Evaluation of Anti-aging and Anti-oxidant properties in *Caenorhabditis elegans* using *Melia azedarach* mediated silver nanoparticles

By

Pavithra M

Roll. No: 20PZO014

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the

Degree of Master of Science in Zoology

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INTRODUCTION

1. INTRODUCTION

1.1. Nanoscience and Nanotechnology

Nanotechnology is a branch of science that combines physics, chemistry, and biology. It has emerged as one of the most significant innovations of our time. It can be defined as a branch of modern science that focuses on the design, synthesis, and manipulation of particles with sizes ranging from 1 to 100 nanometres. The extraordinary advancement of this technology has led the way for the creation of new fundamental and applied frontiers, such as nanoscale structure synthesis and exploratory probing for their application based on their physicochemical and optoelectronic properties. The term 'nano' indicates one billionth or 10^{-9} units. The physicochemical and biological properties of nanoparticles differ in fundamental ways from those of individual atoms and molecules, as well as from those of the bulk materials in this size range. Nanoparticles are one of the levels of economic development in nanotechnology. A nanoparticle (NPs) is a substance that has at least one dimension in the range of 1-100 nm. Due to the development of quantum effects and the origin of phenomena such as superparamagnetic, plasmon resonance, and others, nanoparticles differ from their corresponding bulk materials. The surface area to volume ratio of the nanoparticles is very high. Nanoparticles also exhibit novel qualities as a result of their exact characteristics, such as their small size, large surface area, shape, and particle distribution (Jeevanandam *et al.,* 2018). The reductions in the size of nanoparticles make different physical and chemical properties contrary to their bulk materials. The nanoparticle has a wide range of uses in agriculture, the environment, biotechnology, medicine, textiles, health care, and biomedicine.

The majority of chemical approaches for nanoparticle synthesis are prohibitively expensive and involve the use of toxic and dangerous compounds that pose a variety of biological hazards. However, the synthesis of nanoparticles using plant extracts is the most extensively used approach for sustainable, environmentally friendly nanoparticle production. It also has the added benefit of being widely disseminated, easily accessible, considerably safer to handle, and acting as a source of many metabolites (Ankamwar *et al.,* 2005). The biosynthesis of nanoparticles takes the topmost approach, with reduction or oxidation as the primary chemical step. As physical and chemical processes became more expensive in terms of cost, the necessity for the biosynthesis of nanoparticles. Chemical synthesis methods frequently result in the presence of harmful compounds absorbed on the surface, which might have negative consequences in medical applications. As a result, scientists exploited microbial enzymes and plant extracts in their search for a less expensive way to make nanoparticles. They are responsible for the reduction of metal compounds into their respective nanoparticles due to their antioxidant or reducing capabilities. Green synthesis is preferable to chemical and physical methods because it is environmentally friendly, cost-effective, and readily scaled up for large-scale synthesis, and it does not require the use of high pressure, energy, temperature, or harmful chemicals (Begum *et al.,* 2009).

1.2. Silver nanoparticle

Metal nanoparticles are made from a variety of metal salts, including copper, iron, gold, cerium, zinc, cobalt, and silver. Ag nanoparticles have significant benefits over other metal nanoparticles. AgNPs have been used in a variety of electrical and medical equipment, surgical instruments, bone cementing, surgical masks, and other applications in the last decade (Nisha *et al.,* 2015) Nano-sized particles have been utilized in a wide range of industries, including catalysis and photonics. Silver nanoparticles (AgNPs) are of particular interest among the various metals because of their extraordinary antibacterial nature and localized surface Plasmon resonance capabilities, which endow them with the unique properties of broad-spectrum antimicrobial action (Franci *et al.,* 2015). The main benefit of employing plant extracts for silver nanoparticle manufacturing is that they are practicable, safe, and nontoxic in most circumstances, and they contain a wide spectrum of metabolites that can help reduce silver ions. Plant-assisted reduction, which is aided by the presence of phytochemicals, is the main mechanism for the process (Gomathi, 2020). Among the other Nanomaterials, Silver Nano-particles have the highest level of commercialization. AgNPs have been used as molecular probes, antiangiogenic agents, anticancer agents, and antiproliferative agents due to their biological effects. Antimicrobial properties of silver nanoparticles have been found to be effective against a variety of infections, including respiratory syncytial infection, herpes simplex infection types, and monkeypox infection. Unmistakable silver combinations were therapeutically utilized to reduce skin contaminations in the treatment of consumes (e.g., silver sulfadiazine) and as coatings on various surfaces such as catheters due to their noteworthy clean exercises (Zhang *et al.,* 2016).

1.2.1 Biological and clinical significance of silver nanoparticles

In the recent age of new modern science, the development of improved green chemistry techniques utilizing as well as stabilizing agents for the synthesis of silver nanoparticles with desired morphology and size have become a prime focus for researchers. Without the use of any dangerous or expensive chemical chemicals, biological processes can be used to synthesize silver nanoparticles (Ahmad, 2003). The bio reduction of metal ions by a mixture of biomolecules found in the extracts of certain organisms is both ecologically friendly and chemically complex. Many investigations have shown that such organisms may successfully synthesize silver nanoparticles (Iravani, 2011). Silver has been shown to suppress the growth of microorganisms in medical and industrial processes (Morones *et al.,* 2005). The antimicrobial activity of AgNPs can alter physical properties such as form, size, mass, composition, and synthesis conditions, as well as pH, ions, and macromolecules (Marambio and Hoek, 2010). In vitro, silver nanoparticles were found to bond with calf-thymus DNA (CT-DNA), preventing DNA from working normally and resulting in cell damage. Using electronic absorption and fluorescence spectroscopies, they evaluated the CT-DNA binding capacity of silver nanoparticles made from black tea extract (Ribeiro *et al.,* 2018). Electronic absorption spectroscopy was used to study the interaction between AgNPs and CT-DNA (Rahban *et al.,* 2010). It is discovered that biosynthesized AgNPs interact with CT-DNA (Roy *et al.,* 2015).

1.3. Medicinal herbs

Medicinal herbs are the world's oldest and most widely used type of treatment. According to a study published in the journal "Nature," the world's tree population is estimated to be at 3.04 trillion. It equates to nearly 422 trees for every person (Ehrenberg, 2015). It has been shown to be a source of meals and materials, greatly benefiting our planet and lives. Apart from direct benefits, trees also play important role in medicines. There is a lot number of medicinal plants and trees which possess for curing many diseases. Herbal medicine has long been applied in traditional medicinal systems around the world, particularly in India, where the oldest medical systems such as Ayurveda, Siddha, and Unani rely on plant-based formulations to the extent of 90%.

India is rich in medicinal and herbal plant resources, which serve as both a supply of raw material for therapeutic agents used in traditional and modern medicine, as well as a source of health care for rural India (Ravi and Bharadvaja, 2019). Among other things, it produces hundreds of chemical compounds for defense against insects, fungi, illnesses, and herbivorous mammals. There are numerous phytochemicals that have biological activity, either potential or established.

Meliaceae tree species are recognized for their great products. Timber is highly prized. Because of the existence of biologically active chemicals known as limonoids, bark, sawdust, and wood debris serve as possible sources of extractives that could be used in pest management. Other members of this family are likely to be examined for secondary metabolites and bioactivity in addition to multiplication across physiological barriers against natural disasters.

