area, as well as shape and structure of NPs also contribute to nanotoxicity (Xiao, Shi, Qu, Chu, & Qian, 2019). Primary significant species of the environment such as plants, algae, fishes and bacteria etc. are highly vulnerable to the toxicity of NPs. When ENPs intentionally or accidently enters to the body, it effects the whole organ system and increases their toxicity even during reproduction and the developmental stages of fetus (Prajitha, Athira, & Mohanan, 2019).

Recently, the potential toxicity of engineered nanoparticles that are less than 100 nm in size received higher attention. Larger particles that are greater than 200 nm in size can only be internalized via macrophages and thus produce effects on those cells. Although smaller NPs with a diameter less than 150 nm can be internalized by any cell via pinocytosis. In this regards, small NPs may enter to any cell of the body and can be more toxic as a result of interaction of oxidative stress, inflammation and cell death. In addition to inducing an immune response, the NPs have the potential to be toxic to immune cells (A. K. Singh, Yadav, Pandey, Gupta, &

Singh, 2019) (Malachowski & Hassel, 2020). NPs can cross biological barriers like cell membrane, enters to living organisms and producing cell damage (Madannejad et al., 2019).

NPs exposure have been associated to several toxic effects *in vivo* in a broad range of organisms including developmental abnormalities in zebra fish embryos, hepatic toxicity in mice, deterioration of vertebrate lung function, increased mutagenesis in *Drosophila* and decreased survival in *Daphina* and fish. Additionally, it has also been revealed that ZnO NPs may reach many organs via systemic distribution, and demonstrated toxic effects on organs such as heart, lungs, brain, liver, thymus, kidney, testis, pancreas, spleen and blood etc. (Mir, Qamar, Qadir, Naqvi, & Begum, 2020). One of the main causes of Ag-NPs cytotoxicity is thought to be the production of ROS. The toxicity of AgNPs specifically at the gut level are main concerns in the development and use of these NPs (Zorraquín-Peña, Cueva, Bartolomé, & Moreno-Arribas, 2020). Higher dosage of Ag may have side or toxic effects on human tissues and cells because they are easily agglomerated in tissues (Abbas et al., 2021). It has been demonstrated that excess amount of Ag NPs suppresses the immune system of humans, and start the cytotoxin production which damage the fibrous tissues of the human lung (Li et al., 2020).

2.8 Applications of Ag-ZnO nanocomposites

Ag-ZnO-NCs are less-toxic and low-cost nanocomposites that have been extensively used in numerous medicinal fields like antioxidant (Zare et al., 2019a), anticancer, anti-inflammatory (de Moura et al., 2022), and antibacterial activities (Mtavangu, Machunda, van der Bruggen, & Njau, 2022b). It has also been used in bioimaging and drug delivery applications. Ag-ZnO-NCs possess strong anti-cancerous activity via activating ROS which affects apoptotic factor, as a result, these factors promote apoptosis of cancer cells (Shreema et al., 2022) (Elsayed et al., 2022). Ag-ZnO NCs revealed various cytotoxic effects on A 549, MCF 7 and HCT 116 cell lines, proving dose dependent cytotoxicity (Ambujakshi et al., 2019).

Ag-ZnO-NCs are known as strong anti-bacterial agents due to their high surface area and reactivity that easily stops the pathogenic ability of pathogens. Although, the mechanism of antibacterial activity is still not clear, but it was proposed in various research that Ag-ZnO-NCs produced an excess of ROS like hydroxyl and superoxide, Ag-ZnO NCs are likely to rupture the bacterial membrane, walking out the cytoplasmic content and resulting in the cell death because of enzyme activity or altered membrane protein cause by the generation of ROS radicals.

Ag-ZnO NCs inhibits the replication of DNA at molecular level due to the direct influence of zinc or silver ions or ROS production (M. J. Khan et al., 2021). It has also been reported that both gram positive and gram-negative bacteria can be inhibited with the use of plant derived Ag-ZnO NCs. With the help of various steps, Ag-ZnO NCs can directly interact with the bacterial membrane. First, the Ag-ZnO NCs bind to the negatively charged cell wall of bacteria and release ions which interact electrostatically. The solid interaction between Ag and ZnO nanoparticles split the cell membrane due to the electrons formed by the photo-catalytic reactions of ZnO NPs, and thus exhibited higher antimicrobial activity (Aththanayaka, Thiripuranathar, & Ekanayake, 2022).

Diabetes is a metabolic disorder caused by the inability of body's cells to produce sufficient insulin, resulting in the accumulation of sugar in the blood. This disorder is influenced by many enzymes but two of them α-amylase and α-glucosidase are the primary regulators (K. R. Singh, Nayak, Singh, Singh, & Singh, 2021). Green synthesized Ag-ZnO NCs demonstrated greater antidiabetic and anti-aging characteristics (Y. Iqbal et al., 2021). The increased anti-diabetic activity by green synthesized Ag-ZnO NCs may be due to the presence of higher amount of phytochemicals in plants including phenolic and flavonoids (D. A. S. R. S. Kumar, Murugesan, & Rahiman). The main antiviral mechanism of Ag-ZnO NCs against SARS-CoV-2 is likely to be blocking viral binding, damaging surface proteins or interfering viral entry. Additionally these NCs have the ability to enter the cell cytoplasm, interact nucleic acids, impair virus function and thus prevent the virus transformation from infected cells to non-infected cells (Dolatyari & Rostami, 2022). Being a significant constituent of numerous enzymes, Ag-ZnO NCs have several applications in physiological processes of humans, animals and plants. These NCs play a key role in growth, immune system, antioxidant defense, and hormone secretion and reproduction system. The treated animals with Ag-ZnO revealed better improvement in wound healing after 7 and 14 days when compared with untreated wounds, thus it has a wide role in animal industry (de Moura et al., 2022).

2.9 Aconitum violaceum

The genus *Aconitum* belongs to Ranuculaceae family having almost 400 or more species globally, some of them are medicinally very significant. *Aconitum* is also known as blue rocket, monkshood, aconite, wolf's bane, mousebane, leopard's bane and queen of poisons.

Several studies reported that a number of species have major pharmacological characteristics and great therapeutic to treat numerous diseases (Ali et al., 2021).

Aconitum violaceum is one of them that exhibiting greater association with higher altitude and is highly endangered and threatened species (Bisht et al., 2022) (Dahal, Gurung, & Lachungpa). It's a biennial herbaceous medicinal plant of the northwestern Himalayan regions of Nepal, India and Pakistan. (Hadi et al., 2022). *Aconitum violaceum* belongs to Ranunculaceae family are also known as "the queen of poisons" have been utilized as nerve tonic, to treat high fever, gall bladder disorders, as well as heart diseases. Due to its toxic nature, it has also been used for the treatment of venom poisoning (F. A. Khan et al., 2021a) (Rawat et al., 2021). The roots of *Aconitum violaceum* can be utilized to treat abdominal pain, cough, heart disorders, fever, arthritis, vomiting, neural disorders, nausea and asthma (Abbas et al., 2021). The roots are dissolved in mother's milk and given to babies who suffering from gastrointestinal problems, stomatitis and severe diarrhea (Semwal et al., 2019). Paste of tuber of this plant is applied on insect stings (R. Kumar, Arya, & Chandra Sekar, 2020). Phytochemically, *Aconitum violaceum* is enriched in alkaloids glycosides, flavonoids, terpenoids and saponins (F. A. Khan et al., 2021b). The roots are sweet in taste and can be eaten in raw form. The root powder of this plant species are added to pulses (dal) to enhance flavor and facilitate digestion (P. Chauhan).

Table: 2.2 Taxonomical description of *Aconitum violaceum*

Domain	**Eukaryota**
Kingdom	**Plantae**
Phylum	**Tracheophyta**
Class	**Magnoliopsida**
Order	**Ranunculales**
Family	**Ranunculaceae**
Genus	***Aconitum***
Species	***Aconitum violaceum***

Figure 6: Representative picture of *Aconitum violaceum.*

Objectives:

i. Bio-fabrication of Ag-ZnO-NCs from different parts of *Aconitum violaceum*.
ii. Characterization of Ag-ZnO-NCs via multiple methods like XRD, FTIR, SEM, EDX and HPLC.
iii. In-vitro biological applications of biosynthesized nanocomposites.
iv. Potential of Ag-ZnO-NCs towards HepG2 cell line.

3. Materials & methods

3.1 Plants collection

The *Aconitum violaceum* plant species was collected from a famous tourist's place "TAKHT BAHRAM KHAN" with an altitude of 12000 feet above sea level, in kana valley of district Shangla, Khyber Pakhtunkhwa (KP) Pakistan and was further verified by the Department of Botany University of Malakand Chakdara Dir lower KP Pakistan and the Department of plant sciences Quaid-I-Azam University Islamabad Pakistan.

3.2 Reagents

All the reagents including silver nitrate ($AgNO_3$) and Zinc acetate dehydrate were purchased from Sigma Aldrich Company, Germany. For HPLC analysis, the reference standard of hypaconitine (Batch number: 0798-9406), aconitine (Batch number: 0720-9406), mesaconitine (Batch number; 0799-9203) were purchased from National Institute for the control of Pharmaceutical and Biological product (Beijing, China). The purities of the three chemicals were all above 99.8 %. Dichloromethane, triethylamine, methanol and chloroform were of HPLC grade and commercially obtained. The distilled water was purified by Smart2Pure 6 UV/UF (Thermo Scientific, Langenselbold, Hungary).

3.3 Extract preparation

All parts of the plant (leaves, stem and roots) were properly separated, each part was washed several times with tap water and distilled water to remove dust and was shade dried for 28 days. All the parts were separately converted into small pieces and then grinded via an electrical grinder to convert the pieces into fine powder. 40g of each part powder was mixed in 300ml distilled water in separate conical flasks. Each of the mixtures were continuously stirred via magnetic stirrer for 3 hours at 70 ^{o}C. The prepared extracts were then filtered with double-phase muslin cloth and twice filtered with Whatsman filter paper to separate any solid residue or other particles. The obtained fresh filtrates were then stored for further use.

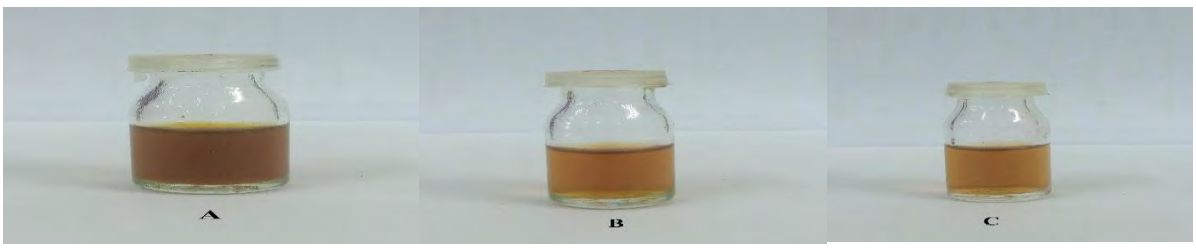


***Figure 7: Aconitum violaceum* plant extracts (A) Leaves, (B) Roots, (C) Stem**

3.4 Biosynthesis of Ag-ZnO nanocomposites

Biosynthesis of Ag-ZnO nanocomposites was done via following sajjadi et al (Sajjadi, Nasrollahzadeh, & Sajadi, 2017) with some modification, where they used the protocol for the fabrication of Ag/Fe_3O_4 NCs. To synthesize Ag-ZnO nanocomposites, 0.3 g silver nitrate and 1 g of zinc acetate dihydrate was taken in 50 ml flasks separately and 35mL extract of each part of the plant was added and kept on magnetic stirrer for 3 hours at 70 ^oC. After the completion of the reactions, the mixtures were allowed to cool down. Once the reaction was completed, the mixtures were centrifuged in order to obtain NCs. The supernatants were discarded and the pellets containing Ag-ZnO were washed trice with DH_2O and once with ethanol via centrifuging for 10 minutes at 10000 rpm. After washing, the obtained pallets were subjected to drying in an incubator at 40 ^oC for 24 hours (Fig: 8).

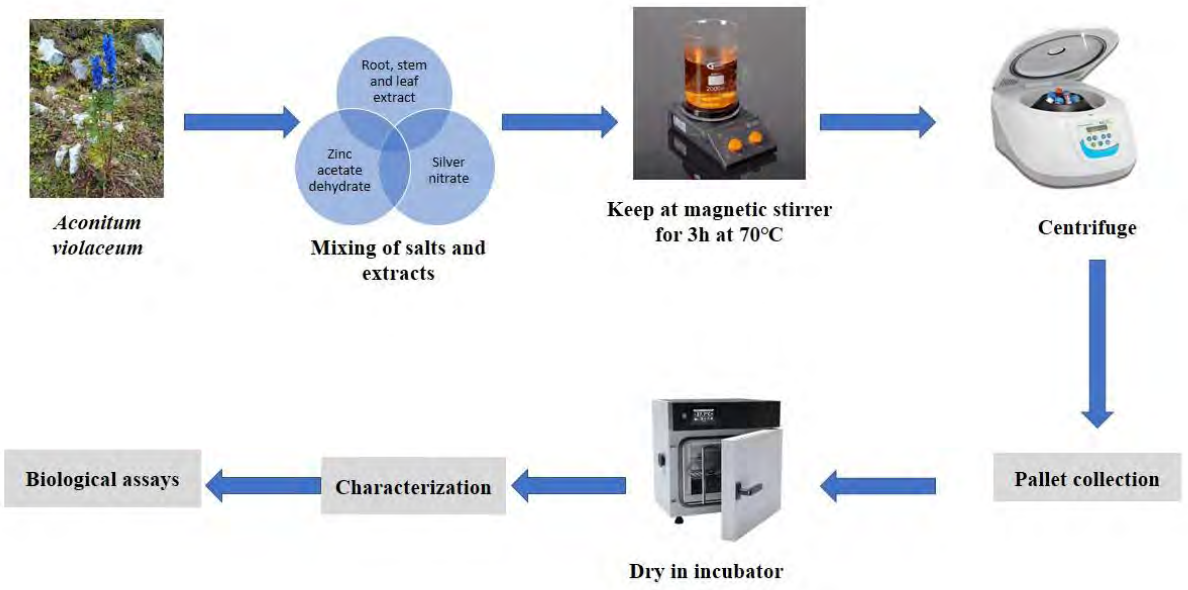


***Figure 8*: Biofabrication of Ag-ZnO-NCs via *Aconitum violaceum* leaf, root and stem extract**

3.5 Characterization

Several methods were utilized to characterize the synthesized Ag-ZnO NCs i.e., XRD, FTIR, SEM, EDX, DLS and HPLC.

3.5.1 XRD (X-ray diffraction)

XRD analysis was used to verify the nature and crystalline size of Ag-ZnO NCs. The principle behind the XRD apparatus (Model-D8 Advance, Germany) is that it has a cathode ray tube that release X-rays towards the samples. The interaction between X-rays with sample produces a constructive interference and diffracted ray. The size of NCs was analyzed via Debye-Scherre equation.

Debye-Scherrer equation was utilized to analyze the size of NCs.

$$D = \frac{\kappa\lambda}{\beta cos\theta}$$

Where D = crystalline size, k = shape of the factor, λ = X-ray wavelength, β = full length at half maximum in radians and θ represents Bragg's angle.

3.5.2 FTIR (Fourier transform infrared) spectroscopy

FTIR (SHIMADZU 8100 M FTIR, Shimadzu, Kyoto, Japan) was carried out in the range of (400-4000 cm^{-1}) to determine and evaluate the functional groups and other biomolecules present on the surface of NCs that involved in the Ag-ZnO synthesis, where they function as stabilizing and reducing agents.

3.5.3 SEM (Scanning electron microscopy)

SEM (JSM-IT 100, Jeol made Japan) was utilized for the size of Ag-ZnO NCs and morphological determination. Samples were mixed in isopropanol for uniform distribution of Ag-ZnO NCs on stubs and to prevent powder from flying off because of vacuum and beam. It was operated on 10KV, and analysis was done via coating a small amount of sample with gold grid.

3.5.4 Energy dispersive x-ray (EDX)

EDX was used to confirm the elemental composition of Ag-ZnO NCs. EDX is generally attached with SEM, same procedure was followed as SEM.

3.5.5 DLS (Dynamic light scattering)

The surface potential and dynamic size distribution of the synthesized NCs was obtained with zeta sizer Nano-ZS (Malvern instruments UK). 1mg of Ag-ZnO NCs of each part were separately dissolved in 1ml deionized water followed by the room temperature.

3.5.6 HPLC (High performance liquid chromatography)

HPLC of bio-fabricated Ag-ZnO NCs was performed via following Sun et al., (Sun et al., 2018). Briefly, an Agilent 1260 Infinity HPLC apparatus (Agilent Technology, CA, USA) was carried out for HPLC. The chromatographic separation was accomplished at 30 ^{o}C on a Hypersil BDS-C18 column (250 x 4.6 nm, 5 µm) (Shandon scientific, cheshire, UK). Methyl alcohol, water, chloroform and triethylamine (100, 50, 3, and 0.15) were the components of the mobile phase that had the flow rate of 1.0 ml/minute. The volume of each sample injection was 2 µl, and 234 nm was the wavelength for detection. The data was analyzed to determine the amount of aconitine, mesaconitine and hypaconitine in each sample.

3.6 Biocompatibility of Ag-ZnO NCs

3.6.1 Lethality of Ag-ZnO NCs against brine shrimp

Lethality of Ag-ZnO NCs was performed in 96 well plates against *Artemia salina* (brine shrimp). In the first step, the eggs of *Artemia salina* eggs were incubated in a plastic tray for 24-48 hours to hatch them. The tray was filled with sterile sea water ($38gL^{-1}$) in addition to sufficient oxygen and dried yeast ($6mgL^{-1}$). After that, ten mature nauplii (phototropic) were taken and 25-200 µg mL^{-1} of Ag-ZnO NCs were added to the well to bring up the final volume up to 300 µL. Doxorubicin was used as a positive control while DMSO (1%) in sea water was used as a negative control. Table curve 2D v5.01 with a less than or equal to 50% mortality rate was utilized for the calculation of lethal concentration (LC50).