1.4. Melia azedarach

It is commonly called *Melia azedarach*, Malai vembu, and Bakain. It is a fast-growing tropical tree that requires almost no maintenance and it is resistant to pests and diseases. Australia, China, India, South America, and Southeast Asia are among the countries that cultivate. Bole is often used as fuelwood and plywood in plantations, while the rest of the plant parts are dumped as trash. Even so, traditional medicinal systems have recorded the use of leaves and fruits for antimalarial, antiparasitic, rheumatic diseases, antipyretic, and blood detoxing properties by tribes (Orwa *et al.,* 2009). Malaria, diabetes, purgative, cough, and skin illness have all been treated with various parts of the plant Melia azedarach, including the leaf, flower, seed, fruit, and young branches (Azam *et al.,* 2013). It exhibits antimicrobial, cytotoxic, antibacterial, antioxidant, analgesic, and anti-inflammatory activities, according to experimental and clinical research (Zahoor *et al.,* 2015).

1.4.1. Characteristics of Melia azedarach

M. azedarach is a small to medium deciduous tree that can grow up to 45 meters in height. It is deciduous with cylindrical bole and thrives in disturbed places, such as near roads and woodland borders. It has the capacity to form dense thickets, preventing native flora from growing. Southeast Asia and northern Australia are native to *M. azedarach.* Bole fluted below with a spreading crown and sparsely branching branches up to 30-60 cm in diameter. When young, the bark is smooth and greenish-brown, but as it ages, it becomes grey and fissured. It grows wild up to 1800 meters in the Himalayan region. It is extremely adaptable and can withstand a wide range of environmental conditions. Deep, fertile, sandy loam solids that support the best growth are ideal for cultivating the plant. It was first imported to the United States in the mid-nineteenth century for ornamental uses. Melia azedarach develops quickly in optimal conditions. During the rainy season, the plant regenerates itself from seeds. Direct sowing, cuttings, and root suckers are all options for artificial propagation. Chinaberry trees are also known as Texas umbrella trees or white cedar trees. Toosendanin is found in the bark of this tree. Green immature berries turn yellow and wrinkle with age. In Chinese medicine, Melia azedarach bark has indeed been boiled and consumed with resultant indigestion, diarrhoea, abdominal discomfort, dizziness, areflexia, coma, blurred vision, muscle soreness, weakness, ataxia, ptosis, numbness, and headache (Kimberlie, 2017).

1.4.2. Uses of *Melia azedarach*

Melia azedarach is a dark wood that is used to make farming equipment, furniture, plywood, boxes, poles, and tool handles. It has potential termite resistance so it is often utilized in cabinetry and construction. Fruit stones are used to construct necklaces and rosaries and make wonderful beads. The tree has been lopped to provide fodder. Unlike neem, Melia azedarach leaves are only mildly bitter; they are occasionally consumed after boiling with vegetables. Leaves can also be utilized as green manure. Insect repellent properties have been found in the leaves, bark, and fruits. Leaves are used to protect book pages and clothing, particularly woolen garments, from pest infestation. Sprays containing leaf extracts, protect plants from grasshoppers and locusts. Carpet beetle larvae are poisonous to alcoholic and petroleum ether preparations of stem bark, which are utilized as holistic biocides.

1.4.3. Medicinal properties

M. azedarach rich in tannic acid has a great potential to synthesize stable AgNPs (Sukirtha *et al.,* 2012). As a consequence, aqueous *M. azedarach* leaf extracts to examine the green production of AgNPs. Its seeds have a pleasant smell and contain around 40% triglyceride oil of fatty acids (Prusti, 2008). The oil is ideal for the development of polymers with film-forming ability, according to the results of Physico-chemical tests, particularly the iodine value (Roop *et al.,* 2005). The plant is extremely useful, particularly in terms of its wood and beautiful appearance, and it is widely planted by farmers in rural regions (Azam *et al.,* 2013). The oil from this seed has yet to be fully utilized, particularly for the production of practical polymers. *M. azedarach* extracts inhibited the activities of nicotinamide adenine dinucleotide phosphate (NADPH) - Cytochrome reductase and Cholinesterase in *Spodopter frugiberda* larvae. Their findings showed that consuming a diet rich in extract inhibited Cholinesterase activity by 31% (Breuer, *et al.,* 2003). More research into the use of *M. azedarach* leaf extract revealed that it is ineffective as a pregnancy preventative (Keshri *et al.,*2003). On the other hand, chloroform extract of roots was discovered to have strong contraceptive efficacy. By assessing changes in the activity of important carbohydrate metabolism enzymes in the rat uterus on day 7 of pregnancy, the antifertility properties of extracts from this plant were studied (Keshri et al 2004). One critical enzyme of the glycolytic pathway was found to be considerably reduced in the uteri of treated rats on day 7 of pregnancy when compared to controls.

1.5. *Caenorhabditis elegans* as a model organism

The nematode Caenorhabditis elegans is a 1 mm long free-living transparent nematode found in temperate soil habitats. It is widely used as a model organism to study developmental biology, neurobiology, and behavioural biology, as well as host-pathogen relationships. It has self-fertilizing hermaphrodites having a reproductive cycle of 2.5–4 days at room temperature and mean longevity of 18–20 days when cultured at 20°C. *C. elegans* can develop into four larval stages (L1–L4) after hatching, or proceed to the dauer larval stage after the L2 larval stage, rather than the L3 larval stage. The dauer larval stage is a developmentally halted dispersion stage that is used to survive in harsh environments. *C. elegans* can recover and moult into the L4 larval stage once the adverse conditions have passed, continuing their regular development (Chen *et al.,* 2017) It has been consistently demonstrated that when human genes are introduced into C. elegans, they replace their *C. elegans* homologs. Many *C. elegans* genes, on the other hand, can act similarly to mammalian genes. It is the first multicellular organism to have its entire genome sequenced. Furthermore, because its genome is eerily similar to that of humans, *C. elegans* has become a popular model organism for studying human diseases. The worm has recently become an important invertebrate model for the explication of the innate immune system (Kim *et al.,* 2002). During the course of host-pathogen interactions, *C. elegans*, the most adaptable model system and best alternative for studying human infections, gave significant insights into various biochemical pathways and signalling molecules.

1.5.1. *C. elegans* in drug discovery

More recently, researchers have looked into using this bacterium as a drug discovery tool. Despite the fact that prior drug screens were labor-intensive and low-throughout, recent advancements in high-throughput liquid processes, imaging systems, and data processing software have made *C. elegans* a feasible alternative for automated high-throughput drug screens. Traditional antibiotic discovery approaches focus on screening compounds that alter bacterial pathogens growth, survival, and identifying compounds with comparable mechanisms of action. The emergence of resistance to these structurally identical medicines necessitates the creation of a new antimicrobial discovery technique (Bumann 2008). The use of live animal models as a screening platform is an excellent alternative for in vitro antibacterial discovery. Furthermore, employing live models has the advantage of validating both the efficacy and toxicity of the screened candidate at the same time (Moy *et al.,* 2006). This simple pathogenicity assay based on feeding allows for high-throughput screening and genetic analysis. *C. elegans* has been shown to be a useful model for screening antiinfectives derived from both chemical and natural sources.

1.5.2. *C. elegans* as a useful model for aging study and stress assay

C. elegans is a useful model for the study of ageing mutations due to its ease of laboratory maintenance, transparent body for anatomical observation, high genetic homology, availability of complete genome sequence, conserved biological molecular responses, high fertility rates, and availability of molecular biology tools such as transgenic, gene knockouts, and RNAi knockdowns (Matsunami, 2018). Furthermore, due to lower experimental costs and their suitability for large throughout screening trials, this organism's short lifespan and tiny size make it ideal for the screening of anti-aging medications. Furthermore, *C. elegans* experiments are free of ethical considerations. Because of these benefits, *C. elegans* has been used to make many major findings in the field of ageing research. In recent decades, the use of *C. elegans* as a model organism to recapitulate most human diseases has proven helpful for in vivo metabolic and genetic studies (Moreno-Arriola *et al.,* 2014). Furthermore, *C. elegans* based ageing research has yielded promising results in discovering molecular signals, transcriptional regulators, and epigenetic alterations linked to lifespan, expanding our understanding of how animals age. Its transparency allows simple microscopy or more complex techniques like SPIM or Light Sheet Microscopy to be used to observe the fate of individual cells. *C. elegans* can be easily screened for effects of novel medications on complicated systems involved in human disease when raised in huge numbers. Because the creature travels through multiple distinct periods of life that may be seen physiologically and genetically, *C. elegans* is particularly useful in the study of ageing processes.

Many human diseases and ageing are exacerbated by oxidative stress. The SKN-1 protein is necessary for oxidative stress tolerance and lifespan in *Caenorhabditis elegans*. It initiates phase II detoxification gene transcription, a conserved oxidative stress response (An *et al.,* 2005). Oxidative stress is considered to play a vital role in the development and progression of neurodegenerative diseases. The multiplicity of signalling cascades, while now recognized as a hallmark of such processes, prevents a thorough understanding of the direct involvement of oxidative stress in neurodegeneration. In addition to its widespread usage as an ageing model, *Caenorhabditis elegans* has been used by certain researchers to examine molecular mediators that either worsen or protect against reactive oxygen species (ROS)-mediated neurodegeneration. *C. elegans* genetic models can be used to explore upstream markers of oxidative stress within interconnected signalling cascades due to their extensively described genome and short life cycle (Chakraborty *et al.,* 2013).

OBJECTIVES OF THE STUDY

2. OBJECTIVES OF THE STUDY

The objectives of this study were to synthesize silver nanoparticles from Melia azedarach seeds and test their efficacy in a variety of biological applications, as well as to assess the anti-aging potential of *Caenorhabditis elegans* as a model organism.

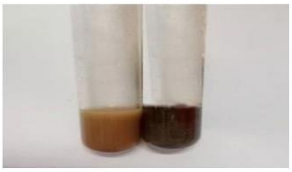
1. To synthesize silver nanoparticles using the *Melia azedarach* seeds extract.
2. To characterize the biosynthesized silver nanoparticles using by UV-Visible (UV-VIS) spectroscopy, Fourier transform infrared spectroscopy (FT-IR), X-Ray Diffraction (XRD), Scanning Electron Microscopy and Energy Dispersive Spectroscopy. (SEM) and (EDAX).
3. To identify the phytochemical compounds in the green synthesized extract.
4. To establish and legalize the experimental model using the *Caenorhabditis elegans.*
5. To evaluate the anti-aging potential of selected *Melia azedarach* synthesized silver nanoparticles in a lifespan assay using *Caenorhabditis elegans.*
6. To evaluate the effect of *Melia azedarach* synthesized silver nanoparticles on the survival rate of *C. elegans* under various stress conditions

Schematic diagram of the research work

Collection of Plant samples

Preparation of Seed extract

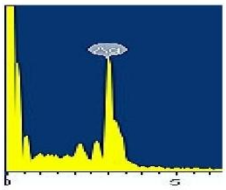
Synthesis of AgNP- Colour changes from brown to black



Characterization MA-AgNPS

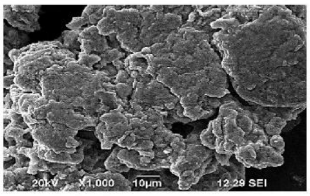
UV-VIS Spectroscopy- 450 nm confirms the formation of AgNPs

EDAX -largest weight percentage of silver Nanoparticles

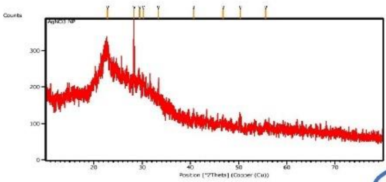


FT-IR Analysis-Alcohols and Phenols, Amides, nitro compounds &Alkyl and Aryl halides

SEM Analysis- poly scattered spherical in shape



XRD Analysis- the peaks at 2θ values are 3.150, 2.686, 2.219 and 1.652



Antioxidant activity- DPPH assay

Absorbance value of ($Y= 0.263x + 58.203$), $R^2 = 0.9708$

Culturing of *C. elegans*

Life span assay- more efficient at 150 µg/ml concentration, increasing the mean lifespan of C. elegans

Heat stress assay- more efficient at 500 µg/ml concentration, increasing the mean resistance of C. elegans

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Aging is one of the natural and biological phenomena which every organism undergoes. The age-related physical and neurological deterioration that results in a decrease in overall organ function was once thought to be a normal part of aging, but the causes of this illness are still unknown. The green synthesis method using plant extracts has attracted great attention to the physical, chemical, and even microorganism-mediated synthesis of silver nanoparticles. It has numerous biological and physicochemical applications. Drug delivery, biosensing, bio-imaging, and biomolecular recognition are all possible applications (Baker *et al.,* 2005). Plant extracts can be utilized to synthesize metallic nanoparticles at a low cost, making them an affordable and practical alternative to large-scale metallic nanoparticle synthesis.

3.1. Nanotechnology:

Nanotechnology is a tremendously powerful technology that has the potential to revolutionize the design and development of a wide range of new products, including medical applications for early illness detection, treatment, and prevention. The goal of the current research is to establish several experimental techniques for the synthesis of nanomaterials in various sizes and shapes. The green synthesis approach was widely adopted in various ways due to its advantages in successfully managing particle size and morphology. Over the recent decades, nanoparticles of worthy metals such as silver showed considerably unique physical, chemical, and biological properties from their inorganic form (Abou El-Nour *et al.,* 2010). silver nanoparticles have been widely utilized in the health industry, food storage, textile coatings, and a variety of environmental applications as antibacterial agents. According to recent discoveries, electromagnetic, optical, and catalytic characteristics of AgNPs are greatly influenced by the shape, size, and size of distribution, which are often varied by ranging the synthetic methods, reducing agents, and stabilizers (Abou El-Nour *et al.,* 2010). Ag NPs can also develop in the gills and liver tissue of fish, impairing their capacity to deal with low oxygen levels and causing oxidative stress (Bilberg *et al.,* 2010; Scown *et al.,* 2010).

There are several consumer goods and applications utilizing silver nanoparticles. According to the Woodrow Wilson Database, there have been 1015 consumer products on sale that contain Nanoparticles, with 259 of them having silver nanoparticles, that have a significant antibacterial effect, making silver Nanoparticles one of the most common and extremely fast-growing classes of nanomaterials in product applications (Fabrega *et al.,* 2011). It is the highly controlled manipulation of matter on atomic, molecular, and supramolecular scales to create functional systems at the molecular level (Caruso *et al.,* 2014). As such, it is the pinnacle of man's insatiable need to comprehend the world and put that knowledge to practical use (Ramsden, 2016). It has the ability to build enormous, intricate structures with nanoscale precision that are quickly evolving, and it employs both top-down reductive and bottom-up additive methods. It has achieved an array of biological machinery that roles at the nanoscale, structures that naturally self-assemble driven by the molecular chemistry of subunit interactions (Booth *et al.,* 2017). Nanotechnology is being employed in biomimetics to design materials that facilitate hard tissue remineralization. Biomimetic materials and processes, in particular, self-assembly of components to produce, replace, or repair oral tissues. Nanoparticles are utilized to change dental implant surfaces in order to influence the host response at the cellular and tissue levels for dental implants and related devices. This technique is utilized to generate nanotextured thin-film and biocompatible coatings for implant surfaces. To optimize the interaction with the surrounding apical tissue, these technologies minimize the thickness of the coating layer and increase the specific surface area and responsiveness (Zafar *et al.,* 2020).