3.6.2 Biocompatibility of Ag-ZnO NCs with hRBCs (Human red blood cells)

After receiving a written consent, the blood samples from 6 healthy males and 6 healthy females (belonged to 28-35 years age group), were taken to determine the hemo-compatibility of Ag-ZnO NCs with RBCs. The procedures involving human participants were carried out taking into considerations with Helsinki Declaration 1964 and its later revisions as well as the ethical standards of national and international research committees. Due to the utilization of human subjects, the experiment was performed while following the above-mentioned considerations. To avoid blood coagulation, EDTA vacutainers were used for blood collection. After RBCs extraction, the erythrocytes and 100 µL Ag-ZnO NCs were added to an Eppendorf tube and then incubated at 35 ^{o}C for 1 hour and performed centrifugation for 10 minutes at 1000 rpm. 96-well plates filled with 100 µL of supernatant and were utilized to determine the release of hemoglobin at 450 nm with the help of Bio Tek ELX 800 Absorbance Microplate Reader (Bio Tek

instruments, France). DMSO was used as a negative control while Triton X-100 was used as a positive control. The resulting data were presented as %hemolysis and calculated with the use of following stated formula:

$$\text{Hemolysis}\,(\%) = \frac{\text{sample absorbance} - \text{negative control absorbance}}{\text{positive control absorbance} - \text{negative control absorbance}} \times 100$$

3.7 Anti-cancerous activities of Ag-ZnO NCs

3.7.1 Measurement of cells viability of HepG2 cells

ATCC (American Type Culture Collection's) HB-8065 human liver cancer cells (HepG2) with the use of dye 3-(4, 5-dimethgylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) to examine the cell viability (MTT). Dulbecco's Modified Eagle Medium was utilized for culturing HepG2 cells. In 96 well plates, 200µL of each sample of Ag-ZnO were added and pre-seeded with HepG2 cells (>90% viability; 200µL per well; 1×10^4 cells per well) for 24 hours. After that 10µL of MTT dye (5 mg mL^{1}) was added and then incubated for 3h. Acidified sodium dodecyl sulfate (10%) was used to dissolve the generated insoluble formazan. To examine the absorbance at 570 nm, the overnight incubated cells were placed on a microplate reader (Platos TR, 496. AMP, AMEDA Labordiagnostik GmbH, Graz, Austria). NTCs (Non-treated cells) were utilized as a control. The equation mentioned below was used to represent the percentage of cell viability.

$$\text{Viability}\,(\%) = \frac{\text{sample absorbance} - \text{control absorbance}}{\text{NTC absorbance} - \text{media absorbance}} \times 100$$

3.7.2 Evaluation of intracellular reactive oxygen and nitrogen species (ROS/RNS) production

The production level of intracellular ROS/RNS production is measured using the fluorescent dye dihydrorhodamine-123 (DHR-123). The method of Nazir et al (Nazir et al., 2019) was followed to measure the production of intracellular ROS/RNS production. Pre-seeded HepG2 in 96-well plate were rinsed twice with PBS (phosphate buffer saline) and then suspended in PBS containing 0.4 µM fluorescent DHR-123. The mixture was then incubated for 10 m at 30 ^{o}C in dark. Finally, A VersaFluor flurometer (Bio-Rad, France) was utilized to measure the fluorescence (λem = 535 nm and λex = 505 nm) of the mixture.

3.7.3 Mitochondrial membrane potential (MMP) measurement

Ag-ZnO resulted in the damage of MMP that was examined by following the protocol of khan et al (A. K. Khan et al., 2021). HepG2 cells were added into the culture medium containing 25 nM 3,3′-dihexyloxacarbocyanine iodide and incubated at 30 ºC for 40 minutes. MMP was reported as RFU (Relative fluorescence unit).

3.7.4 Expression of caspase-3 gene and caspase-3/7 activity

To quantify the expression of caspase-3 gene, first the total RNA was isolated through GeneJET RNA purification kit (Thermo scientific) and then quantified using the Quant-iT RNA assay kit (Invitrogen). First-strand cDNA synthesis kits (Thermo) were used to perform RNA reverse transcription. The PikoReal quantitative PCR was carried out with the DyNAnoColorFlash SYBER Green qPCR Kits (Thermo Fisher). The forward primers for caspase-3 were 5′-TGTTTGCTTCTGAGCC-3′ and the reverse primers were 5′-CACGCCATGTCATCATCAAC-3′ (PCR product size: 210 bp). Pikoreal software was utilized to analyze the data. With the following manufacturer's instructions, the Apo-One Homogeneous Caspase-3/7 Assay kits (Promega) were utilized to measure *in vitro* caspase-3/7 activity. All the data were performed in triplicate

4. Results and discussion

Previous studies have been demonstrated that the plant components such as proteins, amides, flavonoids, phenolics, tannins, saponins etc. worked as capping, reducing and stabilizing agents for the synthesis of nanoparticles. Due to these reasons, it was expected that the presence of above-mentioned constituents in *Aconitum violaceum* may be used as reducing and capping agents in the biofabrication of Ag-ZnO NCs. Therefore, the extracts of various parts (leaves, stem, and roots) of the plant (*Aconitum violaceum*) with high phenolics and flavonoids contents were utilized for the first time to biosynthesize Ag-ZnO NCs. The flasks were covered with aluminum foil due to sensitivity of $AgNO_3$ to light. At the end of the process, changes occurrence in colors from yellow-brown to black-brown indicated the biosynthesis of Ag-ZnO NCs. The Biosynthesized Ag-ZnO NCs were then analyzed through various approaches like XRD, FTIR, SEM, EDX, DLS and HPLC.

4.1 X-ray Diffraction (XRD)

Structure, purity and crystalline nature of leaf, stem and root-mediated biosynthesized Ag-ZnO NCs were confirmed through XRD pattern shown in fig: 9, in which both minor and major peaks were examined on 20-80^o at 2θ^o. Results revealed the diffraction peaks at 38.17^o, 44.25^o, 64.42 and 77.42^o corresponds to lattice plans (111), (200), (220) and (311) respectively. Previous studies revealed hexagonal structure for ZnO using the standard data (JCPDS-80-0075) and showed center cubic for AgNPs using (JCPDS 87-0717) (Yeganeh-Faal, Bordbar, Negahdar, & Nasrollahzadeh, 2017). Same results were confirmed by previous studies in which *Tetradenia riperia* leaf extract was used for the synthesis of Ag-ZnO NCs. The average grain size of biofabricated Ag-ZnO NCs was determined by Deby-Scherrer equation (Mtavangu et al., 2022b).

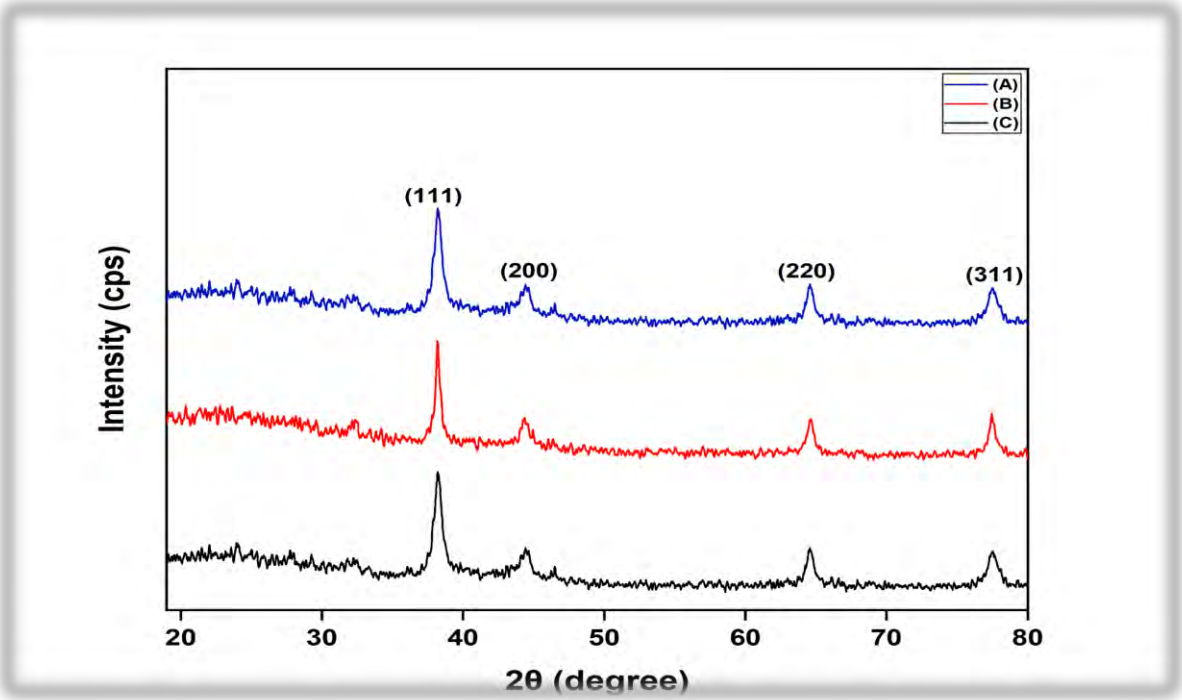
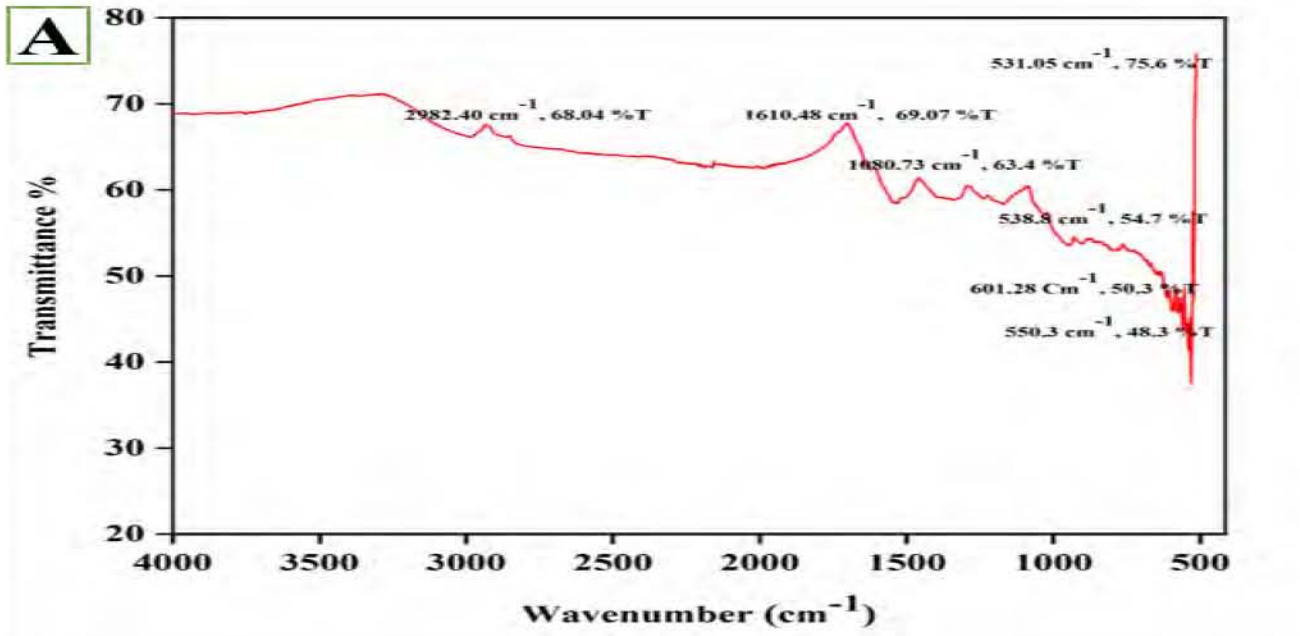


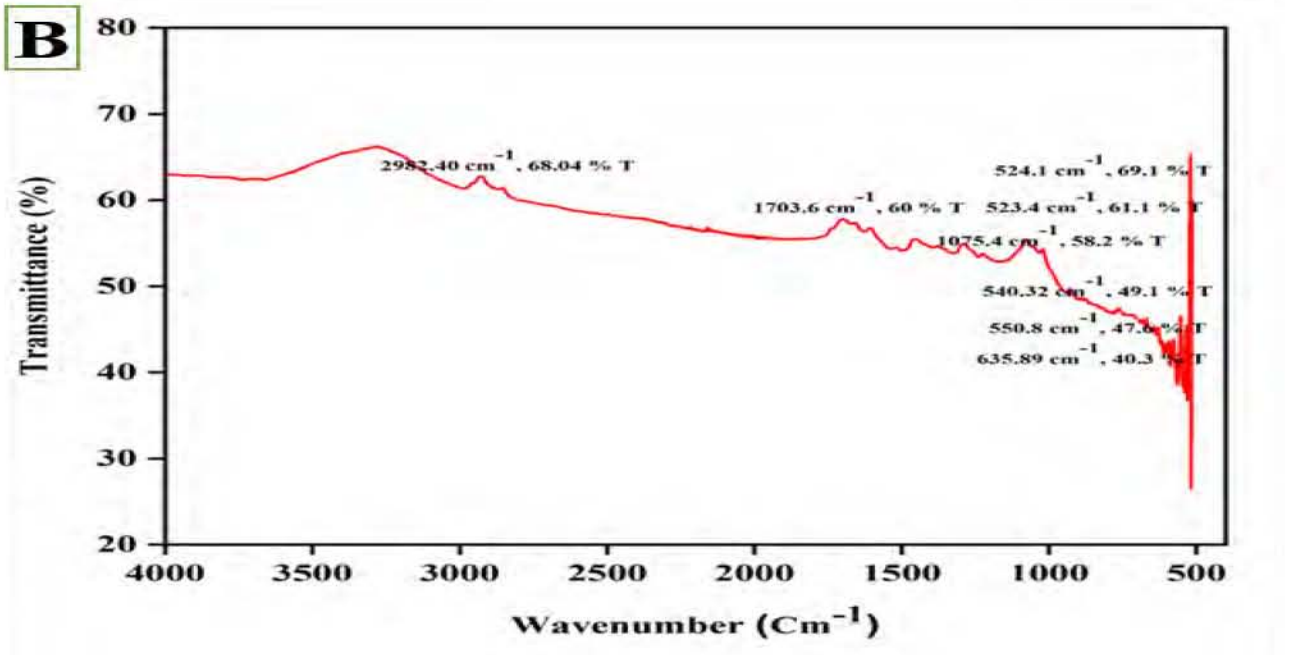
Figure 9: **XRD pattern of Ag-ZnO NCs. (A) leaf-mediated, (B) Root mediated and (C) Stem-mediated Ag-ZnO NCs**

4.2 Fourier transform infrared spectroscopy (FTIR)

FTIR is a broadly used approach to characterize NPs at molecular level. This approach was performed for Ag-ZnO NCs to identify the biomolecules in leaf, root and stem extracts of *Aconitum violaceum* which are responsible for reduction, capping and stabilization. This tool detects the attached functional groups of biomolecules via measuring the vibrational frequencies of bonds in molecule. FTIR was performed for the synthesized Ag-ZnO NCs at range 4000-500 cm^{-1}. Fig: 10 (A) indicates the FTIR spectra for leaf-mediated Ag-ZnO NCs showed various at peaks 2982 cm^{-1}, 1610 cm^{-1}, 1080 cm^{-1}, 601 cm^{-1}, and 550 cm^{-1}, 538 cm^{-1}, 531 cm^{-1}, and the peaks for root-mediated NCs (B) were observed at 2982 cm^{-1}, 1703 cm^{-1}, 1075 cm^{-1}, 635 cm^{-1}, 550 cm^{-1}, 540 cm^{-1}, 524 cm^{-1}, and 523 cm^{-1} whereas peaks for stem-mediated NCs (C) were observed at 2982 cm^{-1}, 1604 cm^{-1}, 1170 cm^{-1}, 610 cm^{-1}, 543 cm^{-1}, 540 cm^{-1}, 528 cm^{-1}. Peak at 2982 cm^{-1} is due to carboxyl and aromatic hydrocarbons (Chireh, Naseri, & Ghiasvand, 2019) and peak at 1610 $cm^{-1}$are responsible for stretching vibration modes of heptazine heterocyclic ring (C_6N_7) (S. Iqbal et al., 2021).

Presence of peak at 1610 at cm^{-1} in higher frequency range is due to O-H stretching from Zn-OH bonds and the intensive absorption band at 550–560 cm^{-1} is assigned as a result of Zn-O stretching vibration (Bouzid, Faisal, Harraz, Al-Sayari, & Ismail, 2015). Peaks between 2091 and 2443 cm^{-1} relate to weak C=C stretching of alkynes. The =C-H bends of alkenes is represented by 599 and 621 wavenumber cm^{-1} (Anjum, Nawaz, Ahmad, Hano, & Abbasi, 2022). Peak at 1621.80 cm^{-1} is attributed to the O-H bending mode and peak at 2982 cm^{-1} can be attributed to C-H stretching (Jafarirad, Taghizadeh, & Divband, 2020). The peaks at the fingerprint regions are at 510-540 cm^{-1} representing Cu-O stretching. Previous studies reported that these peaks verify the creation of CuO. The functional groups demonstrate the possible participation of phenols, carboxyl acid and amine groups in bio-reducing and stabilizing of Ag-ZnO NCs. Overall, in the synthesis of Ag-ZnO NCs, FTIR data revealed the participation of carboxyl groups, polyphenols and amides as capping of nanoparticles (Anjum et al., 2021).