3.2. Approaches in Nanobiotechnology

Nanotechnology refers to atomic, molecular, and macromolecular scale research and development that leads to the controlled manipulation and study of structures and devices with length scales ranging from 1 to 100 nanometres. The small size of nanoparticle surface tolerance increased solubility, and multifunctionality provides new study opportunities for biologists. Nanomaterials have unique features that allow them to engage with complicated biological functions in unprecedented ways, acting at the size of biomolecules. Cross-disciplinary researchers can design and construct multifunctional nanoparticles that can target, diagnose, and treat diseases like cancer in this fast-emerging field (McNeil, 2005). Nanotechnology is discussed in depth in a variety of sectors, including health and medical, electronics, energy, and the environment. Nanoparticle uses in medicine delivery, protein, peptide delivery, and cancer treatment are discussed. The use of carbon nanotubes, dendrimers, nanocrystals, nanowires, and other nano systems in cancer therapy is discussed. Nanotechnology advancements aid in the treatment of neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. Nanotechnology in tuberculosis therapy, operative dentistry, ophthalmology, surgery, imaging, tissue engineering, antibiotic resistance, and immune response are all clinical applications of nanotechnology (Nikalje, 2015). The agronomic application of nanotechnology in plants has the potential to disrupt traditional plant production systems by allowing for the controlled release of agrochemicals and the delivery of biomolecules to specified targets. Increased disease resistance, nutrient utilization, and crop output could all benefit from a better knowledge of the interactions between nanoparticles (NPs) and plant responses, including their absorption, localization, and activity (Wang *et al.,* 2016). DNA nanotechnology is a field that takes this molecule out of its biological environment and uses its information to create structural motifs and then connect them. This field has had a significant impact on nanoscience and nanotechnology, and its capacity to manipulate molecular self-assembly has been revolutionary (Seeman and Sleiman, 2017). For plant genetic transformation, nanotechnology-based gene delivery systems have been developed. This nano strategy demonstrates high transformation efficiency, biocompatibility, appropriate preservation of external nucleic acids, and plant regeneration capacity. However, the nanomaterial-mediated gene-delivery method in plants is still in its early stages, and there are numerous obstacles to overcome before it can be widely used and the creation of nanomaterial-based gene-delivery methods were examined (Yan *et al.,* 2022).

3.3. Synthesis of Nanoparticles

The production of metallic nanoparticles is a popular topic in nanotechnology in the terms of "application research". Metallic nanoparticles can be synthesized using a variety of chemical and physical methods. These processes, however, have a number of drawbacks, including the use of toxic solvents, the production of hazardous by-products, and excessive energy consumption (Thakkar *et al.,* 2010). Chemical or biological methods can be used to make nanoparticles. Metallic nanoparticles come in a variety of forms, including gold, silver, alloys, and magnetic nanoparticles (Hasan, 2015). Nanotechnology research has produced a variety of ways for synthesizing NPs from a variety of materials, including metals, semiconductors, ceramics, metal oxides, polymers, and other materials. NPs have distinct physicochemical, structural, and morphological properties that are significant in a wide range of applications in the electronic, optoelectronic, optical, electrochemical, environmental, and biological domains, depending on their origin and synthesis methods (Dhand *et al.,* 2015). The plants have been widely examined for their ability to synthesize metal nanoparticles, and this method has been identified as a green and effective technique to further exploit microorganisms as convenient nano factories.

Organic, inorganic and carbon-based particles of nanometric scale have better qualities than bigger sizes of respective materials. Because of their small size, nanoparticles have improved features such as high reactivity, strength, surface area, sensitivity, and stability. For research and commercial purposes, nanoparticles are synthesized using a variety of technologies that are divided into three categories such as physical, chemical, and mechanical processes, all of which have advanced significantly over time (Ealia and Saravanakumar, 2017). The synthesis of nanoparticles is a relatively new challenge in terms of sustainability. This field has received a great deal of interest in recent years because of its capacity to build safer, more energy-efficient, and less toxic synthesis methods. These pathways have been linked to the sensible use of a variety of chemicals in nanoparticle preparations and synthetic approaches (Duan *et al.,* 2015). The form, size, polydispersity, and surface chemistry of nanoparticles all influence their properties. Nanoparticles must be manufactured with carefully regulated features in order to be used in chemical sensing, medical diagnostics, catalysis, thermoelectrics, photovoltaics or pharmaceutics. As nanoparticle syntheses sometimes include many chemicals are carried out under interdependent experimental settings, this is a time-consuming, arduous, and resource-intensive task. Machine learning (ML) is a promising method for the rapid creation of efficient nanoparticle synthesis protocols, as well as the synthesis of new nanoparticle types (Tao *et al.,* 2021). The biosynthesis of nanoparticles is progressively paving the way to biological methods of sustainable and environmental nature. Despite the massive potentiality of microbes and plants as sources, biological synthesis can be used as a green alternative to conventional methods for nanoparticle synthesis (Srivastava *et al.,* 2021). During nanoparticle synthesis, wang *et al.,* 2022 use liquid-phase transmission electron microscopy to directly view the self-assembly of platinum nanoparticles into close-packed supraparticles over tens of seconds. The reduction of an aqueous platinum precursor by an electron beam produced monodisperse 2–3 nm platinum nanoparticles that self-assembled into 3D supraparticles in tens of seconds, some of which had crystalline ordered domains. Supraparticle production was driven by weak attractive van der Waals forces balanced by short ranging repulsive steric interactions, as indicated by modifying interparticle interactions (e.g., electrostatic, steric interactions) by changing precursor chemistry. Nanoparticle surface levels increased on supraparticles were orders of magnitude quicker than nanoparticle attachment, allowing nanoparticles to reach high coordination binding sites unhindered by incoming particles, according to growth kinetic studies and an interparticle interaction model.

3.4. Green synthesis of silver nanoparticles

The green synthesis method using plant extract has received more attention than physical and chemical, or even microorganism-based silver nanoparticle synthesis. Plant extracts can be utilized for synthesizing metallic nanoparticles at a low cost, and hence can be used as a useful substitute for large-scale metallic nanoparticle synthesis. The advantages of the green synthesis procedure for the manufacturing of SNPs using plants include a rapid, environmentally sustainable, non-pathogenic, cost-effective protocol and a single-step synthesis process. Green compounds provide various advantages over harmful chemicals, including low energy consumption and moderate operating conditions (Mie *et al.,* 2014; Raja *et al.,* 2017). The microbial production of AgNPs from yeast extract of *Saccharomyces cerevisiae* was described by Roy *et al.,* 2015. The photocatalytic degradation of AgNPs was mediated by Cordia dichotoma leaf extract, which was studied by UV-vis, SEM, TEM, FTIR, DLS, and XRD. CD-AgNPs were also tested for photocatalytic degradation of azo dyes like MB and CR, as well as antibacterial activity against *E. coli* and *Pseudomonas aeruginosa* (Kumari *et al.,* 2016). *Murraya koenigi* leaf extract was utilized as a reducing and capping agent for photo-induced green synthesis of AgNPs (Kumar *et al.,* 2017). The green produced AgNPs were spherical in shape, measuring 8.6 nm in TEM and 1.8 nm in AFM. The functional groups found on the surface of AgNPs derived from aqueous *M. koenigi* leaf extract are investigated using FTIR between 4000 and 400 nm. Devadiga *et al.,* 2017 made AgNPs from aqueous leaf extract of Terminalia catappa, and agro-waste. Due to capping compounds found in the *T. catappa* extract, monodispersed, crystalline, quasi-spherical AgNPs with a diameter of 11 nm were produced. This technique produced highly stable AgNPs, as evidenced by a zeta potential of -36.7 mV. It was found to have potent antibacterial properties. *Durio sibehimix* aqueous seed extract mediated green synthesis process of AgNPs obtain as photocatalytic, cytotoxic effects, and antibacterial properties. The surface plasmon resonance peak at 420 nm confirmed the green synthesis of AgNPs. The characterization of NPs revealed that they are spherical and rod-shaped, with a size range of 20-75 nm. Zeta potential was discovered to be the -15.41 mV. Zibethim AgNPs demonstrated photocatalytic activity against MB, strong antibacterial activity, and brine shrimp cytotoxicity (Sumitha *et al*, 2018).

UV-vis spectra indicated the presence of AgNPs, with an absorbance peak at 450 nm. The spherical shape was revealed by TEM and XRD examination, with an average size distribution of 10 nm and a face-centered cubic crystal lattice. Methylene blue was used to test the photocatalytic activity of *S. molesta* extract mediated AgNPs in the sun, and these NPs demonstrated efficiency in degrading the dye within a few hours of exposure. Ravichandran *et al.,* 2019 proposed the green production of AgNPs from aqueous *Parkia speciosa* leaf extract by AgNO3 bioreduction. UV Visual spectroscopy verified green synthesis, with an SPR peak at 410.5 nm. Different physicochemical factors such as pH, time, temperature, AgNO3 concentration and the volume of leaf extract were spectrophotometrically tuned in this work. SEM, TEM, and DLS investigation revealed that the average size of AgNPs was 31 nm, 35 nm, and 155.3 nm, respectively. XRD and EDX measurements revealed the presence of Ag in green-produced NPs. Under solar irradiation, green produced AgNPs showed strong photocatalytic activity against the azo dye MB, antibacterial activity against *E. coli, S. aureus, Pseudomonas aeruginosa,* and *Bacillus subtilis*, and antioxidant activity against the DPPH free radical. The waste extract of *Brassica oleracea* was also used in the green synthesis of AgNPs. UV-vis spectroscopy was used to optimize pH, temperature, extract, and AgNO3 concentration by optimizing the experiment. TEM, SEM, XRD, FT-IR, SAED, XPS, and BET were used to characterize green-produced AgNPs. These investigations validated the production of spherical AgNPs in the 5-50 nm region. By serving as capping and reducing agents, functional groups found in the extract of *B. oleracea* are responsible for the green synthesis of AgNPs, according to FTIR studies. AgNPs were tested for MB dye degradation and mercury ion detection under sun irradiation. NPs were discovered to be extraordinarily capable of sensing Hg2+ ions at concentrations of up to 0.1 mg/l (Kadam *et al.,* 2020).

3.5. Biological application of synthesized AgNPs

Silver nanoparticles are utilized in a wide range of fields, including medical, food, health care, consumer, and industrial applications, due to their excellent physical and chemical properties. Nanomaterials have been examined as possible instruments for the progress of diagnostic biosensors, medication, gene delivery and biomedical imaging due to nanoscale effects and increased surface area.

Oxidative stress is caused by an imbalance between radical-generating and radical-scavenging systems, which leads to increased synthesis of reactive oxygen species (ROS) or diminished antioxidant defense activity. As a results, there is a need to find medicinal plants with antioxidant properties that may be used as an alternative source of medicine to treat disorders caused by free radical production. Plants and their parts have been shown to have antioxidant action due to polyphenol content in several studies (Morakinyo *et al.,* 2011). According to the findings, the scavenging effect increased as the amount of food consumed rose. The production, shape, and bioactivities of the AgNPs provided were nearly the same between tissue experiments, indicating that freeze-drying the leaf material could be useful in preserving them for future Nanoparticle amalgamation. Overall, biosynthesized AgNPs have shown a broad spectrum of antibacterial helplessness, pointing to promising antimicrobial specialists with potential biological applications.

Among the many possible uses of AgNPs, their intriguing implications in wound treatment, tissue scaffolding, and protective clothing applications have recently received a lot of attention and effort (Gudikandula *et al.,* 2017; Mokhena and Luyt, 2017). Maintaining the nanoscale size of AgNPs, enhancing their dispersion and stability, and preventing aggregation are some important elements connected to their particular antibacterial characteristics (Mokhena and Luyt, 2017). Many investigations have shown that the anti-pathogenic action of AgNPs is superior to that of silver ions (Li, W. R., Sun *et al.,* 2017). The worrying and rising phenomenon of pathogenic drug-resistant incidence is a major source of concern for the global healthcare system. As a result, AgNPs are showing promise for developing unique and effective biocompatible nanostructured materials for novel antibacterial applications based on nanotechnology (Premkumar *et al.,* 2018). AgNPs are one of the most widely employed metallic nanoparticles in modern antimicrobial applications due to their inherent broad bactericidal activity against both

Gram-negative and Gram-positive bacteria, as well as their physicochemical features (Shao *et al.,* 2018). AgNPs interact with the bacterial membrane and permeate the cell, causing severe disruptions in cell function, structural damage, and cell death (yan *et al.,* 2018).

AgNPs are crucial in the development and implementation of new biomedical techniques (Kalaivani *et al.,* 2018). AgNPs have recently been thoroughly examined in many human malignant cell lines, including endothelium cells, IMR-90 lung fibroblasts, U251 glioblastoma cells, and MDA-MB-231 breast cancer cells, for their promising anticancer activities (Thapa *et al.,* 2017). AgNPs have the inherent potential to bind to mammalian cells and easily permeate them through energy-driven internalization routes (Pongrac *et al.,* 2018). Another appealing feature of AgNPs is their unique fluorescence, which makes them ideal candidates for use in X-ray irradiation applications for detection and dose enhancement (Mattea *et al.,* 2017). At the moment, targeted therapies or the combination of therapy and diagnosis, is the most important, appealing and demanding method accepted by healthcare practitioners and researchers in terms of successful and individualized cancer therapy (Vedelago., 2018). AgNPs are also plasmonic structures that have the ability to scatter and absorb light in specific locations. AgNP-derived dispersed light can be used for imaging after selective uptake into malignant cells, whilst absorbed light can be employed for selective hyperthermia (Sharma *et al.,* 2015).

3.6. Regulation of Aging

Aging is defined as a progressive functional impairment at the molecular, cellular, tissue, and organismal levels. The processes that ordinarily maintain health and stress resistance deteriorate dramatically as people age, leading to decrepitude, frailty and mortality. Aging is defined as the post-maturational degeneration of cells and organisms through time, as well as a greater vulnerability to challenges and the incidence of age-related disorders as well as a decreasing ability to survive. Increased creation of reactive oxygen species (ROS) and oxidative damage, as well as incomplete housekeeping and a buildup of altered ROS-hyper producing organelles in aged cells. Autophagy has been demonstrated to be the only line of defense against the accumulation of harmful mitochondria and peroxisomes, that its function declines with age, and that it determines cell and individual lifetime (Cuervo, 2008).

The basis of age-related deterioration is changes in transcriptional networks and chromatin state. These epigenomic changes are not only detected as people age, but they also have a significant impact on cellular function and stress tolerance, contributing to the aging process (Booth and Brunet, 2016). Oral aging is a current focus of several organizations including the Federation Dentaire Internationale, the World Health Organization, and the American and Japanese Dental Associations. Despite the fact that the prevalence of periodontal disease continues to rise as the population ages, one of the WHO's goals is for each individual to keep more than 20 teeth by the age of 80.

Every species has a unique lifespan that is established by its evolutionary history and influenced by a variety of factors, including biological mechanisms. In humans, the aging process is aided by the steady accumulation of cellular metabolic products and significant DNA damage. Aging is considered to be associated with the low-grade inflammatory phenotype in mammals and is the result of autophagic capacity impairing called 'Housekeeping activities' in the cells, resulting in protein aggregation, mitochondrial dysfunction and oxidative stress. Delay in stem-cell proliferation, which is linked to the aging, may have an impact on a living being's maintenance and survival, but excessive proliferation could deplete stem-cell reserves. The link between delayed cell proliferation and wound healing and the emergence of periodontal diseases and treatment response requires more research. Periodontal disorders, and eventually tooth loss, are hypothesized to be caused by the effects of systemic diseases, drugs, psychological effects, and diminished motivation or capacity in performing oral hygiene routines in elderly people (Kanasi *et al.,* 2016). Aging is strongly linked to many disorders, including Alzheimer's disease, atherosclerosis, heart disease, Type 2 diabetes, and cancer, according to growing data. Many advancements have been achieved in the previous decade in studies focusing on the role of inflammation in disease progression. However, the mechanisms which influence pathological alterations and disease progression remain unknown (Xia *et al.,* 2016).