A
Transmittance %
Wavenumber (cm-1)
2982.40 cm-1, 68.04 %T
1610.48 cm-1, 69.07 %T
1080.73 cm-1, 63.4 %T
538.8 cm-1, 54.7 %T
601.28 Cm-1, 50.3 %T
550.3 cm-1, 48.3 %T
531.05 cm-1, 75.6 %T


B
Transmittance (%)
Wavenumber (Cm-1)
2982.40 cm-1, 68.04 % T
1703.6 cm-1, 60 % T
1075.4 cm-1, 58.2 % T
635.89 cm-1, 40.3 % T
524.1 cm-1, 69.1 % T
523.4 cm-1, 61.1 % T
540.32 cm-1, 49.1 % T
550.8 cm-1, 47.6 % T

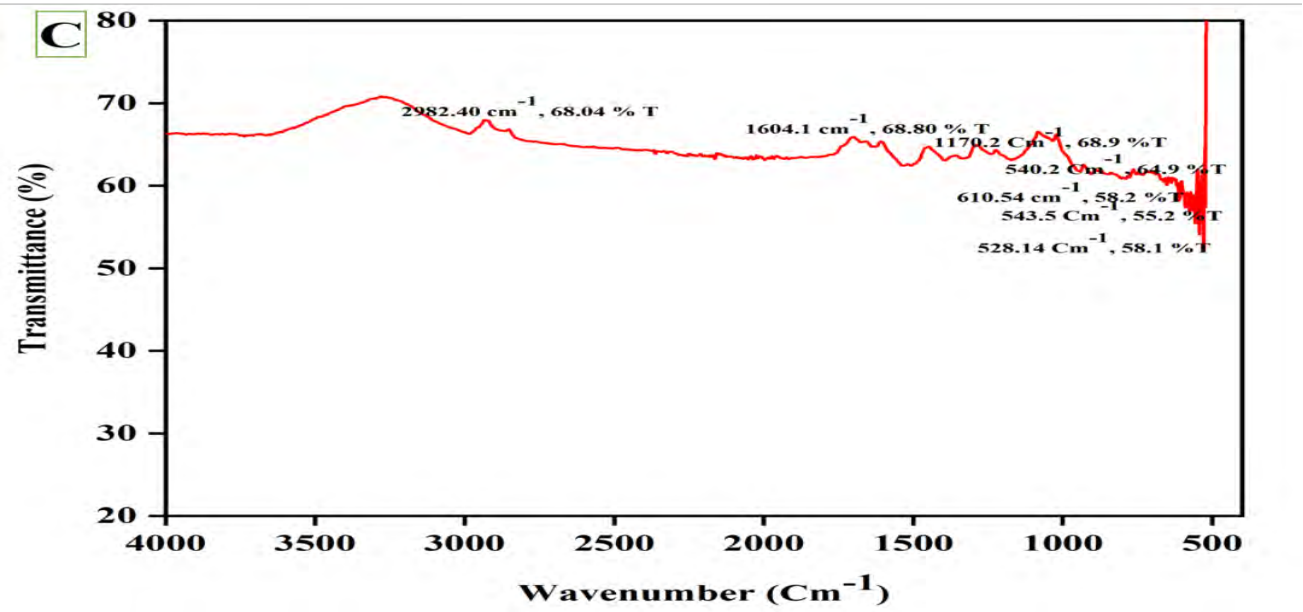


***Figure 10*: FTIR patterns of biofabricated (A) leaf-mediated Ag-ZnO NCs (B) Root-mediated Ag-ZnO NCs and (C) stem-mediated Ag-ZnO NCs of Aconitum violaceum**

4.3 SEM (Scanning Electron Microscopy)

The morphology and size of biosynthesized Ag-ZnO NCs from leaf, root and stem extract of *Aconitum violaceum* were evaluated through SEM. Surface morphology of Ag-ZnO NCs exhibits spherical structure with some degree of aggregation (fig: 11). The images were taken at different magnifications i.e., 10 µm, and 20 µm. The micrographs illustrated that the shapes of nanocomposites are spherical either more or less clustered with some agglomerated particles. The average size of NCs were calculated via SEM images using Image J software is 54 nm (leaf-mediated Ag-ZnO NCs), 66 nm (root-mediated Ag-ZnO NCs) and 52 nm (Stem-mediated Ag-ZnO NCs). Similar results were reported by previous studies (Iqbal et al., 2022). Moreover, the morphology of nanoparticles also depends on interaction of capping and stabilizing agents in extract.

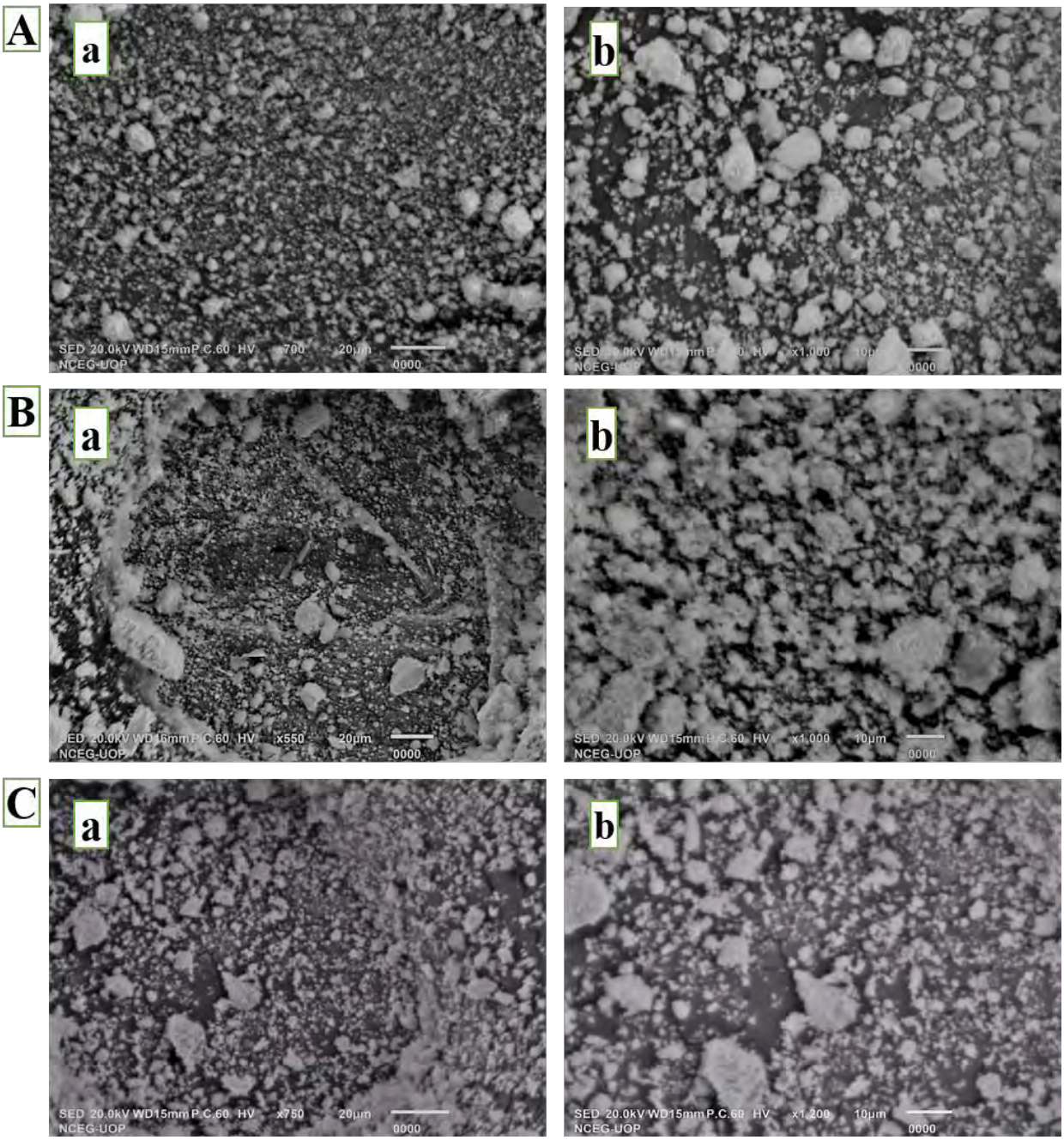
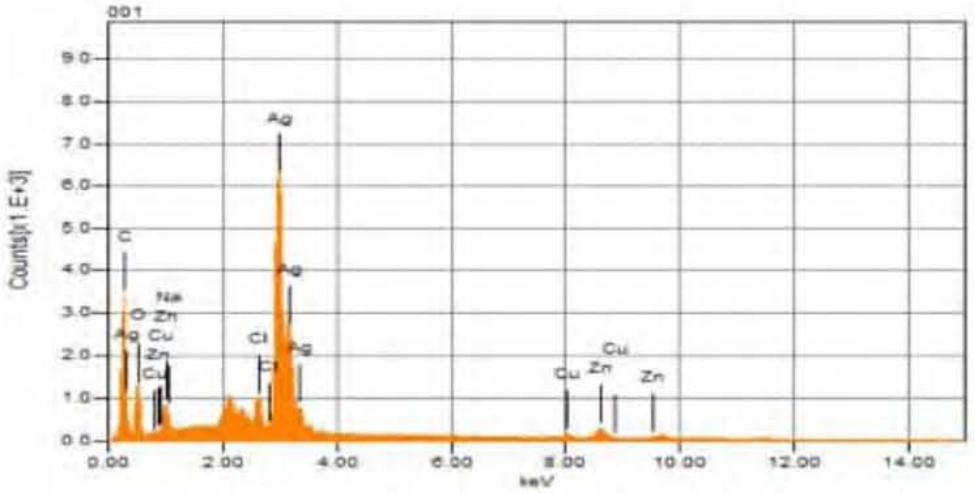


Figure 11:SEM micrographs of (A) Leaf-mediated Ag-ZnO NCs (a) shows micrograph at 20µg (b) shows micrograph at 10µg, (B) Root-mediated Ag-ZnO NCs (a) shows micrograph at 20µg (b) shows micrograph at 10µg, (C) Stem-mediated Ag-ZnO NCs (a) shows micrograph at 20µg (b) shows micrograph at 10µg

4.4 Energy dispersive X-ray (EDX)

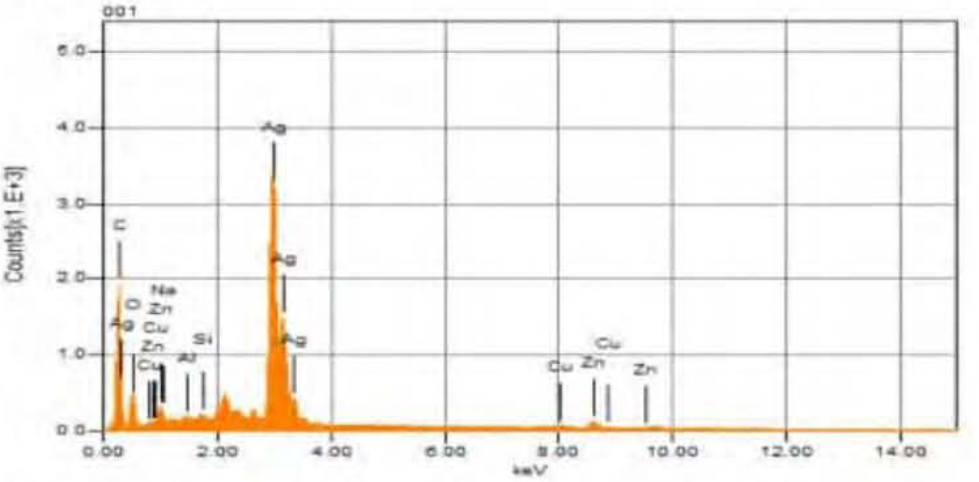
EDX analysis revealed elemental composition of Ag-ZnO NCs from *Aconitum violaceum*. The EDX spectrum of all 3 samples shows strong signal at 3KeV establishing Ag in Ag-ZnO NCs. Fig: 12 shows the total weight estimated for (A) leaf-mediated Ag-ZnO NCs was 48.64 % for Ag, 5.82 % Zn and 20.5 for O followed by low percentage of the rest of all other elements such as C, Cu, Cl and Na. Correspondingly, the estimated weight for (B) root-mediated Ag-ZnO NCs was 55.8 % for Ag, 4.87% for Zn and 16.5% for O followed by low percentage of the rest of all other elements such as C, Cu, Cl and Na etc. which is a very small amount. Similarly, the estimated weight for (C) stem-mediated Ag-ZnO NCs was 63.34 % for Ag, 4.28 % for Zn and 15.84% for O followed by low percentage of the rest of all elements such as C, Cu, Cl and Na etc. Total mass percentage noted for (A) leaf-Ag-ZnO was 74.96, for (B) root-mediated Ag-ZnO was 77.17 and (C) stem-mediated Ag-ZnO was 83.46. The estimated values showed that Ag-ZnO NCs synthesized with good purity. Our results corresponds with previous published studies by (Noohpisheh et al., 2020).

A

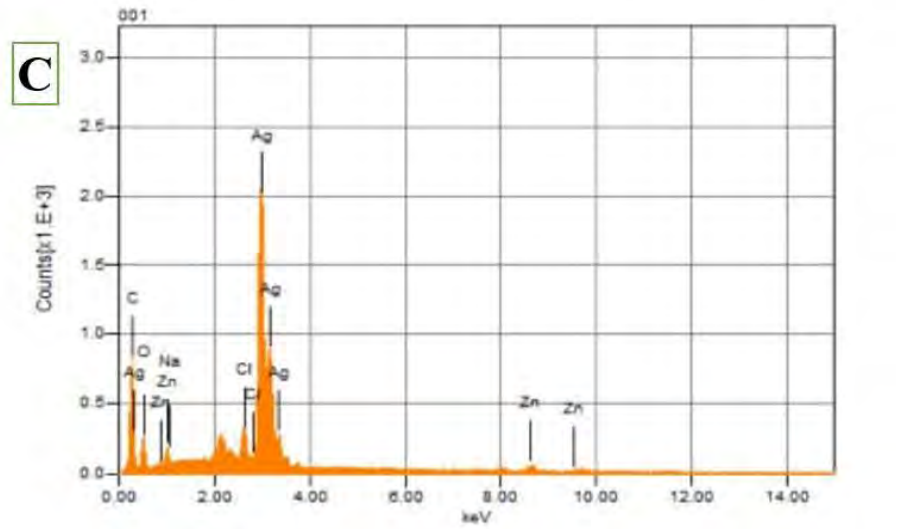


Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	20.63	46.99	0.02	84062	0.0194311	K
O	20.65	35.31	0.09	33460	0.0353445	K
Na	0.70	0.84	0.04	5121	0.0021973	K
Cl	1.64	1.27	0.02	29034	0.0116272	K
Cu	1.90	0.82	0.06	7047	0.0126354	K
Zn	5.82	2.44	0.09	18192	0.0389052	K
Ag	48.64	12.33	0.12	506053	0.2905291	L
Total	100.00	100.00				

B



Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	19.94	49.42	0.02	46856	0.0212721	K
O	16.58	30.85	0.12	12018	0.0249335	K
Na	0.52	0.67	0.06	1744	0.0014695	K
Al	0.33	0.37	0.03	2092	0.0013545	K
Si	0.27	0.29	0.03	1864	0.0011658	K
Cu	1.67	0.78	0.08	2878	0.0101353	K
Zn	4.87	2.22	0.12	7109	0.0298616	K
Ag	55.83	15.41	0.19	269960	0.3043948	L
Total	100.00	100.00				



Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	15.84	45.02	0.03	19761	0.0169561	K
O	14.10	30.09	0.16	5513	0.0216161	K
Na	0.49	0.73	0.08	902	0.0014366	K
Cl	1.94	1.87	0.04	8707	0.0129429	K
Zn	4.28	2.24	0.16	3402	0.0270072	K
Ag	63.34	20.05	0.27	166994	0.3558808	L
Total	100.00	100.00				

Figure 12: **EDX analysis of Ag-ZnO NCs from (A) leaf-mediated Ag-ZnO NCs, (B) root-mediated Ag-ZnO NCs and (C) Stem-mediated Ag-ZnO NCs**

4.5 DLS (Dynamic light scattering)

Surface potential and size distributions were evaluated through DLS approach. Samples were dispersed in DH_2O followed by sonication to keep PDI (Polydispersity Index) lower than 0.5 that is highly recommended for analysis. High intensity peaks for (A) (a) leaf-mediated Ag-ZnO were observed at 96.6nm and average particle size measured as 60.21nm (Fig: 13 (A) (a)). In the same way, high intensity peaks for (B) (a) leaf-mediated Ag-ZnO were observed at 133.5 nm and average particle was measured at 94.68nm. (Fig: 13 (B) (a)). Similarly, the high intensity peaks for (C) (a) stem-mediated Ag-ZnO NCs were observed at 126.6nm and average particle size was measured 68.64nm. (Fig: 13 (C) (a)).

In all samples the size of particles were slightly higher than SEM size due to poor dispersion. This was due to DLS calculating the hydrodynamic size (i.e., size and surface water molecule). Zeta potential for (A) (b) leaf-mediated Ag-ZnO measured was -26.6 mV.In the same way zeta potential for (B) (b) root-mediated Ag-ZnO measured was -19.9 mV.

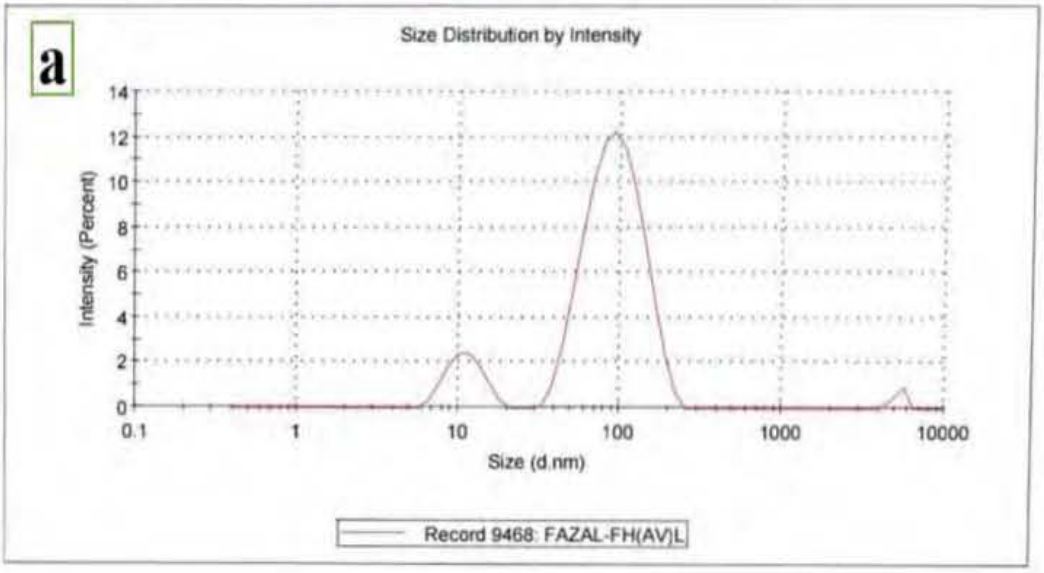
Similarly, zeta potential for (C) (b) stem-mediated Ag-ZnO measured was -21.1 mV as shown in (Fig: 13 (C) (b)). In all samples, the negative potential was due to the use of plant extract, and it also indicated stability of particle in aqueous medium. Suspension that reveled ≥15mV potential are stable thus Ag-ZnO NCs were reflected to be stable and well dispersed.

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	60.21	Peak 1:	96.64	87.1	37.15
PdI:	0.499	Peak 2:	11.29	11.4	2.738
Intercept:	0.868	Peak 3:	5236	1.5	451.7

Result quality : Refer to quality report

a



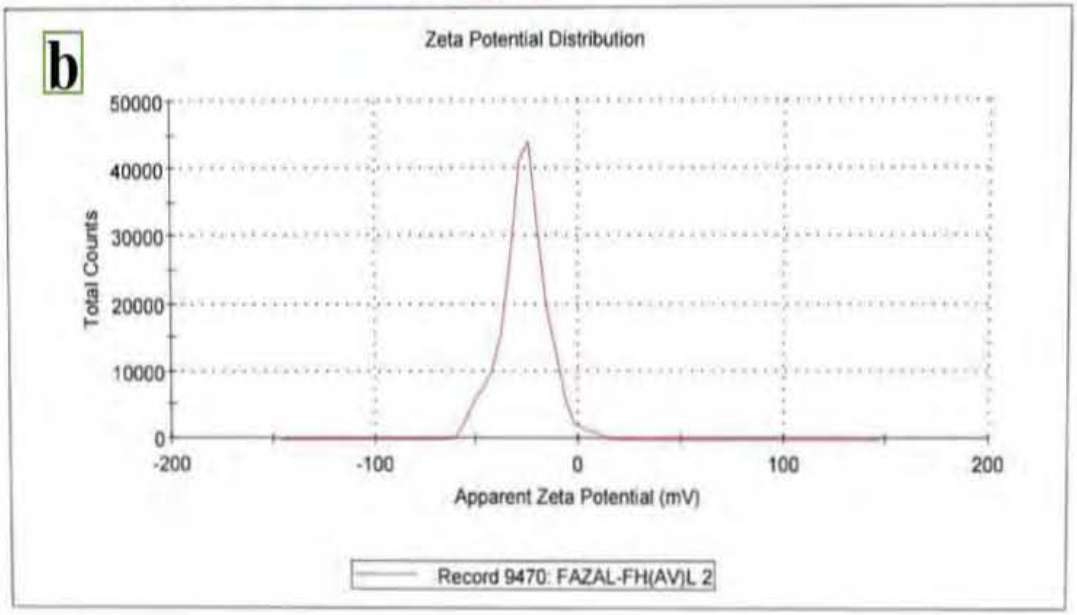
A

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-26.6	Peak 1:	-26.6	100.0	11.4
Zeta Deviation (mV):	11.4	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.165	Peak 3:	0.00	0.0	0.00

Result quality : See result quality report

b

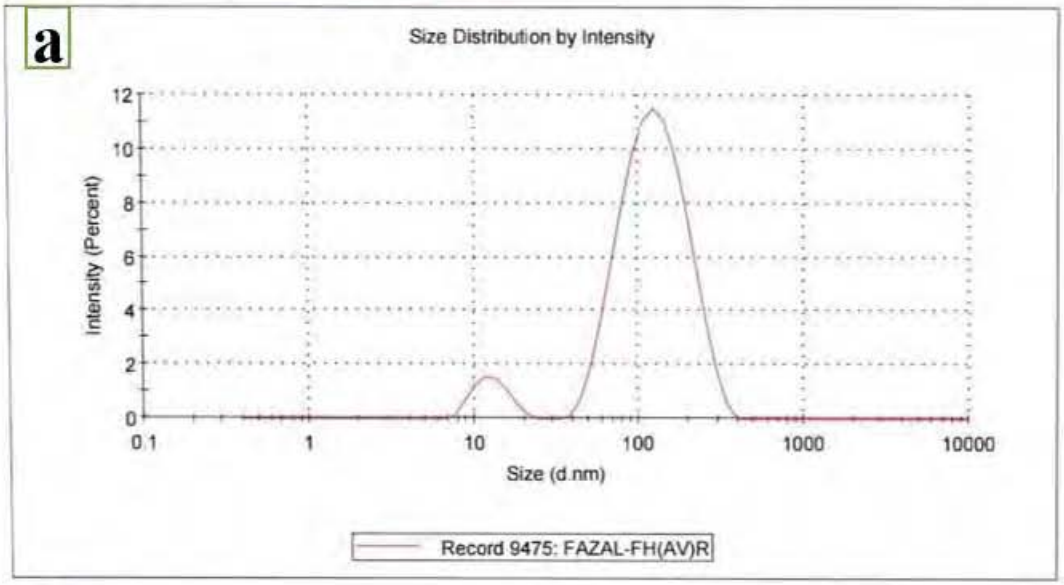


Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	94.68	Peak 1:	133.5	93.1	58.69
PdI:	0.334	Peak 2:	12.91	6.9	3.007
Intercept:	0.876	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

a



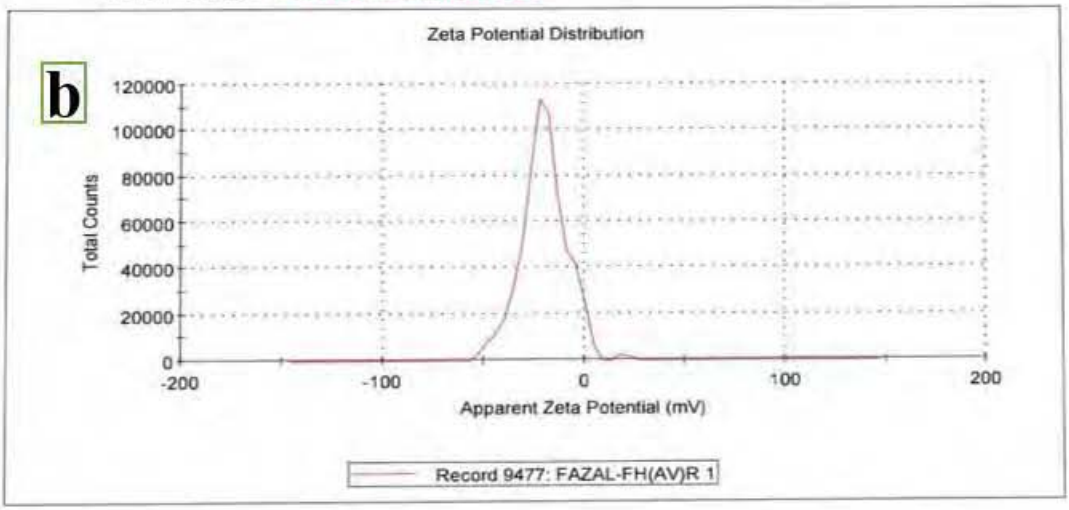
B

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-19.9	Peak 1:	-20.2	99.3	11.0
Zeta Deviation (mV):	11.4	Peak 2:	19.7	0.7	2.20
Conductivity (mS/cm):	0.147	Peak 3:	0.00	0.0	0.00

Result quality : See result quality report

b



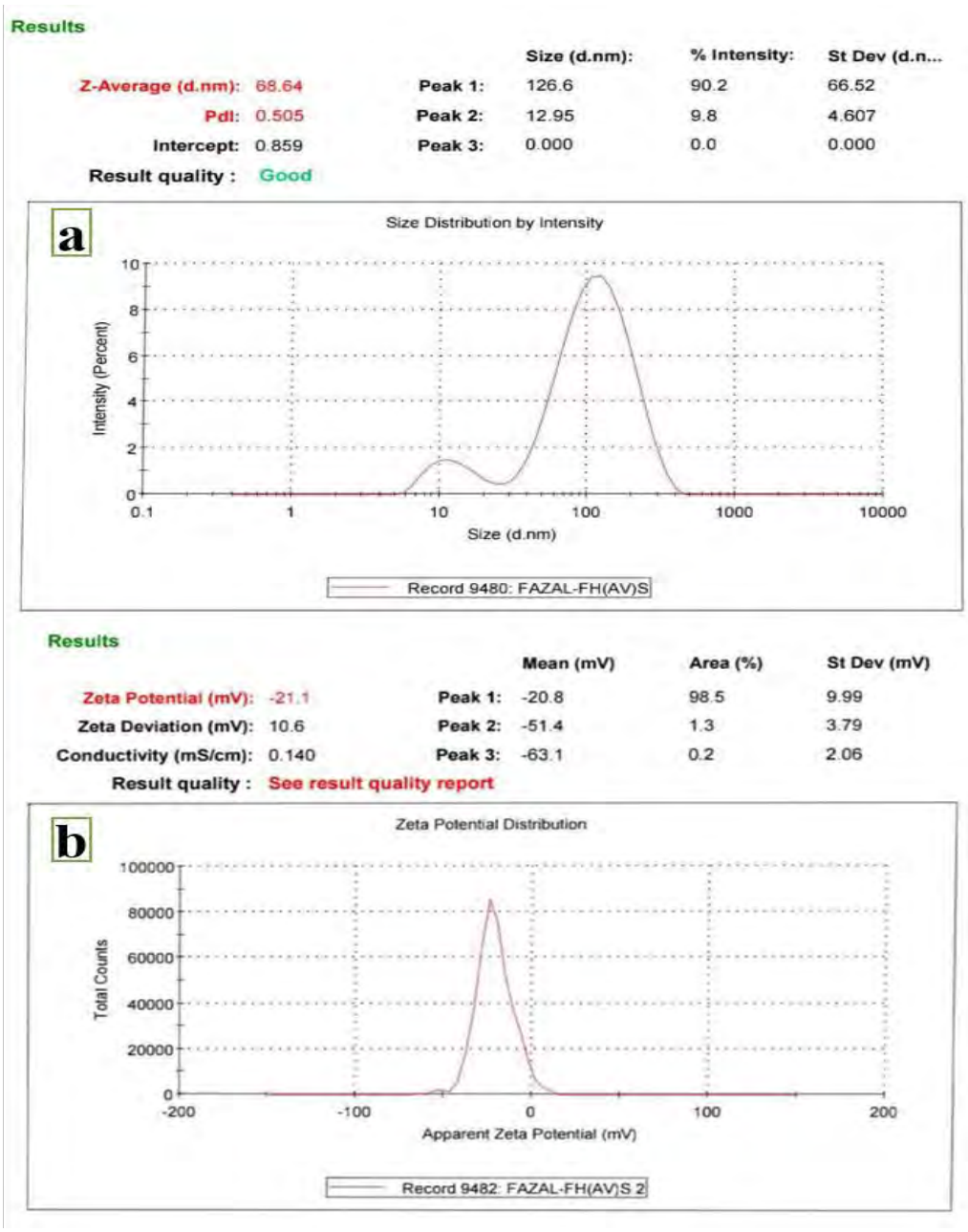
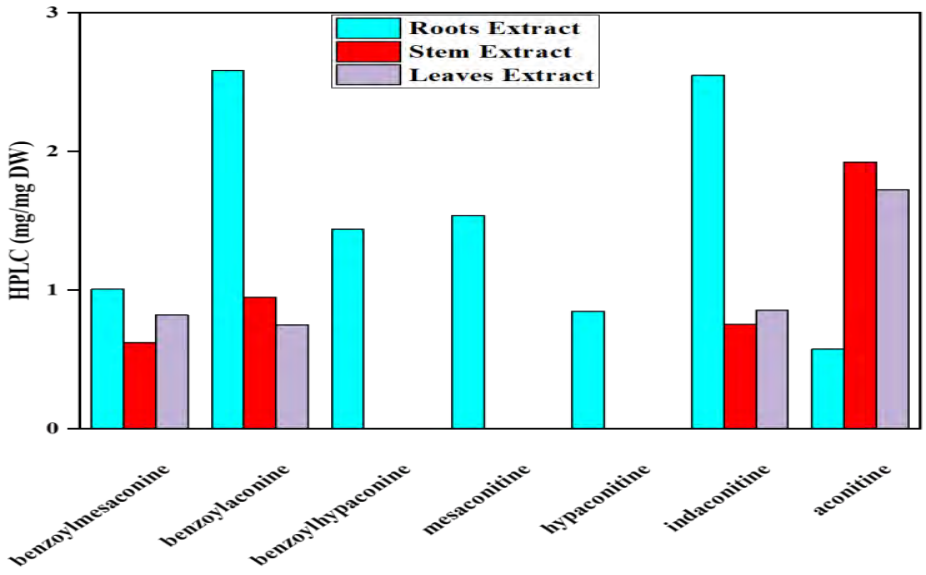


Figure 13: **Zeta size=(A)(a) leaf-mediated Ag-ZnO NCs, (B)(a) root-mediated Ag-ZnO NCs, (C)(a) stem-Ag-ZnO NCs. Zeta potential=(A)(b) leaf-mediated Ag-ZnO NCs, (B)(b) Ag-ZnO NCs, (C)(b) stem-mediated Ag-ZnO NCs**

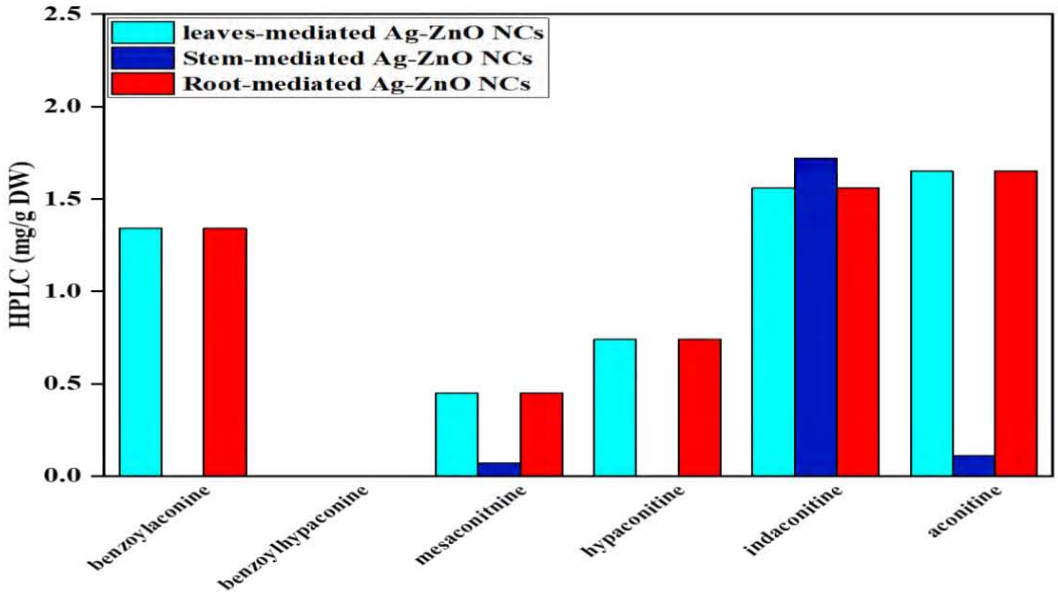
4.6 HPLC profiling of *Aconitum violaceum* extract and its mediated Ag-ZnO NCs.

HPLC analysis was performed to quantify phytochemicals in *Aconitum violaceum* extract and its mediated Ag-ZnO NCs. HPLC fingerprinting for root extract revealed seven chromatographic peaks, 1. Benzoylmesaconine, 2. Benzoylaconine, 3. Benzoylhypaconine, 4. Mesaconitine, 5. Hypaconitine, 6. Indaconitine, 7. Aconitine. Similarly, HPLC fingerprinting for stem and leaf extracts revealed four compounds (Benzoylmesaconine, Benzoylaconine, Indaconitine, Aconitine). Similar compounds were also observed in Ag-ZnO NCs. In leaf-mediated Ag-ZnO NCs Benzoylmesaconine was not detected. Similarly, Benzoylmesaconine, Benzoylhypaconine were not detected in root-mediated Ag-ZnO and Benzoylmesaconine, Benzoylaconine were not indicated in stem-mediated Ag-ZnO NCs. An interesting observation was made during NCs analysis that some compounds were present in extracts while not quantified in all leaves, root and stem mediated NCs. The absence of compounds in NCs indicated that the compound might be not involved in the capping of NCs. The changes in composition and disappearance of phytochemicals also indicate specificity of capping agents. Further studies are required to understand the exact mechanism of NCs synthesis and capping. Moreover, all these compounds have a wide range of biological activities like Antioxidant, anti-inflammatory, anticancer, antibacterial, antiviral, immunomodulatory and other biological applications. Cell viability and anticancer assays represents the major applications of *Aconitum violaceum* (Sabir et al., 2016).