3.7. Oxidative stress

Oxidative stress occurs when the amount of reactive oxygen species (ROS) in the body is overridden by the availability and activity of antioxidants. Increased production of free radicals or decreased antioxidant activity disrupts this balance. It is critical to develop methods and identify acceptable biomarkers for assessing oxidative stress in vitro cells. It's crucial because determining the role of stress in life style-related disorders necessitates accurate measurement. Older oxidative stress markers, such as thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA), are gradually being replaced by newer ones are isoprostanes and their metabolites, or allantoin (Czerska *et al.,* 2015). The oxidative stress theory of aging is predicated on the assumption that age-related functional deficits are caused by RONS-induced damage buildup. At the same oxidative stress has a role in a variety of age-related disorders, including sarcopenia and frailty (i.e., cardiovascular diseases (CVDs), chronic obstructive pulmonary disease (COPD), chronic renal disease, neurodegenerative diseases, and cancer). Various forms of oxidative stress biomarkers have been developed, and they may provide vital information on therapy success, directing the selection of the most effective drugs regimens for patients, and, acting on a specific therapeutic target. Antioxidant therapy could improve the natural history of various diseases, given the crucial role of oxidative stress in the pathogenesis of many clinical ailments and aging. However, more research is needed to determine the true efficacy of these therapeutic interventions (Liguori *et al.,* 2018). Reactive oxygen species (ROS) appear to have differential effects on cancer evolution, either initiating/stimulating carcinogenesis and encouraging cancer cell transformation/proliferation or triggering cell death. To cope with high ROS levels, tumor cells alter sulfur-based metabolism, NADPH production, and antioxidant transcription factor activity. During the start phase, genetic alterations activate antioxidant transcription factors or increase NADPH via the pentose phosphate pathway, allowing cells to survive in the presence of high ROS levels. Tumour cells adapt to oxidative stress during development and metastasis by generating NADPH in a variety of mechanisms, including activation of AMPK, the PPP, and reductive glutamine and folate metabolism (Hayes *et al.,* 2020).

These oxidants were oxidized with the biological macromolecules in their immediate vicinity, impairing cellular processes and resulting in oxidative stress. To defend themselves from OS, aerobes have evolved both enzymatic and nonenzymatic antioxidant defense. Hormones, as a mechanism of biological coordination, regulate tissue physiological processes through regulating metabolism, but any variation in their cause pathologic conditions. While antioxidant hormones like melatonin, insulin, estrogen, and progesterone exist, thyroid hormone, corticosteroids, and catecholamines cause free radical production and OS, and the role of testosterone in causing OS is debatable (Chainy and Sahoo, 2020). Exogenous oxidants like cigarette smoke and air pollution, as well as endogenous creation of reactive oxygen species by inflammatory and structural cells in the lungs, cause increased oxidative stress in COPD patients' lungs. Mitochondrial oxidative stress may play a significant role in COPD. With the inactivation of various antioxidant enzymes and the transcription factors Nrf2 and FOXO, which regulate multiple antioxidant genes, antioxidant defences are also reduced. Increased oxidative stress in the body can exacerbate comorbidities and lead to skeletal muscle weakness. Oxidative stress amplifies chronic inflammation, induces fibrosis and emphysema, develops corticosteroid resistance, accelerates lung ageing, causes DNA damage and stimulates creation of autoantibodies. This shows that using antioxidants to alleviate oxidative stress or increasing endogenous antioxidants could be a viable technique for treating COPD's underlying pathogenetic mechanisms. Most COPD clinical investigations have used glutathione-generating antioxidants including N-acetylcysteine which have been shown to lessen exacerbations in COPD patients, albeit it is unclear whether this is due to their antioxidant or mucolytic effects. Dietary antioxidants have yet to be proven therapeutically beneficial in the treatment of COPD (Barnes, 2020).

A relationship between oxidative stress and hypertension has been shown, although it remains enigmatic in humans. While early research focused on superoxide's inactivation of nitric oxide our knowledge of important reactive oxygen species and how they change complicated signaling pathways to induce hypertension has grown dramatically. Recent advances in identifying the primary and secondary sources of reactive oxygen species, as well as the posttranslational oxidative modifications they cause on protein targets important for redox signaling, their interactions with endogenous antioxidant systems, and the role of inflammasome activation and endoplasmic reticular stress in the development of hypertension. The role of oxidative stress in several organ systems in hypertension is discussed, as well as new animal models that have defined the importance of certain proteins and clinical studies that have provided light on how these processes and pathways are altered in human hypertension (Griendling *et al.,* 2021). The progressive loss of function in tissues and organs is one of the hallmarks of aging. According to the aging theory, age-related functional losses are caused by the accumulation of reactive oxygen species (ROS), which causes tissue damage and malformations. Cardiovascular diseases, chronic obstructive pulmonary disease (COPD), chronic renal disease, neurological diseases, and cancer are among the diseases and disorders induced by OS. OS, which is caused by ROS, is neutralized by a variety of enzymatic and non-enzymatic antioxidants, preventing damage to cells, tissues, and organs. Prolonged OS, on the other hand, lowers the antioxidant status of cells by lowering the activities of reductants and antioxidative enzymes, resulting in a variety of clinical diseases (Hajam *et al.,* 2022).

3.8 *Caenorhabditis elegans* as model organism

The worm *Caenorhabditis elegans* is an excellent experimental model for studying the impact of the microbiome on the host and vice versa. Emile Maupas, a biologist, discovered the worm *Rhabditis elegans* in the soil more than a century ago. Following further research and phylogenic studies, the species was renamed *Caenorhabditis elegans*. However, it wasn't until 1963 that Sydney Brenner, already well-known for his work on DNA, RNA, and the genetic code, argued that model organisms could be the future of biological research. Brenner believed that biological research needed a model system that could grow in large numbers in the lab, was inexpensive to maintain and had a basic body plan, therefore he picked the nematode *Caenorhabditis elegans* to fit that role. Since then, *Caenorhabditis elegans* has become one of the most widely used model organisms in aging research (Tissenbaum, 2015). Several genetic illnesses caused by mitochondrial abnormalities have been identified in recent decades, with varying onset and symptomatology ranging from neuromuscular degeneration to cancer syndromes. Unfortunately, most of these serious, life-threatening illnesses have only symptomatic and no curative treatments available at this time. Model organisms, particularly the nematode *Caenorhabditis elegans*, have made significant contributions in recent years to understanding the pathophysiology and treatment of various diseases, thanks to its sequenced and largely conserved genome and basic but well-characterized nervous system (Maglioni and Ventura, 2016). Unlike cell-based toxicity research, *Caenorhabditis elegans* toxicity assays provide data from a whole animal with fully functional digestive, reproductive, endocrine, sensory, and neuromuscular systems. Toxicity ranking screens in *Caenorhabditis elegans* have been demonstrated to be as accurate as mouse LD50 rankings in predicting rat LD50 rankings. In addition, there have been numerous reports of *Caenorhabditis elegans* and mammals sharing the same method of toxic action. These strong correlations support the use of *Caenorhabditis elegans* assays in early safety testing and as a component of tiered or integrated toxicity testing methodologies, but they do not imply that nematodes can substitute mammalian data for hazard assessment (Hunt, 2017).