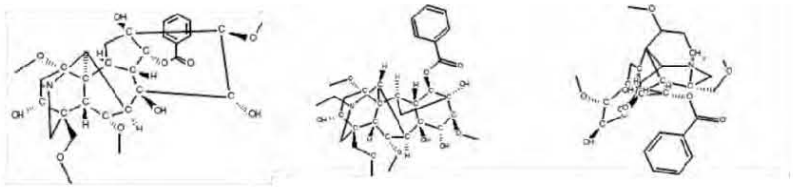
A)



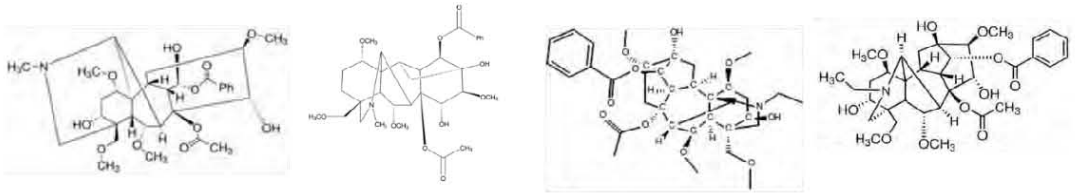
B)



C)



1. Benzoylmesaconine 2. Benzoylaconine 3. Benzoylhypaconine



4. Mesaconitine 5. Hypaconitine 6. Indaconitine 7. Aconitine

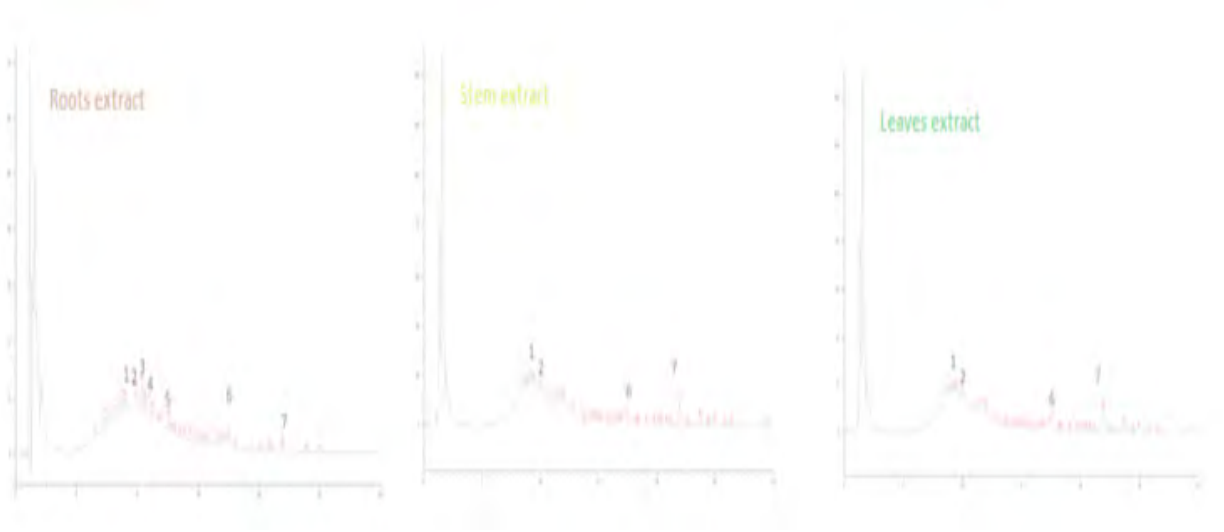


Figure 14: (a) HPLC fingerprinting of *Aconitum violaceum* (leaves, roots, stem) extract (b) HPLC fingerprinting of *Aconitum violaceum* (leaves, roots, and stem) mediated Ag-ZnO NCs, (c) Structures of isolated phytochemicals from *Aconitum violaceum* extract, (d) HPLC chromatogram of the roots, stem and leaves extracts

4.7 Cytotoxic assays

4.7.1 Brine shrimp assay

Brine shrimp lethality assay was carried out to determine the lethality of plant bioactive compounds and green synthesized Ag-ZnO NCs. This provides information on environmental and aquatic biosafety. *Artemia salina* was used in these studies as it is reported to be one of the most beneficial organisms. The LC50 value for root extract was 4.98 ±1.02ug/ml while root-mediated Ag-ZnO NCs was 2.13±0.38ug/ml and the value for leaves extract was 5.09±1.23ug/ml where the leaves-mediated Ag-ZnO NCs was 1.79±0.29ug/ml, value for stem extract was 5.02±1.21ug/ml and stem-mediated Ag-ZnO NCs value was 2.04±0.42ug/ml. These values showed that the NCs are moderately toxic as compared to plant extracts. A comparison between plant extract and Ag-ZnO NCs is shown in fig: 15. Current results correlates with previous reported studies reported by Anjum et, al (Anjum et al., 2022). These results demonstrate the unseen value of bio-fabricated Ag-ZnO NCs for anticancer, antibacterial and antioxidant applications etc.

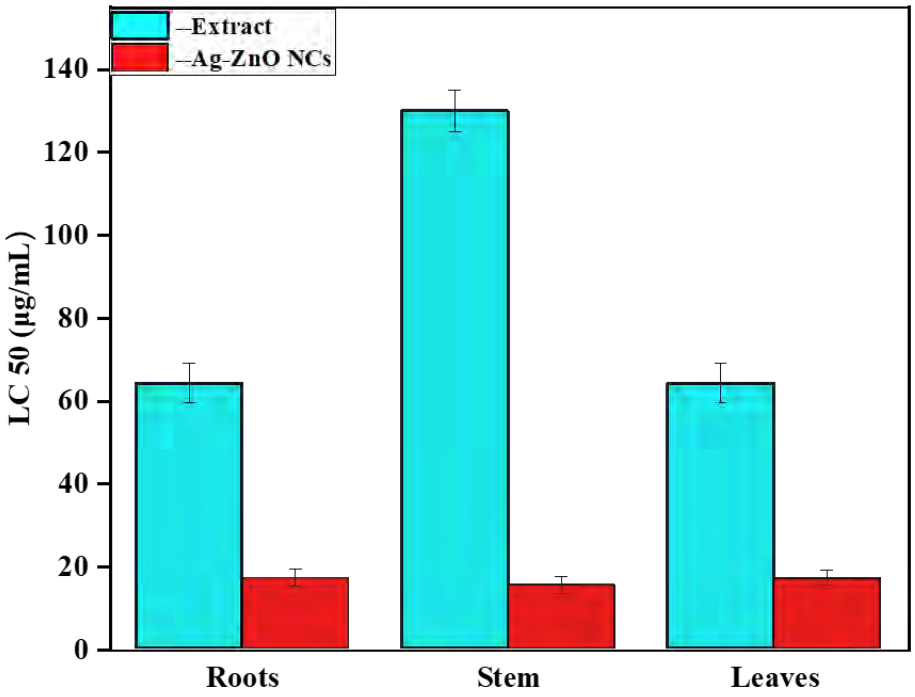


***Figure 15: Aconitum violaceum* extracts and their-mediated Ag-ZnO NCs cytotoxicity towards Brine shrimp**

4.7.2 Hemolysis activity

It is reported that the toxicity of nanomaterials on blood can be studied with hemolysis activity where the RBCs and nanomaterials are combined to check the hemolysis. According to "American Society for Testing and Materials Designation," nanomaterials with hemolysis rates >5% are thought to be hemolytic, 2-5% are thought to be slightly hemolytic, and 2% are thought to be non-hemolytic. The comparison of Ag-ZnO NCs (root, leaf and stem-mediated) with plant extract (root, leaf and stem) revealed that Ag-ZnO NCs are more hemolytic than plant extract. Ag-ZnO NCs demonstrated higher hemolytic activity than their extracts. Root-mediated Ag-ZnO NCs revealed hemolytic activity with 6.6±0.10% where the extract of root showed slightly hemolytic activity with 3.75±0.26% upto 200ug/ml. Leaf-mediated Ag-ZnO NCs revealed hemolytic activity with 5.75±0.08% where the extract of leaf showed slightly hemolytic activity with 2.78±0.26% upto 200ug/ml.

Similarly, stem-mediated Ag-ZnO NCs revealed hemolytic activity with 6.63±0.10% where the extract of stem showed slightly hemolytic activity with 2.88±0.27% upto 200ug/ml (fig: 16). At this concentration our Ag-ZnO NCs are not safe for human use because the hemolysis rates are >5% which are hemolytic. This can be reduced with the reduction in concentration (as up to 100ug/ml), which may reduce the toxicity and can be utilized as a substitute drug as 5% hemolysis for biomaterials is acceptable. Previous investigations revealed that magnetite/silver nanoparticles demonstrated small hemolysis 1% up to 200µg mL^{-1} concentration while its observed that increase in concentration increased the hemolysis (Ramírez-Acosta, Cifuentes, Cruz, & Reyes, 2020).

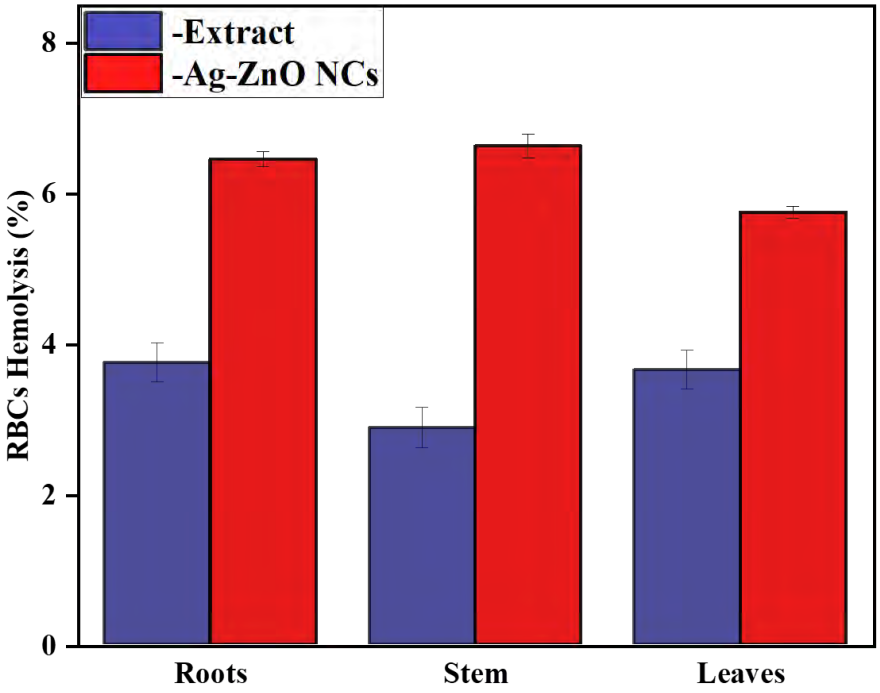


***Figure 16*: Hemolysis activity of *Aconitum violaceum* extracts and their-mediated Ag-ZnO NCs**

4.8 Anti-cancerous activities of plant extracts and Ag-ZnO NCs

4.8.2 MTT assay:

The MTT assay is one of the commonly used assays for evaluating anticancer activities indicating cell viability, proliferation and cytotoxicity. MTT assay was carried out to test the viability of HepG2 cells after treating with plant extract and their mediated Ag-ZnO NCs. Loss of cell viability by root-mediated Ag-ZnO NCs (28.0±3.4%), root extract (42.5±2.8%) as compared to NTCs which were 100% viable. In the same way, loss of cell viability by leaf-mediated Ag-ZnO NCs (30.0±2.4%), leaf extract (58.7±5.8%) as compared to NTCs which were 100% viable. Similarly, cell viability loss by stem-mediated Ag-ZnO NCs (32.1±3.4%), stem extract (60.7.5±6.6%) as compared to NTCs which were 100% viable, (figure 17). Previously, Ag-ZnO NCs were fabricated by laser ablation technique and showed its higher concentrations were cytotoxic against HCT116 and HeLa cancer cells (Elsayed et al., 2022).

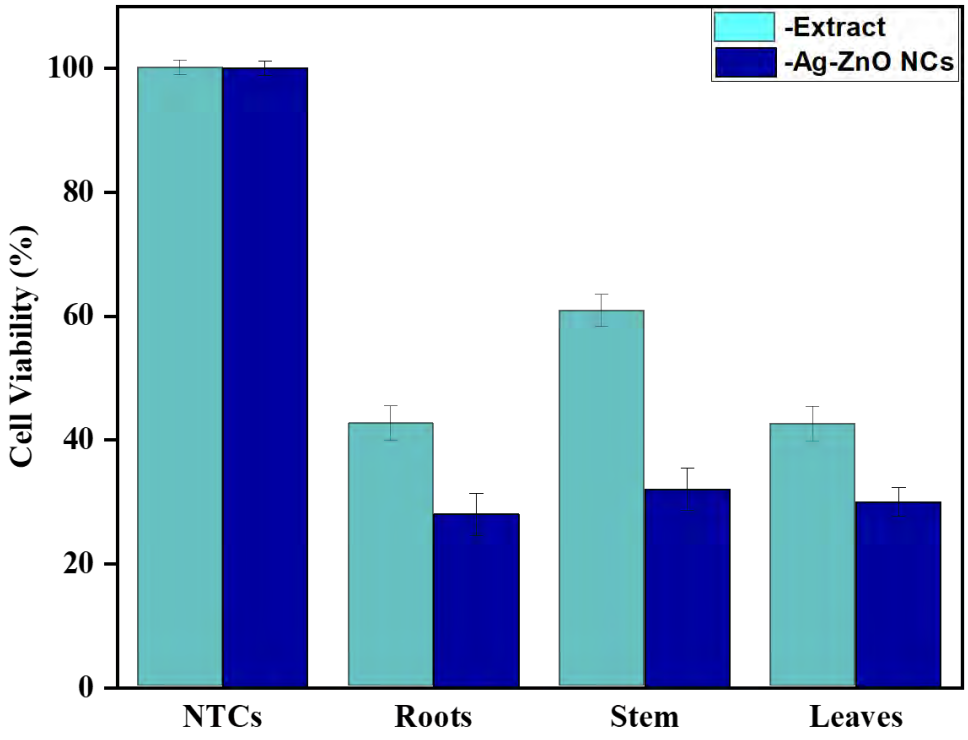


Figure 17: **Cell viability of HepG2 cell lines treated with *Aconitum violaceum* extracts and the extracts-mediated Ag-ZnO NCs and NTCs**

4.8.3 Intracellular ROS/RNS production

ROS/RNS production can be increased with an increase in loss of cell viability. The increase in ROS production directs the use of Ag-ZnO NCs to fight cancer. The unusual level of ROS causes disruption of cellular components like DNA damage, lipid peroxidation and apoptosis of cell. The agents responsible for ROS triggering can be used as therapeutic agent for killing cancer cells. The observed ROS production for root-mediated Ag-ZnO NCs was (6245.33±229.06 RFU) and root extract (3530.33±344.523 RFU). In the same way, the demonstrated ROS production for leaf-mediated Ag-ZnO NCs was (5612.00±249.96 RFU) and leaf extract (1788±202.78 RFU). Similarly, the examined ROS production for stem-mediated Ag-ZnO NCs (6073.33±142.00 RFU) and stem extract (1888.33±201.78 RFU).

The results showed that all root, leaf and stem-mediated Ag-ZnO NCs demonstrated higher ROS production as compared to their respective extracts as well as reveled highest production of ROS compared to NTCs (944.00±82.46 RFU) (fig: 18). Previous studies demonstrated by Sivamaruthi *et al.* that *Terminalia chebula*-mediated Ag-Pd nanoparticles ROS production in A549 lung cancer cells (Sivamaruthi, Ramkumar, Archunan, Chaiyasut, & Suganthy, 2019). These results provide insight into the increase in intracellular stress via NPs as a mode to fight cancer.

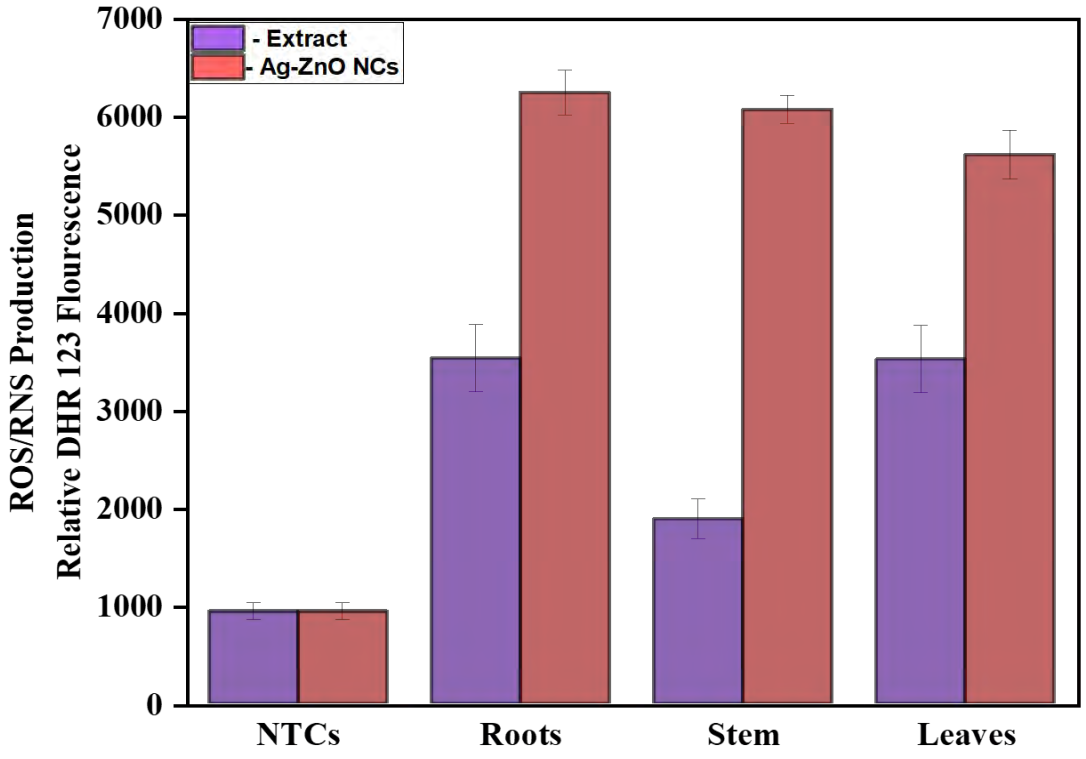


Figure 18: **ROS/RNS production of HepG2 cell lines treated with *Aconitum violaceum* extracts, their mediated-Ag-ZnO NCs and NTCs**

4.8.4 CASPASE-3 gene expression and CASPASE 3/7 gene activity

Caspases are the enzymes which cause programmed cell death as a result of damage to outer mitochondrial membrane acting as primary effectors of apoptosis. In addition, they also have non-apoptotic roles causing tumor relapse and tumor angiogenesis (Tang, Xia, Liang, Huo, & Wei, 2020). Caspase 3 gene expression was calculated for root-mediated Ag-ZnO NCs (632.82±23.30%) and root extract (383.68±24.67%). In the same way, caspase 3 gene expression was calculated for leaf-mediated Ag-ZnO NCs (595.82±10.06%) and leaf extract (172.30±25.36%). Similarly, caspase 3 gene expression was calculated for stem-mediated Ag-ZnO NCs (663.8±28.86%) and stem extract (182.50±25.46%) compared to NTCs (100%).

The subsequent rising in caspase-3/7 activity was also investigated associating with the expression of caspase-3 gene. Increase in caspase 3/7 activities result in apoptosis and are considered as useful for cancer therapy. Caspase 3/7 gene activity was resulted for root-mediated Ag-ZnO NCs (797.50±27.53 RFU/mg) and root extract (372.30±29.09 RFU/mg). In the same way, caspase 3/7 gene activity resulted for leaf-mediated Ag-ZnO NCs (687.44±13.24 RFU/mg) and leaf extract (260.6±16.04 RFU/mg). Similarly, caspase 3/7 gene activity resulted for stem-mediated Ag-ZnO NCs (821.80±50.51) and stem extract (250.6±16.00) compared to NTCs (100%). Previous studies demonstrated by Baharara *et al.* that the initiation of apoptosis via increased caspase-3/9 activity in HeLa cells by AuNPs fabricated from the extract of *Zataria multiflora* (Baharara, Ramezani, Divsalar, Mousavi, & Seyedarabi, 2016). Researchers also demonstrated strong caspase 3/7 activity in A-549 cells grown with 10 ppm AgNPs, showing the cell death via apoptosis reported by Sambale *et al* (Sambale et al., 2015). These high caspase3/7 activity of synthesized Ag-ZnO NCs from *Aconitum violaceum* extracts show anticancer activity that can be used is an alternate to chemotherapeutic agents.

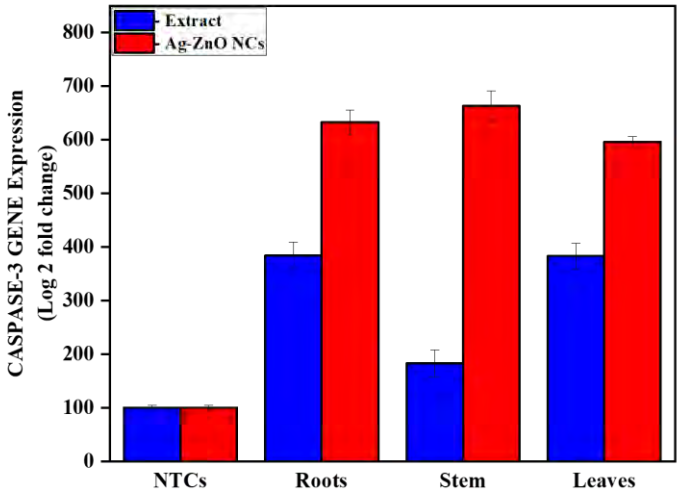


Figure 19: (a) CASPASE 3 gene expression of HepG2 cell lines treated with *Aconitum violaceum*-mediated Ag-ZnO NCs, *Aconitum violaceum* extract and NTCs

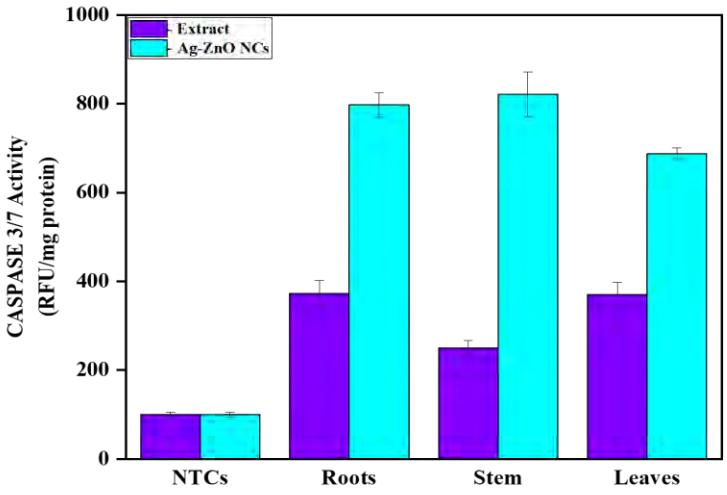


Figure 20: (a) CASPASE 3 gene expression of HepG2 cell lines treated with *Aconitum violaceum*-mediated Ag-ZnO NCs, *Aconitum violaceum* extract and NTCs (b) CASPASE 3/7 activity of HepG2 cell lines treated with *Aconitum violaceum*-mediated Ag-ZnO NCs, *Aconitum violaceum* extract, and NTCs

4.8.5 Mitochondrial Membrane potential (MMP)

Damage to mitochondria is significant in determining the fate of cell particularly programmed cell death/apoptosis. Mitochondrial damage includes loss of membrane permeability of mitochondria, mitochondrial swelling, mitochondrial splitting and apoptic protein releasing (Hussain, 2019). One important anti-cancerous approach is the loss of MMP in response to NPs. In this work, MMP was significantly reduced in HepG2 cells after exposure to greenly fabricated Ag-ZnO NCs.

Root-mediated Ag-ZnO NCs resulted (801.67±113.38 RFU) and root extract (1704±82.29 RFU). Leaf-mediated Ag-ZnO NCs resulted (862.87±87.84 RFU) and leaf extract (2122±128.99 RFU). Stem-mediated Ag-ZnO NCs resulted (826.33±33.29 RFU) and stem extract (2211±129.99 RFU) compared to NTCs (2965±7455 RFU). Among all maximum loss of MMP was noted with root-mediated Ag-ZnO NCs (801.67±113.38 RFU) compared with their extracts (1704±82.29 RFU) and NTCs (2965±7455 RFU) shown in fig: (21). Leaf and stem-mediated Ag-ZnO NCs also revealed better response when compared to their extracts and NTCs. These results demonstrated that Ag-ZNO NCs are strongly effective in disrupting MMP. Our results correlates with previous findings by (Anjum et al., 2022).

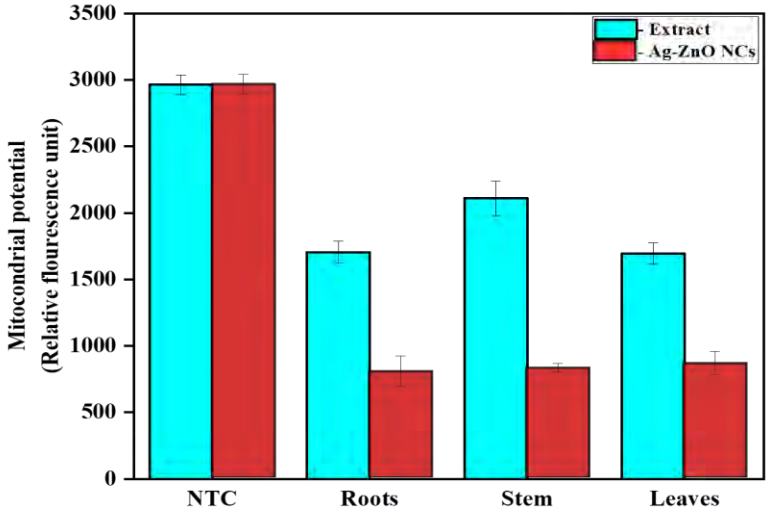


Figure 21: **Mitochondrial membrane potential (MMP) of HepG2 cell lines treated with *Aconitum violaceum* mediated Ag-ZnO NCs, extracts and NTCs**

5. Conclusion

In current era, NPs are the tools for research almost in every field due to their wide range of applications. NPs are most widely applicable in the field of medical sciences therefore our studies involves the manipulation of green synthesized NCs from roots, leaves and stem of *Aconitum violaceum* with their respective extracts. Biosynthesis of Ag-ZnO NCs is non-toxic, cheap and more environmental friendly. The biosynthesized Ag-ZnO NCs exhibited well defined phytochemical properties, spherical morphology and size range between 60-72 nm. FTIR analysis showed the functional groups of bioactive compounds in medicinal plants. HPLC analysis detected various phytochemical compounds including benzoylmesaconine, benzoylaconine, benzoylhypaconine, mesaconine, hypaconine, indaconine, aconine that have anticancer activities. Their activities were further confirmed by Caspase gene activity, ROS production, mitochondrial potential and viability test. Biosafety was also tested on brine shrimp and human RBCs. Ag-ZnO NCs demonstrated enhanced anticancer activity on HepG2 cells. However, further studies are recommended to be conducted on Ag-ZnO NCs for exploring their biomedical applications in both *in vivo* and *in vitro* levels.

References

Abbas, Z., Kousar, S., Aziz, M. A., Pieroni, A., Aldosari, A. A., Bussmann, R. W., . . . Abbasi, A. M. (2021). Comparative assessment of medicinal plant utilization among Balti and Shina communities in the periphery of Deosai National Park, Pakistan. *Biology, 10*(5), 434.

Abid, N., Khan, A. M., Shujait, S., Chaudhary, K., Ikram, M., Imran, M., . . . Maqbool, M. (2021). Synthesis of nanomaterials using various top-down and bottom-up approaches, influencing factors, advantages, and disadvantages: A review. *Advances in Colloid and Interface Science*, 102597.

Agarwal, H., Nakara, A., & Shanmugam, V. K. (2019). Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: A review. *Biomedicine & Pharmacotherapy, 109*, 2561-2572.

Ahmad, S. A., Das, S. S., Khatoon, A., Ansari, M. T., Afzal, M., Hasnain, M. S., & Nayak, A. K. (2020). Bactericidal activity of silver nanoparticles: A mechanistic review. *Materials Science for Energy Technologies, 3*, 756-769.

Alavi, M., & Varma, R. S. (2021). Phytosynthesis and modification of metal and metal oxide nanoparticles/nanocomposites for antibacterial and anticancer activities: Recent advances. *Sustainable Chemistry and Pharmacy, 21*, 100412.

Ali, S., Chouhan, R., Sultan, P., Hassan, Q. P., & Gandhi, S. G. (2021). A comprehensive review of phytochemistry, pharmacology and toxicology of the genus Aconitum L. *Advances in Traditional Medicine*, 1-22.

Ambujakshi, N., Raveesha, H., Manohara, S., Dhananjaya, N., Pratibha, S., & Shivakumara, C. (2019). Chonemorpha grandiflora extract mediated synthesis of Ag-ZnO nanoparticles for its anticancer, electrical and dielectric applications. *Materials Research Express, 6*(9), 095068.

Anjum, S., Khan, A. K., Qamar, A., Fatima, N., Drouet, S., Renouard, S., . . . Hano, C. (2021). Light Tailoring: Impact of UV-C Irradiation on Biosynthesis, Physiognomies, and Clinical Activities of Morus macroura-Mediated Monometallic (Ag and ZnO) and Bimetallic (Ag–ZnO) Nanoparticles. *International journal of molecular sciences, 22*(20), 11294.

Anjum, S., Nawaz, K., Ahmad, B., Hano, C., & Abbasi, B. H. (2022). Green synthesis of biocompatible core–shell (Au–Ag) and hybrid (Au–ZnO and Ag–ZnO) bimetallic nanoparticles and evaluation of their potential antibacterial, antidiabetic, antiglycation and anticancer activities. *RSC advances, 12*(37), 23845-23859.

Arya, S., Mahajan, P., Mahajan, S., Khosla, A., Datt, R., Gupta, V., . . . Oruganti, S. K. (2021). influence of processing parameters to control morphology and optical properties of Sol-Gel synthesized ZnO nanoparticles. *ECS Journal of Solid State Science and Technology, 10*(2), 023002.

Asadian, E., Ghalkhani, M., & Shahrokhian, S. (2019). Electrochemical sensing based on carbon nanoparticles: A review. *Sensors and Actuators B: Chemical, 293*, 183-209.

Ates, B., Koytepe, S., Ulu, A., Gurses, C., & Thakur, V. K. (2020). Chemistry, structures, and advanced applications of nanocomposites from biorenewable resources. *Chemical Reviews, 120*(17), 9304-9362.

Aththanayaka, S., Thiripuranathar, G., & Ekanayake, S. (2022). Emerging advances in biomimetic synthesis of nanocomposites and potential applications. *Materials Today Sustainability*, 100206.

Baharara, J., Ramezani, T., Divsalar, A., Mousavi, M., & Seyedarabi, A. (2016). Induction of apoptosis by green synthesized gold nanoparticles through activation of caspase-3 and 9 in human cervical cancer cells. *Avicenna journal of medical biotechnology, 8*(2), 75.

Basnet, P., Samanta, D., Chanu, T. I., Mukherjee, J., & Chatterjee, S. (2019). Tea-phytochemicals functionalized Ag modified ZnO nanocomposites for visible light driven photocatalytic removal of organic water pollutants. *Materials Research Express, 6*(8), 085095.

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., & Rizzolio, F. (2019). The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine. *Molecules, 25*(1), 112.

Belusso, L. C., Lenz, G. F., Fiorini, E. E., Pereira, A. J., Sequinel, R., Bini, R. A., . . . Schneider, R. (2019). Synthesis of silver nanoparticles from bottom up approach on borophosphate glass and their applications as SERS, antibacterial and glass-based catalyst. *Applied Surface Science, 473*, 303-312.

Berhanu, S., Habtamu, F., Tadesse, Y., Gonfa, F., & Tadesse, T. (2022). Fluorescence Sensor Based on Polyaniline Supported Ag-ZnO Nanocomposite for Malathion Detection. *Journal of Sensors, 2022*.

Bhutto, W. A., Soomro, A. M., Nizamani, A. H., Saleem, H., Khaskheli, M. A., Sahito, A. G., . . . Saleem, S. (2019). Controlled growth of zinc oxide nanowire arrays by chemical vapor deposition (CVD) method. *Int. J. Comput. Sci. Netw. Secur, 19*, 135-141.

Bisht, M., Sekar, K. C., Mukherjee, S., Thapliyal, N., Bahukhandi, A., Singh, D., & Dey, D. (2022). Influence of Anthropogenic Pressure on the Plant Species Richness and Diversity Along the Elevation Gradients of Indian Himalayan High-Altitude Protected Areas. *Front. Ecol. Evol, 10*, 751989.

Bohan, A. J., Salman, G. K., & Ahmed, D. S. (2019). INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES.

Bouzid, H., Faisal, M., Harraz, F. A., Al-Sayari, S. A., & Ismail, A. A. (2015). Synthesis of mesoporous Ag/ZnO nanocrystals with enhanced photocatalytic activity. *Catalysis Today, 252*, 20-26.

Chandra, H., Kumari, P., Bontempi, E., & Yadav, S. (2020). Medicinal plants: Treasure trove for green synthesis of metallic nanoparticles and their biomedical applications. *Biocatalysis and Agricultural Biotechnology, 24*, 101518.

Chauhan, A., Verma, R., Kumari, S., Sharma, A., Shandilya, P., Li, X., . . . Kumar, R. (2020). Photocatalytic dye degradation and antimicrobial activities of Pure and Ag-doped ZnO using Cannabis sativa leaf extract. *Scientific reports, 10*(1), 1-16.

Chauhan, P. Ethnobotanical studies of Wild edible plants used by ethnic people in Pabbar valley, district Shimla, Himachal Pradesh.

Chen, X., Yang, Z.-T., Wang, N., Gao, X., Wang, G., Song, C., . . . Cui, L. (2021). Fabrication of Ag/ZnO hollow nanospheres and cubic TiO2/ZnO heterojunction photocatalysts for RhB degradation. *Nanotechnology Reviews, 10*(1), 1349-1358.

Chikkanna, M. M., Neelagund, S. E., & Rajashekarappa, K. K. (2019). Green synthesis of zinc oxide nanoparticles (ZnO NPs) and their biological activity. *SN Applied Sciences, 1*, 1-10.

Chireh, M., Naseri, M., & Ghiasvand, S. (2019). Enhanced photocatalytic and antibacterial activities of RGO/LiFe5O8 nanocomposites. *Journal of Photochemistry and Photobiology A: Chemistry, 385*, 112063.

Chitradevi, T., Lenus, A. J., & Jaya, N. V. (2019). Structure, morphology and luminescence properties of sol-gel method synthesized pure and Ag-doped ZnO nanoparticles. *Materials Research Express, 7*(1), 015011.

Crivellaro, S., Guadagnini, A., Arboleda, D. M., Schinca, D., & Amendola, V. (2019). A system for the synthesis of nanoparticles by laser ablation in liquid that is remotely controlled with PC or smartphone. *Review of Scientific Instruments, 90*(3), 033902.

da Silva, B. L., Abuçafy, M. P., Manaia, E. B., Junior, J. A. O., Chiari-Andréo, B. G., Pietro, R. C. R., & Chiavacci, L. A. (2019). Relationship between structure and antimicrobial activity of zinc oxide nanoparticles: An overview. *International journal of nanomedicine, 14*, 9395.

Dahal, S., Gurung, K., & Lachungpa, D. RAPID BIODIVERSITY SURVEY OF FOREST ECOSYSTEM OF SIKKIM, INDIA: AN APPROACH FOR BIODIVERSITY CONSERVATION. *PERSPECTIVES ON BIODIVERSITY OF INDIA*, 492.

Dang-Bao, T., Favier, I., & Gómez, M. (2021). Metal nanoparticles in polyols: bottom-up and top-down syntheses and catalytic applications. *Nanoparticles in Catalysis: Advances in Synthesis and Applications*, 99-122.

Dawadi, S., Katuwal, S., Gupta, A., Lamichhane, U., Thapa, R., Jaisi, S., . . . Parajuli, N. (2021). Current research on silver nanoparticles: Synthesis, characterization, and applications. *Journal of nanomaterials, 2021*.

de Moura, F. B. R., Ferreira, B. A., Muniz, E. H., Justino, A. B., Silva, A. G., de Azambuja Ribeiro, R. I. M., . . . Espindola, F. S. (2022). Antioxidant, anti-inflammatory, and wound healing effects of topical silver-doped zinc oxide and silver oxide nanocomposites. *International Journal of Pharmaceutics, 617*, 121620.

Deepak, P., Amutha, V., Kamaraj, C., Balasubramani, G., Aiswarya, D., & Perumal, P. (2019). Chemical and green synthesis of nanoparticles and their efficacy on cancer cells *Green synthesis, characterization and applications of nanoparticles* (pp. 369-387): Elsevier.

Defnet, P. A., & Zhang, B. (2021). Collision, Adhesion, and Oxidation of Single Ag Nanoparticles on a Polysulfide-Modified Microelectrode. *Journal of the American Chemical Society, 143*(39), 16154-16162.

Deshmukh, S. P., Patil, S., Mullani, S., & Delekar, S. (2019). Silver nanoparticles as an effective disinfectant: A review. *Materials Science and Engineering: C, 97*, 954-965.

Deyab, M. (2020). Anticorrosion properties of nanocomposites coatings: A critical review. *Journal of Molecular Liquids, 313*, 113533.