With the rapid advancement of nanotechnology, the number of biosafety evaluation studies of nanoparticles (NPs) employing various biological models is increasing. As a full model organism, the nematode *Caenorhabditis elegans* has become an essential in vivo alternative test system for assessing the danger of Nanoparticles, particularly at the ambient level. According to the findings of qualitative and quantitative evaluations, investigations of nanoscientific research using *Caenorhabditis elegans* are steadily increasing. The entire conclusion and analysis of nanoparticles harmful effects in *Caenorhabditis elegans*, on the other hand, are restricted and chaotic (Wu *et al.,* 2019). The application of a SOD-1 reporter, as well as the Hyper and GRX biosensor strains to monitor changes in the cellular redox state, was used to measure the induction of antioxidant defences. Both types of Ag caused a rise in sod-1 expression, increased H2O2 levels, and a redox imbalance in the cellular GSSG/GSH system. AgNO3 generated ROS-related effects in many organs, including the pharynx, intestinal cells, and muscular tissues, according to microscopy analysis of the strains. NM300K, on the other hand, caused localised ROS generation and oxidative stress in tissues around the intestinal lumen. This suggests that Ag from AgNO3 exposure was easily distributed throughout the body, whereas Ag or ROS from NM300K exposure was restricted primarily to the luminal tissues. Concentrations that resulted in an increase in ROS generation and alterations in the GSSG/GSH ratio were consistent with physiological adverse effects.

However, at the lowest Ag doses, sod-1 was not generated, despite nephrotoxicity being seen. While both types of Ag induced oxidative stress, developmental impairment, and nephrotoxicity, the findings show that ROS generation plays a different role in the harmful effects of AgNO3 versus NM300K (Rossbach *et al.,* 2020). With an increase in industrial applications, silver nanoparticles have become more common in the environment. However, investigations on their possible health hazards are insufficient, particularly in terms of neurotoxic consequences. The neurotoxic effects of 20nm AgNPs with/without polyvinylpyrrolidone (PVP) coating at low concentrations (0.01–10 mgL1) on *Caenorhabditis elegans* during longer-term exposure (prolonged exposure for 48 h and chronic exposure for 6 days). The findings revealed that nematode survival was harmed by exposure to AgNPs, with the longest and relative average life spans being lowered.

Neurotoxicity was observed in locomotion (head thrashes, body bends, pharyngeal pumping frequency, and defecation interval) and sensory perception (chemotaxis assay and thermotaxis assay), as well as impaired dopaminergic, GABAergic, and cholinergic neurons, with the exception of glutamatergic neurons, in a dose- and time-dependent manner. Further research revealed that low-dose AgNPs (0.01–0.1 mgL1) exposure boosts GABAergic and dopamine receptors in *Caenorhabditis elegans* at the genetic level, whereas greater doses (1–10 mgL1) had the reverse effect, implying that AgNPs could cause neurotoxicity by decreasing neurotransmitter transport (Zhang *et al.,* 2021). The reproductive toxicity of common nanomaterials in *Caenorhabditis elegans*, such as metal-based nanomaterials (silver nanoparticles, gold NPs, zinc oxide NPs, copper oxide NPs), carbon-based nanomaterials (graphene oxide, multi-walled carbon nanotubes, fullerene nanoparticles), polymeric NPs, silica NPs, quantum dots), and the mechanisms involved This research sheds light on the harmful consequences of current nanoparticles on the reproductive system of humans. We also discuss how nanomaterials physicochemical features (e.g., size, charge, surface modification, and shape) affect their reproductive toxicity. Overall, employing *Caenorhabditis elegans* as a platform to develop quick detection techniques and prediction methodologies for nanomaterial reproductive toxicity is likely to close the gap between biosafety assessment and deployment of nanomaterials (Yao *et al.,* 2022).

3.9 *Melia azedarach*

Melia azedarach Linn is an indigenous species to the Indian subcontinent, containing several medicinal properties and has been in use for centuries. The plant has traditionally been used for anti-helminthic, anti-fungal, anti-malarial, anti-bacterial, and antioxidant effects. It has antiviral and anti-infertility properties in its leaf extract (Nathan *et al.,* 2006; Kaneria *et al.,* 2009; Jabeen *et al.,* 2011). The antioxidant activity and phytochemical analyses of *Melia azedarach* leaf extracts were studied. The study found that the ethanolic leaves extract of *Melia azedarach* contained the most phenolic compounds and had the best anti-oxidant efficacy compared to petroleum ether and aqueous extracts. *Melia azedarach* has a high scavenging ability could be attributed to the presence of hydroxyl groups in the phenolic compounds (Ahmed *et al.,* 2012). The seed extracts of *Melia azedarach* had substantial antibacterial action against pathogens. In comparison to all other extracts, ethyl acetate extract showed the most inhibition.

M. azedarach seed extracts could be effective antibiotics in the treatment of both gram-positive and gram-negative human pathogenic illnesses (Khan *et al.,* 2011).

3.9.1. Green synthesized silver nanoparticles using Melia azedarach

Carpinella *et al.,* 2005 reported the biosynthesis of silver nanoparticles (AgNPs) from Melia azedarach, as well as their cytotoxicity in vitro against HeLa cells and in vivo against Dalton's ascites lymphoma (DAL) mice. In vitro cytotoxicity of biosynthesized AgNPs against the human epithelial cancer cell line, HeLa revealed a dose-response relationship. The lethal dose (LD50) of AgNPs against the HeLa cell line was reported to be 300 g/ml. Only increasing concentrations of both AgNPs and 5-FU caused cytotoxicity in the normal continuous cell line human breast lactating, donor 100 (HBL 100). In addition, acridine orange and ethidium bromide (AO and EB) staining demonstrated that induction of apoptosis occurred in the DAL mice model in vivo. In the disc diffusion assay for human pathogenic gram-positive bacteria Bacillus cereus (34 mm) and gram-negative bacteria *Escherichia coli* (37 mm), MA-AgNPs demonstrated a greater zone of inhibition than MA-extract, showing their better antibacterial efficacy. On human dermal fibroblast cells, a cell scratch assay demonstrated potential wound healing ability. MA-AgNPs (400 g/mL) showed high antidiabetic efficacy (85.75 percent) and antioxidant activity (80.33 percent) as determined by -amylase (85.75 percent) and -glucosidase (80.33 percent) inhibition assays and DPPH (63.83 percent) and ABTS (63.61 percent) radical scavenging assays. MA-AgNPs had no toxicity against human liver cells (CCL-13) as evaluated by MTS, optical microscopy, and CMFDA dye techniques (Chinnasamy *et al.,* 2019).

The leaf extract of Melia azedarach was used as a reducing agent of silver ions to silver nanoparticles. UV–Visible spectroscopy found an absorption peak at 482 nm, which was used to monitor bio-reduction. The features of manufactured particles were identified using scanning electron microscopy and energy-dispersive X-ray spectroscopy. The particles were spherical and ranged in size from 34 to 48 nm. The antibacterial activity of silver nanoparticles was tested against common bacteria in silver-based antibacterial products (Mehmood *et al.,* 2017). Jebril et al 2020 observed that the addition of AgNPs at a concentration of 60 ppm greatly reduced Verticillium dahlia growth. In an in vivo trial, however, the addition of 20 ppm AgNPs lowers the degree of Verticillium wilt and relative vascular discoloration by 87 and 97 percent, respectively, when compared to inoculated and untreated controls. The antifungal action demonstrated in this study offers up a new dimension for biosynthesized AgNPs. Nanosized and nanostructured silver-based materials are important in modern biomedicine and biotechnology. Their amazing core ingredient for producing novel and performance-enhanced nanomaterials for biomedicine substantially influences their potential for such specific applications. Furthermore, the beneficial interactions of nano-silver with biological living structures, as well as their nontoxic effects on healthy human cells, define their potential for a wide range of biomedical applications, such as detection and diagnosis platforms, anti-infective and anti-cancer therapy, pharmaceutical and ageing studies.