Dheyab, M. A., Aziz, A. A., Khaniabadi, P. M., Jameel, M. S., Ahmed, N. M., & Ali, A. T. (2021). Distinct advantages of using sonochemical over laser ablation methods for a rapid-high quality gold nanoparticles production. *Materials Research Express, 8*(1), 015009.

Dolatyari, M., & Rostami, A. (2022). Strong anti-viral nano biocide based on Ag/ZnO modified by amodiaquine as an antibacterial and antiviral composite. *Scientific reports, 12*(1), 19934.

Ebadi, A., & Mozaffari, M. (2020). Ag/ZnO Nanocomposites: Preparation, Characterization and Investigation of the Catalytic Activity for Oxidation of Toluene in Liquid Phase. *Journal of Nanostructures, 10*(1), 1-8.

Egbuna, C., Parmar, V. K., Jeevanandam, J., Ezzat, S. M., Patrick-Iwuanyanwu, K. C., Adetunji, C. O., . . . Akram, M. (2021). Toxicity of nanoparticles in biomedical application: nanotoxicology. *Journal of Toxicology, 2021*.

Elsayed, K. A., Alomari, M., Drmosh, Q., Alheshibri, M., Al Baroot, A., Kayed, T., . . . Al-Alotaibi, A. L. (2022). Fabrication of ZnO-Ag bimetallic nanoparticles by laser ablation for anticancer activity. *Alexandria Engineering Journal, 61*(2), 1449-1457.

Fahmy, H. M., Mosleh, A. M., Abd Elghany, A., Shams-Eldin, E., Serea, E. S. A., Ali, S. A., & Shalan, A. E. (2019). Coated silver nanoparticles: Synthesis, cytotoxicity, and optical properties. *RSC advances, 9*(35), 20118-20136.

Garemark, J., Yang, X., Sheng, X., Cheung, O., Sun, L., Berglund, L. A., & Li, Y. (2020). Top-down approach making anisotropic cellulose aerogels as universal substrates for multifunctionalization. *ACS nano, 14*(6), 7111-7120.

Gu, W., Lv, J., Quan, B., Liang, X., Zhang, B., & Ji, G. (2019). Achieving MOF-derived one-dimensional porous ZnO/C nanofiber with lightweight and enhanced microwave response by an electrospinning method. *Journal of Alloys and Compounds, 806*, 983-991.

Gudkov, S. V., Burmistrov, D. E., Serov, D. A., Rebezov, M. B., Semenova, A. A., & Lisitsyn, A. B. (2021). A mini review of antibacterial properties of ZnO nanoparticles. *Frontiers in Physics, 9*, 641481.

Guilger-Casagrande, M., & Lima, R. d. (2019). Synthesis of silver nanoparticles mediated by fungi: a review. *Frontiers in bioengineering and biotechnology, 7*, 287.

Habte, L., Shiferaw, N., Mulatu, D., Thenepalli, T., Chilakala, R., & Ahn, J. W. (2019). Synthesis of nano-calcium oxide from waste eggshell by sol-gel method. *Sustainability, 11*(11), 3196.

Hadi, A., Singh, S., Rafiq, S., Nawchoo, I. A., Wagay, N. A., Mahmoud, E. A., . . . Yessoufou, K. (2022). In Vitro Propagation of Aconitum violaceum Jacq. ex Stapf through Seed Culture and Somatic Embryogenesis. *Horticulturae, 8*(7), 599.

Hamrayev, H., Shameli, K., & Korpayev, S. (2021). Green Synthesis of Zinc Oxide Nanoparticles and Its Biomedical Applications: A Review. *Journal of Research in Nanoscience and Nanotechnology, 1*(1), 62-74.

Harish, V., Ansari, M. M., Tewari, D., Gaur, M., Yadav, A. B., García-Betancourt, M.-L., . . . Barhoum, A. (2022). Nanoparticle and Nanostructure Synthesis and Controlled Growth Methods. *Nanomaterials, 12*(18), 3226.

Hasan, S. (2015). A review on nanoparticles: their synthesis and types. *Res. J. Recent Sci, 2277*, 2502.

Hassan, T., Salam, A., Khan, A., Khan, S. U., Khanzada, H., Wasim, M., . . . Kim, I. S. (2021). Functional nanocomposites and their potential applications: A review. *Journal of Polymer Research, 28*, 1-22.

Hulla, J., Sahu, S., & Hayes, A. (2015). Nanotechnology: History and future. *Human & experimental toxicology, 34*(12), 1318-1321.

Hussain, S. (2019). Measurement of nanoparticle-induced mitochondrial membrane potential alterations. *Nanotoxicity: Methods and Protocols*, 123-131.

Ijaz, I., Gilani, E., Nazir, A., & Bukhari, A. (2020). Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. *Green Chemistry Letters and Reviews, 13*(3), 223-245.

Iqbal, J., Andleeb, A., Ashraf, H., Meer, B., Mehmood, A., Jan, H., . . . Fazal, H. (2022). Potential antimicrobial, antidiabetic, catalytic, antioxidant and ROS/RNS inhibitory activities of Silybum marianum mediated biosynthesized copper oxide nanoparticles. *RSC advances, 12*(22), 14069-14083.

Iqbal, S., Bahadur, A., Javed, M., Hakami, O., Irfan, R. M., Ahmad, Z., . . . Abd-Rabboh, H. S. (2021). Design Ag-doped ZnO heterostructure photocatalyst with sulfurized graphitic C3N4 showing enhanced photocatalytic activity. *Materials Science and Engineering: B, 272*, 115320.

Iqbal, T., Azhar, S., Zafar, M., Kiran, H., Kebaili, I., & Alrobei, H. (2021). Synthesis and characterization of Ag-ZnO nano-composites for investigation of variations in the germination of peanut and kidney beans. *Applied Nanoscience, 11*(12), 2767-2777.

Iqbal, Y., Malik, A. R., Iqbal, T., Aziz, M. H., Ahmed, F., Abolaban, F. A., . . . Ullah, H. (2021). Green synthesis of ZnO and Ag-doped ZnO nanoparticles using Azadirachta indica leaves: Characterization and their potential antibacterial, antidiabetic, and wound-healing activities. *Materials Letters, 305*, 130671.

Ismail, A., Saputri, L., Dwiatmoko, A., Susanto, B., & Nasikin, M. (2021). A facile approach to synthesis of silica nanoparticles from silica sand and their application as superhydrophobic material. *Journal of Asian Ceramic Societies, 9*(2), 665-672.

Jafarirad, S., Taghizadeh, P. M., & Divband, B. (2020). Biosynthesis, characterization and structural properties of a novel kind of Ag/ZnO nanocomposites in order to increase its biocompatibility across human A549 cell line. *BioNanoScience, 10*, 42-53.

Jaji, N.-D., Lee, H. L., Hussin, M. H., Akil, H. M., Zakaria, M. R., & Othman, M. B. H. (2020). Advanced nickel nanoparticles technology: From synthesis to applications. *Nanotechnology Reviews, 9*(1), 1456-1480.

Jamkhande, P. G., Ghule, N. W., Bamer, A. H., & Kalaskar, M. G. (2019). Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications. *Journal of drug delivery science and technology, 53*, 101174.

Jayapriya, M., Premkumar, K., Arulmozhi, M., & Karthikeyan, K. (2020). One-step biological synthesis of cauliflower-like Ag/MgO nanocomposite with antibacterial, anticancer, and catalytic activity towards anthropogenic pollutants. *Research on Chemical Intermediates, 46*, 1771-1788.

Jeremiah, S. S., Miyakawa, K., Morita, T., Yamaoka, Y., & Ryo, A. (2020). Potent antiviral effect of silver nanoparticles on SARS-CoV-2. *Biochemical and biophysical research communications, 533*(1), 195-200.

Jeyaraj, M., Gurunathan, S., Qasim, M., Kang, M.-H., & Kim, J.-H. (2019). A comprehensive review on the synthesis, characterization, and biomedical application of platinum nanoparticles. *Nanomaterials, 9*(12), 1719.

Kalantari, K., Mostafavi, E., Afifi, A. M., Izadiyan, Z., Jahangirian, H., Rafiee-Moghaddam, R., & Webster, T. J. (2020). Wound dressings functionalized with silver nanoparticles: promises and pitfalls. *Nanoscale, 12*(4), 2268-2291.

Kaur, K., & Sidhu, A. K. (2021). Green synthesis: An eco-friendly route for the synthesis of iron oxide nanoparticles. *Frontiers in Nanotechnology, 3*, 655062.

Khan, A. K., Renouard, S., Drouet, S., Blondeau, J.-P., Anjum, I., Hano, C., . . . Anjum, S. (2021). Effect of UV irradiation (A and C) on casuarina equisetifolia-mediated biosynthesis and characterization of antimicrobial and anticancer activity of biocompatible zinc oxide nanoparticles. *Pharmaceutics, 13*(11), 1977.

Khan, F. A., Khan, S., Khan, N. M., Khan, H., Khan, S., Ahmad, S., . . . Aziz, R. (2021a). Antimicrobial and Antioxidant Role of the Aerial Parts of Aconitum violaceum. *Journal of the Mexican Chemical Society, 65*(1), 84-93.

Khan, F. A., Khan, S., Khan, N. M., Khan, H., Khan, S., Ahmad, S., . . . Aziz, R. (2021b). Cómo citar Buscar Antimicrobial and Antioxidant Role of the Aerial Parts of Aconitum violaceum Journal of the Mexican Chemical Society, vol. 65, no. 1, 2021 Sociedad Química de México AC. *Journal of the Mexican Chemical Society, 65*(1).

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry, 12*(7), 908-931.

Khan, M. J., Tahir, K., El-Zahhar, A. A., Arooj, A., Al-Abdulkarim, H. A., Saleh, E. A. M., . . . Khan, A. U. (2021). Facile synthesis of silver modified zinc oxide nanocomposite: An

efficient visible light active nanomaterial for bacterial inhibition and dye degradation. *Photodiagnosis and Photodynamic Therapy, 36*, 102619.

Khan, Y., Sadia, H., Ali Shah, S. Z., Khan, M. N., Shah, A. A., Ullah, N., . . . Khedher, N. B. (2022). Classification, synthetic, and characterization approaches to nanoparticles, and their applications in various fields of nanotechnology: a review. *Catalysts, 12*(11), 1386.

Kim, M., Osone, S., Kim, T., Higashi, H., & Seto, T. (2017). Synthesis of nanoparticles by laser ablation: A review. *KONA Powder and Particle Journal*, 2017009.

Kolahalam, L. A., Viswanath, I. K., Diwakar, B. S., Govindh, B., Reddy, V., & Murthy, Y. (2019). Review on nanomaterials: Synthesis and applications. *Materials Today: Proceedings, 18*, 2182-2190.

Krasno, S., & Swathi, K. (2018). A review on types of nanocomposites and their applications. *Int J Adv Res Ideas Innov Technol, 4*(6), 235-236.

Krishnia, L., Thakur, P., & Thakur, A. (2022). Synthesis of Nanoparticles by Physical Route. *Synthesis and Applications of Nanoparticles*, 45.

Kumar, D. A. S. R. S., Murugesan, S. S. S., & Rahiman, A. K. Antidiabetic Activity of Phytosynthesized Ag/CuO Nanocomposites Using Murraya koenigii and Zingiber officinale Extracts.

Kumar, R., Arya, D., & Chandra Sekar, K. (2020). Diversity, utilization patterns and conservation of ethnomedicinal plants in Pindari Valley, Uttarakhand Himalaya. *Asia Pac. J. Re*, 1-11.

Kuruppu, A. I., Paranagama, P., & Goonasekara, C. L. (2019). Medicinal plants commonly used against cancer in traditional medicine formulae in Sri Lanka. *Saudi Pharmaceutical Journal, 27*(4), 565-573.

Kwiczak-Yiğitbaşı, J., Laçin, Ö., Demir, M., Ahan, R. E., Şeker, U. Ö. Ş., & Baytekin, B. (2020). A sustainable preparation of catalytically active and antibacterial cellulose metal nanocomposites via ball milling of cellulose. *Green Chemistry, 22*(2), 455-464.

Lee, K. X., Shameli, K., Yew, Y. P., Teow, S.-Y., Jahangirian, H., Rafiee-Moghaddam, R., & Webster, T. J. (2020). Recent developments in the facile bio-synthesis of gold nanoparticles (AuNPs) and their biomedical applications. *International journal of nanomedicine, 15*, 275.

Leon, L., Chung, E. J., & Rinaldi, C. (2020). A brief history of nanotechnology and introduction to nanoparticles for biomedical applications *Nanoparticles for Biomedical Applications* (pp. 1-4): Elsevier.

Li, Y., Yang, Y., Qing, Y. a., Li, R., Tang, X., Guo, D., & Qin, Y. (2020). Enhancing ZnO-NP antibacterial and osteogenesis properties in orthopedic applications: A review. *International journal of nanomedicine*, 6247-6262.

Liao, C., Li, Y., & Tjong, S. C. (2019). Bactericidal and cytotoxic properties of silver nanoparticles. *International journal of molecular sciences, 20*(2), 449.

Liao, G., Fang, J., Li, Q., Li, S., Xu, Z., & Fang, B. (2019). Ag-Based nanocomposites: synthesis and applications in catalysis. *Nanoscale, 11*(15), 7062-7096.

Liu, J., Wang, Y., Ma, J., Peng, Y., & Wang, A. (2019). A review on bidirectional analogies between the photocatalysis and antibacterial properties of ZnO. *Journal of Alloys and Compounds, 783*, 898-918.

Ma, L., Zhang, Q., Li, J., Lu, X., Gao, C., Song, P., & Xia, L. (2021). Ag–ZnO Nanocomposites Are Used for SERS Substrates and Promote the Coupling Reaction of PATP. *Materials, 14*(4), 922.

Madannejad, R., Shoaie, N., Jahanpeyma, F., Darvishi, M. H., Azimzadeh, M., & Javadi, H. (2019). Toxicity of carbon-based nanomaterials: Reviewing recent reports in medical and biological systems. *Chemico-biological interactions, 307*, 206-222.

Majumder, S., Basnet, P., Mukherjee, J., & Chatterjee, S. (2020). *Bio-capped facile synthesis of silver zinc oxide for photocatalytic degradation of Rhodamine 6G under visible-light irradiation.* Paper presented at the AIP Conference Proceedings.

Malachowski, T., & Hassel, A. (2020). Engineering nanoparticles to overcome immunological barriers for enhanced drug delivery. *Engineered Regeneration, 1*, 35-50.

Matussin, S., Harunsani, M. H., Tan, A. L., & Khan, M. M. (2020). Plant-extract-mediated SnO2 nanoparticles: synthesis and applications. *ACS Sustainable Chemistry & Engineering, 8*(8), 3040-3054.

Mir, A. H., Qamar, A., Qadir, I., Naqvi, A. H., & Begum, R. (2020). Accumulation and trafficking of zinc oxide nanoparticles in an invertebrate model, Bombyx mori, with insights on their effects on immuno-competent cells. *Scientific reports, 10*(1), 1-14.

MODAN, E. M., & PLĂIAȘU, A. G. (2020). Advantages and disadvantages of chemical methods in the elaboration of nanomaterials. *The Annals of "Dunarea de Jos" University of Galati. Fascicle IX, Metallurgy and Materials Science, 43*(1), 53-60.

Moein, M. M., Abdel-Rehim, A., & Abdel-Rehim, M. (2019). Recent applications of molecularly imprinted sol-gel methodology in sample preparation. *Molecules, 24*(16), 2889.

Molla, M. A. I., Furukawa, M., Tateishi, I., Katsumata, H., & Kaneco, S. (2019). Studies of effects of calcination temperature on the crystallinity and optical properties of Ag-doped ZnO nanocomposites. *Journal of Composites Science, 3*(1), 18.

Montaseri, H., Kruger, C. A., & Abrahamse, H. (2021). Inorganic nanoparticles applied for active targeted photodynamic therapy of breast cancer. *Pharmaceutics, 13*(3), 296.

Mousavi-Kouhi, S. M., Beyk-Khormizi, A., Amiri, M. S., Mashreghi, M., & Yazdi, M. E. T. (2021). Silver-zinc oxide nanocomposite: From synthesis to antimicrobial and anticancer properties. *Ceramics International, 47*(15), 21490-21497.

Mtavangu, S. G., Machunda, R. L., van der Bruggen, B., & Njau, K. N. (2022a). In situ facile green synthesis of Ag–ZnO nanocomposites using Tetradenia riperia leaf extract and its antimicrobial efficacy on water disinfection. *Scientific reports, 12*(1), 1-14.

Mtavangu, S. G., Machunda, R. L., van der Bruggen, B., & Njau, K. N. (2022b). In situ facile green synthesis of Ag–ZnO nanocomposites using Tetradenia riperia leaf extract and its antimicrobial efficacy on water disinfection. *Scientific Reports, 12*(1), 15359.

Mughal, S. S., & Hassan, S. M. (2022). Comparative study of AgO nanoparticles synthesize via biological, chemical and physical methods: a review. *American Journal of Materials Synthesis and Processing, 7*(2), 15-28.

Muhammad, W., Ullah, N., Haroon, M., & Abbasi, B. H. (2019). Optical, morphological and biological analysis of zinc oxide nanoparticles (ZnO NPs) using Papaver somniferum L. *RSC advances, 9*(51), 29541-29548.

Muñoz-Fernandez, L., Alkan, G., Milošević, O., Rabanal, M. E., & Friedrich, B. (2019). Synthesis and characterisation of spherical core-shell Ag/ZnO nanocomposites using single and two–steps ultrasonic spray pyrolysis (USP). *Catalysis Today, 321*, 26-33.

Mushtaq, M., Hassan, S. M., & Mughal, S. S. (2022a). Synthesis, Characterization and Biological Approach of Nano Oxides of Calcium by Piper nigrum. *American Journal of Chemical Engineering, 10*(4), 79-88.

Mushtaq, M., Hassan, S. M., & Mughal, S. S. (2022b). Synthesis, Characterization and Biological Approach of Nano Oxides of Calcium by Piper nigrum. *American Journal of Chemical Engineering, 10*(4), 79.

Nagajyothi, P., Muthuraman, P., Tettey, C., Yoo, K., & Shim, J. (2021). In vitro anticancer activity of eco-friendly synthesized ZnO/Ag nanocomposites. *Ceramics International, 47*(24), 34940-34948.

Narayanan, K. B., Sakthivel, N., & Han, S. S. (2021). From chemistry to biology: Applications and advantages of green, biosynthesized/biofabricated metal-and carbon-based nanoparticles. *Fibers and Polymers, 22*(4), 877-897.

Nasrollahzadeh, M., Sajadi, S. M., Sajjadi, M., & Issaabadi, Z. (2019). An introduction to nanotechnology *Interface science and technology* (Vol. 28, pp. 1-27): Elsevier.

Nasrollahzadeh, M., Sajjadi, M., Sajadi, S. M., & Issaabadi, Z. (2019). Green nanotechnology *Interface science and technology* (Vol. 28, pp. 145-198): Elsevier.

Nazir, M., Tungmunnithum, D., Bose, S., Drouet, S., Garros, L., Giglioli-Guivarc'h, N., . . . Hano, C. (2019). Differential production of phenylpropanoid metabolites in callus cultures of Ocimum basilicum L. with distinct in vitro antioxidant activities and in vivo protective effects against UV stress. *Journal of agricultural and food chemistry, 67*(7), 1847-1859.

Nemiwal, M., Zhang, T. C., & Kumar, D. (2021). Recent progress in g-C3N4, TiO2 and ZnO based photocatalysts for dye degradation: Strategies to improve photocatalytic activity. *Science of the total environment, 767*, 144896.

Nikolova, M., Slavchov, R., & Nikolova, G. (2020). Nanotechnology in medicine. *Drug discovery and evaluation: methods in clinical pharmacology*, 533-546.

Noohpisheh, Z., Amiri, H., Farhadi, S., & Mohammadi-Gholami, A. (2020). Green synthesis of Ag-ZnO nanocomposites using Trigonella foenum-graecum leaf extract and their antibacterial, antifungal, antioxidant and photocatalytic properties. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 240*, 118595.

Omanović-Mikličanin, E., Badnjević, A., Kazlagić, A., & Hajlovac, M. (2020). Nanocomposites: A brief review. *Health and Technology, 10*, 51-59.

Panchal, P., Paul, D. R., Sharma, A., Choudhary, P., Meena, P., & Nehra, S. (2020). Biogenic mediated Ag/ZnO nanocomposites for photocatalytic and antibacterial activities towards disinfection of water. *Journal of colloid and interface science, 563*, 370-380.

Pant, M., Singh, R., Negi, P., Tiwari, K., & Singh, Y. (2021). A comprehensive review on carbon nano-tube synthesis using chemical vapor deposition. *Materials Today: Proceedings, 46*, 11250-11253.

Parashar, M., Shukla, V. K., & Singh, R. (2020a). Metal oxides nanoparticles via sol–gel method: a review on synthesis, characterization and applications. *Journal of Materials Science: Materials in Electronics, 31*, 3729-3749.

Parashar, M., Shukla, V. K., & Singh, R. (2020b). Metal oxides nanoparticles via sol–gel method: a review on synthesis, characterization and applications. *Journal of Materials Science: Materials in Electronics, 31*(5), 3729-3749.

Patil, N., Bhaskar, R., Vyavhare, V., Dhadge, R., Khaire, V., & Patil, Y. (2021). Overview on methods of synthesis of nanoparticles. *International Journal of Current Pharmaceutical Research, 13*(2), 11-16.

Peng, J., Lu, T., Ming, H., Ding, Z., Yu, Z., Zhang, J., & Hou, Y. (2019). Enhanced photocatalytic ozonation of phenol by Ag/ZnO nanocomposites. *Catalysts, 9*(12), 1006.

Phumthum, M., Balslev, H., & Barfod, A. S. (2019). Important medicinal plant families in Thailand. *Frontiers in Pharmacology, 10*, 1125.

Pradeep, H., Bindu, M., Suresh, S., Thadathil, A., & Periyat, P. (2022). Recent trends and advances in polyindole-based nanocomposites as potential antimicrobial agents: a mini review. *RSC advances, 12*(13), 8211-8227.

Prajitha, N., Athira, S., & Mohanan, P. (2019). Bio-interactions and risks of engineered nanoparticles. *Environmental research, 172*, 98-108.

Qin, B., Ma, H., Hossain, M., Zhong, M., Xia, Q., Li, B., & Duan, X. (2020). Substrates in the synthesis of two-dimensional materials via chemical vapor deposition. *Chemistry of Materials, 32*(24), 10321-10347.

Rahman, K., Khan, S. U., Fahad, S., Chang, M. X., Abbas, A., Khan, W. U., . . . Khan, D. (2019). Nano-biotechnology: a new approach to treat and prevent malaria. *International journal of nanomedicine, 14*, 1401.

Ramírez-Acosta, C. M., Cifuentes, J., Cruz, J. C., & Reyes, L. H. (2020). Patchy core/shell, magnetite/silver nanoparticles via green and facile synthesis: routes to assure biocompatibility. *Nanomaterials, 10*(9), 1857.

Ratan, Z. A., Haidere, M. F., Nurunnabi, M., Shahriar, S. M., Ahammad, A., Shim, Y. Y., . . . Cho, J. Y. (2020). Green chemistry synthesis of silver nanoparticles and their potential anticancer effects. *Cancers, 12*(4), 855.

Rawat, J. M., Rawat, B., Pandey, S., Anand, J., Rai, N., & Das, M. P. (2021). Regeneration of transformed plants of Aconitum violaceum from Agrobacterium rhizogenes mediated hairy root cultures. *Webology, 18*(1), 1561-1568.

Rezaei, F., & Dinari, M. (2021). Novel covalent organic polymer-supported Ag nanoparticles as a catalyst for nitroaromatics reduction. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 618*, 126441.

Sabir, S., Arshad, M., Hussain, M., Sadaf, H. M., Imran, M., Yasmeen, F., & Chaudhari, S. K. (2016). A probe into biochemical potential of Aconitum violaceum: A medicinal plant from Himalaya. *Asian Pacific Journal of Tropical Disease, 6*(6), 502-504.

Saeed, M., Alshammari, Y., Majeed, S. A., & Al-Nasrallah, E. (2020). Chemical vapour deposition of graphene—Synthesis, characterisation, and applications: A review. *Molecules, 25*(17), 3856.

Safdar, A., & Bibi, Y. (2020). Medicinal Uses and Conservation Status of Aconitum violaceum. *Journal of Plant and Environment, 2*(1), 19-23.

Sahani, S., & Sharma, Y. C. (2021). Advancements in applications of nanotechnology in global food industry. *Food Chemistry, 342*, 128318.

Sajid, M., & Płotka-Wasylka, J. (2020). Nanoparticles: Synthesis, characteristics, and applications in analytical and other sciences. *Microchemical Journal, 154*, 104623.

Sajjadi, M., Nasrollahzadeh, M., & Sajadi, S. M. (2017). Green synthesis of Ag/Fe3O4 nanocomposite using Euphorbia peplus Linn leaf extract and evaluation of its catalytic activity. *Journal of colloid and interface science, 497*, 1-13.

Sakib, A. A. M., Masum, S. M., Hoinkis, J., Islam, R., & Molla, M. A. I. (2019). Synthesis of CuO/ZnO nanocomposites and their application in photodegradation of toxic textile dye. *Journal of Composites Science, 3*(3), 91.

Sambale, F., Wagner, S., Stahl, F., Khaydarov, R., Scheper, T., & Bahnemann, D. (2015). Investigations of the toxic effect of silver nanoparticles on mammalian cell lines. *Journal of Nanomaterials, 16*(1), 6-6.

Semwal, D. K., Chauhan, A., Kumar, A., Aswal, S., Semwal, R. B., & Kumar, A. (2019). Status of Indian medicinal plants in the International Union for Conservation of Nature and the future of Ayurvedic drugs: Shouldn't think about Ayurvedic fundamentals? *Journal of integrative medicine, 17*(4), 238-243.

Shah, S., Khan, S., Bussmann, R. W., Ali, M., Hussain, D., & Hussain, W. (2020). Quantitative ethnobotanical study of Indigenous knowledge on medicinal plants used by the tribal communities of Gokand Valley, District Buner, Khyber Pakhtunkhwa, Pakistan. *Plants, 9*(8), 1001.

Shameem, M. M., Sasikanth, S., Annamalai, R., & Raman, R. G. (2021). A brief review on polymer nanocomposites and its applications. *Materials Today: Proceedings, 45*, 2536-2539.

Sharma, P., Jang, N.-Y., Lee, J.-W., Park, B. C., Kim, Y. K., & Cho, N.-H. (2019). Application of ZnO-based nanocomposites for vaccines and cancer immunotherapy. *Pharmaceutics, 11*(10), 493.

Sharwani, A. A., Narayanan, K. B., Khan, M. E., & Han, S. S. (2022). Photocatalytic degradation activity of goji berry extract synthesized silver-loaded mesoporous zinc oxide (Ag@ ZnO) nanocomposites under simulated solar light irradiation. *Scientific reports, 12*(1), 1-18.

Shnoudeh, A. J., Hamad, I., Abdo, R. W., Qadumii, L., Jaber, A. Y., Surchi, H. S., & Alkelany, S. Z. (2019). Synthesis, characterization, and applications of metal nanoparticles *Biomaterials and bionanotechnology* (pp. 527-612): Elsevier.

Shreema, K., Mathammal, R., Kalaiselvi, V., & Gopi, S. (2022). Effective study of antibacterial and anticancer profiling: Nyctanthes arbor-tristis functionalized silver–zinc oxide nanocomposite. *Chemical Papers*, 1-12.

Simbine, E. O., Rodrigues, L. d. C., Lapa-Guimaraes, J., Kamimura, E. S., Corassin, C. H., & Oliveira, C. A. F. d. (2019). Application of silver nanoparticles in food packages: a review. *Food Science and Technology, 39*, 793-802.

Singh, A. K., Yadav, T. P., Pandey, B., Gupta, V., & Singh, S. P. (2019). Engineering nanomaterials for smart drug release: Recent advances and challenges. *Applications of targeted nano drugs and delivery systems*, 411-449.

Singh, K. R., Nayak, V., Singh, J., Singh, A. K., & Singh, R. P. (2021). Potentialities of bioinspired metal and metal oxide nanoparticles in biomedical sciences. *RSC advances, 11*(40), 24722-24746.

Singh, P., Shandilya, P., Raizada, P., Sudhaik, A., Rahmani-Sani, A., & Hosseini-Bandegharaei, A. (2020). Review on various strategies for enhancing photocatalytic activity of graphene based nanocomposites for water purification. *Arabian Journal of Chemistry, 13*(1), 3498-3520.

Singh, T. A., Das, J., & Sil, P. C. (2020). Zinc oxide nanoparticles: A comprehensive review on its synthesis, anticancer and drug delivery applications as well as health risks. *Advances in Colloid and Interface Science, 286*, 102317.

Singh, V., Yadav, P., & Mishra, V. (2020). Recent advances on classification, properties, synthesis, and characterization of nanomaterials. *Green synthesis of nanomaterials for bioenergy applications*, 83-97.

Sivamaruthi, B. S., Ramkumar, V. S., Archunan, G., Chaiyasut, C., & Suganthy, N. (2019). Biogenic synthesis of silver palladium bimetallic nanoparticles from fruit extract of Terminalia chebula–In vitro evaluation of anticancer and antimicrobial activity. *Journal of Drug Delivery Science and Technology, 51*, 139-151.

Stanley, R., Jebasingh, J. A., Stanley, P. K., Ponmani, P., Shekinah, M., & Vasanthi, J. (2021). Excellent Photocatalytic degradation of Methylene Blue, Rhodamine B and Methyl Orange dyes by Ag-ZnO nanocomposite under natural sunlight irradiation. *Optik, 231*, 166518.

Sun, W., Yan, B., Wang, R., Liu, F., Hu, Z., Zhou, L., . . . Tong, P. (2018). In vivo acute toxicity of detoxified Fuzi (lateral root of Aconitum carmichaeli) after a traditional detoxification process. *EXCLI journal, 17*, 889.

Tăbăran, A.-F., Matea, C. T., Mocan, T., Tăbăran, A., Mihaiu, M., Iancu, C., & Mocan, L. (2020). Silver nanoparticles for the therapy of tuberculosis. *International journal of nanomedicine, 15*, 2231.

Taha, A., Ben Aissa, M., & Da'na, E. (2020). Green synthesis of an activated carbon-supported Ag and ZnO nanocomposite for photocatalytic degradation and its antibacterial activities. *Molecules, 25*(7), 1586.

Tang, Q., Xia, H., Liang, W., Huo, X., & Wei, X. (2020). Synthesis and characterization of zinc oxide nanoparticles from Morus nigra and its anticancer activity of AGS gastric cancer cells. *Journal of Photochemistry and Photobiology B: Biology, 202*, 111698.

Umapathi, A., Kumawat, M., & Daima, H. K. (2022). Engineered nanomaterials for biomedical applications and their toxicity: a review. *Environmental Chemistry Letters, 20*(1), 445-468.

Upadhayay, V. K., Khan, A., Singh, J., & Singh, A. V. (2019). Splendid Role of Nanoparticles as Antimicrobial Agents in Wastewater Treatment *Environmental Contaminants: Ecological Implications and Management* (pp. 119-136): Springer.

Veeman, D., Shree, M. V., Sureshkumar, P., Jagadeesha, T., Natrayan, L., Ravichandran, M., & Paramasivam, P. (2021). Sustainable development of carbon nanocomposites: synthesis and classification for environmental remediation. *Journal of nanomaterials, 2021*, 1-21.

Verma, R., Pathak, S., Srivastava, A. K., Prawer, S., & Tomljenovic-Hanic, S. (2021). ZnO nanomaterials: Green synthesis, toxicity evaluation and new insights in biomedical applications. *Journal of Alloys and Compounds, 876*, 160175.

Wang, J., Li, Y., & Nie, G. (2021). Multifunctional biomolecule nanostructures for cancer therapy. *Nature Reviews Materials, 6*(9), 766-783.

Wang, S., Huang, Q., Liu, X., Li, Z., Yang, H., & Lu, Z. (2019). Rapid antibiofilm effect of Ag/ZnO nanocomposites assisted by dental LED curing light against facultative anaerobic oral pathogen Streptococcus mutans. *ACS Biomaterials Science & Engineering, 5*(4), 2030-2040.

Wang, X., & Li, J. (2021). Sol-gel fabrication of Ag-Coated ZnO quantum dots nanocomposites with excellent photocatalytic activity. *Optical Materials, 118*, 111235.

Xiao, Y., Shi, K., Qu, Y., Chu, B., & Qian, Z. (2019). Engineering nanoparticles for targeted delivery of nucleic acid therapeutics in tumor. *Molecular Therapy-Methods & Clinical Development, 12*, 1-18.

Xie, X., Sun, T., Xue, J., Miao, Z., Yan, X., Fang, W., . . . Tang, L. (2020). Ag nanoparticles cluster with pH-triggered reassembly in targeting antimicrobial applications. *Advanced Functional Materials, 30*(17), 2000511.

Xu, L., Yi-Yi, W., Huang, J., Chun-Yuan, C., Zhen-Xing, W., & Xie, H. (2020). Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics, 10*(20), 8996.

Yadav, V. B., Gadi, R., & Kalra, S. (2019). Clay based nanocomposites for removal of heavy metals from water: A review. *Journal of environmental management, 232*, 803-817.

Yan, J. (2021). A review of sintering-bonding technology using Ag nanoparticles for electronic packaging. *Nanomaterials, 11*(4), 927.

Yang, Z., Ma, C., Wang, W., Zhang, M., Hao, X., & Chen, S. (2019). Fabrication of Cu2O-Ag nanocomposites with enhanced durability and bactericidal activity. *Journal of colloid and interface science, 557*, 156-167.

Yaqoob, A. A., Umar, K., & Ibrahim, M. N. M. (2020). Silver nanoparticles: various methods of synthesis, size affecting factors and their potential applications–a review. *Applied Nanoscience, 10*, 1369-1378.

Yarahmadi, M., Maleki-Ghaleh, H., Mehr, M. E., Dargahi, Z., Rasouli, F., & Siadati, M. H. (2021). Synthesis and characterization of Sr-doped ZnO nanoparticles for photocatalytic applications. *Journal of Alloys and Compounds, 853*, 157000.

Yeganeh-Faal, A., Bordbar, M., Negahdar, N., & Nasrollahzadeh, M. (2017). Green synthesis of the Ag/ZnO nanocomposite using Valeriana officinalis L. root extract: application as a reusable catalyst for the reduction of organic dyes in a very short time. *Iet Nanobiotechnology, 11*(6), 669-676.

Yuan, X., Zhang, X., Sun, L., Wei, Y., & Wei, X. (2019). Cellular toxicity and immunological effects of carbon-based nanomaterials. *Particle and fibre toxicology, 16*(1), 1-27.

Zare, M., Namratha, K., Alghamdi, S., Mohammad, Y. H. E., Hezam, A., Zare, M., . . . Ramakrishna, S. (2019a). Novel green biomimetic approach for synthesis of ZnO-Ag nanocomposite; antimicrobial activity against food-borne pathogen, biocompatibility and solar photocatalysis. *Scientific reports, 9*(1), 8303.

Zare, M., Namratha, K., Alghamdi, S., Mohammad, Y. H. E., Hezam, A., Zare, M., . . . Ramakrishna, S. (2019b). Novel green biomimetic approach for synthesis of ZnO-Ag nanocomposite; antimicrobial activity against food-borne pathogen, biocompatibility and solar photocatalysis. *Scientific reports, 9*(1), 1-15.

Zhou, P., Xia, Z., Qi, C., He, M., Yu, T., & Shi, L. (2021). Construction of chitosan/Ag nanocomposite sponges and their properties. *International Journal of Biological Macromolecules, 192*, 272-277.

Zhu, X., Wang, J., Yang, D., Liu, J., He, L., Tang, M., . . . Wu, X. (2021). Fabrication, characterization and high photocatalytic activity of Ag–ZnO heterojunctions under UV-visible light. *RSC advances, 11*(44), 27257-27266.

Zorraquín-Peña, I., Cueva, C., Bartolomé, B., & Moreno-Arribas, M. V. (2020). Silver nanoparticles against foodborne bacteria. Effects at intestinal level and health limitations. *Microorganisms, 8*(1), 132.