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1. Collection of plant samples

The Seeds of *Melia azedarach* (L.) were collected (Plate.1) in Coimbatore, (10.9634° N, 76.9730° E) Tamil Nadu, India. The plant sample was identified by the Botanical Survey of India, Agricultural University, Coimbatore, Tamil Nadu. (Authentication No: BSI/SRC/5/23/2022/Tech/21).

Melia azedarach belongs to the kingdom Plantae and order belongs to Sapindales. The seeds were allowed to dry for five days at room temperature. The dried seeds were finely ground by using a mortar and pestle and made into a fine powder (Plate.3).

Plate: 1. *M. azedarach* tree Plate: 2. *M. azedarach* Seeds Plate: 3. *M. azedarach* powder

4.2. Preparation of seed extract

The seed extracts were carried out by adding 20 g of ground powder in a 200 ml Erlenmeyer flask filled with 500 ml distilled water. The contents are boiled on a hot plate for 30 minutes, stirring occasionally. The suspension was left to cool and filtered through Whatman no. 1 filter paper. This filtrate was used for the present study. The prepared seed extract was placed in the refrigerator to be used in future experiments.

4.3. Preparation of silver nitrate solution

A concentration of 0.02 mM silver nitrate solution was made by dissolving 3.3974 g of silver nitrate in 100 ml double distilled water and used for the green synthesis of silver nanoparticles.

4.4. Biosynthesis of silver nanoparticles

0.2 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *M. azedarach* extract was added into 90 ml of the aqueous solution of 0.2 mM silver nitrate for reduction into Ag+ ions and incubated overnight at room temperature. The reaction mixture was kept in the dark to reduce the photoactivation of silver nitrate. The Bio-reduction of silver nitrate into silver nanoparticles can be confirmed by visual observation of the transition of colour changes from brown to black (Plate. 4). The obtained *M. azedarach* silver nanoparticle (MA-AgNPs) was stored at 4°C for further analysis.

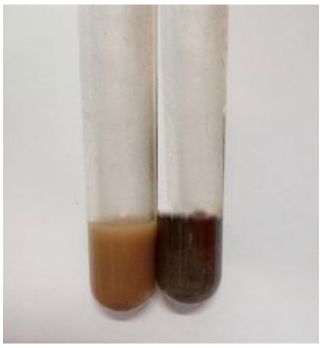


Plate: 4. Synthesis of MA-AgNPs

4.5. Characterization of synthesized silver nanoparticles

The synthesized SNPs of aqueous plant extract of *M. azedarach* were characterized based on their shape, size, and surface area following the standard techniques.

4.5.1. UV-Visible spectroscopy

The formation of MA-AgNPs solution was monitored using UV-Visible absorption Spectroscopy carried out in the range of 300-500nm, which gives the result of surface plasmon resonance formed for the metal. Absorption spectroscopy in the UV-visible band has long been used to characterize nanoparticles.

4.5.2. X-RAY diffraction (XRD) analysis

X-ray diffraction analysis (XRD) is being used to analyze the crystalline/ amorphous structure of green synthesized silver nanoparticles. The process conditions were at a voltage of 45 keV and a current of 20 mA with Cu-Ka radiation as an X-ray source in the range of 20–80 at the 2 h angular position. The aliquot was drop-coated onto a glass plate by placing a small number of aliquots on the plate and allowing them to dry before applying a thick coat of aliquots to the plate for XRD measurement (Madhan Kumar, 2021). The average particle crystalline size of the AgNPs was calculated using Debye Scherer's formula: $D = k\lambda/\beta \cos\theta$ where, K - constant, λ - wavelength of the X-ray, β - Whole width half maximum of the XRD peak (radians), θ - Bragg's angle of the XRD peak.

4.5.3. Fourier transform infrared spectroscopy (FT-IR)

FT-IR is used to identify the organic functional groups that are responsible for reducing metal ions to metal nanoparticles. The interaction between Ag and protein molecules is measured using FTIR measurements. As a result, when an infrared light interacts with a silver nanoparticle sample, the chemical bonds lengthen. The FT-IR result was matched with the normal library search of Infrared charts, indicating the contracting and bending nature of nanoparticles as shown by a peak.

4.5.4. Scanning electron microscopy (SEM) with Energy dispersive X-Ray (EDAX) analysis

In the current study, a Scanning electron microscope (SEM) was used to characterize the size and morphology of the nanoparticles. SEM has a magnification ranging from 20X to approximately 30,000X with a spatial resolution of 50 to 100 nm and for the imaging, a sample of 0.5 mg MA-AgNPs was dusted on one side of the double-sided adhesive carbon conducting tape. The tape was then mounted on an 8 mm dia aluminium stub. The sample was observed at different magnifications and the images were picturized. EDAX is an X-ray technique used to characterize the rudimentary construction of metals. The information got from the EDX examination show tops that match the segments of the genuine structure of the example to be assessed. High energy particles assault more grounded segments, electrons joined to an inner shell can be dispatched and a hole is created. The EDS plot identifies these particular X-rays noticing their similar solidarity to other frequencies.

4.6. Phytochemical analysis of MA-AgNPs

The major phytochemicals present in the *M. azedarach* silver nanoparticle (MA-AgNPs) were identified by following the standard protocol with slight modification (Ali *et al.,* 2018), (Archana *et al.,* 2012), (Chandraker and Shukla, 2021).

4.6.1. Test for alkaloids

- **Mayer's test:** According to this test method, 2 ml of concentrated HCl was added to 2 ml of the MA-AgNPs followed by the addition of a few drops of Mayer's reagent. The presence of alkaloids in that sample was confirmed by the formation of white precipitate or the appearance of green colour.
- **Hager's test:** In 2 ml of the sample, a few drops of Hager's reagent were added. The presence of alkaloids was revealed by the presence of bright yellow precipitate.
- **Dragendroff's test:** The small amount of sample was treated with Dragendroff's reagent (sodium iodide, basic bismuth carbonate, glacial acetic acid, and ethyl acetate) and the presence of an orange-brown precipitate was noted.

4.6.2. Test for carbohydrates

- **Benedict's test:** A few drops of the test solution and an equal volume of Benedict's reagent were boiled together. The presence of sugars was confirmed by the formation of a brick-red precipitate.
- **Fehling's test:** A few drops of test solution were combined with an equal volume of Fehling's solution. The presence of reducing sugars was confirmed by the formation of a brick-red precipitate.

4.6.3. Test for flavonoids

- **$FeCl_3$ test:** A small amount of the sample was mixed with a few drops of neutral ferric chloride solution and noted for the development of intense green colour.
- **Alkaline reagent test:** 1 ml of 2N NaOH solution was added to 1 ml of samples. The yellow colour appearance confirmed the presence of flavonoids in the sample.

4.6.4. Test for glycosides

- **Keller Killani test:** 1 ml glacial acetic acid was added to 1 ml of sample, which were then cooled. After cooling, 2 drops of $FeCl_3$ were added to the test tube, followed by a careful application of conc.H2SO4 along the test tube's walls. The presence of glycosides was revealed by a reddish-brown colour ring that formed at the intersection of two layers.

4.6.5. Test for proteins

- **Biuret test:** The sample was treated with an equivalent volume of 40% sodium hydroxide and two drops of 1% copper sulphate solution. The presence of proteins was indicated by the pink or purple colour.
- **Warming test:** In a boiling water bath, the test solution was boiled. The presence of proteins was revealed by the appearance of coagulation.

4.6.6. Test for sterols and triterpenoids

- **Salkowski's test:** 5 ml chloroform was added to 2 ml MA-AgNPs, then 1 ml conc.H2SO4 was carefully added along the tube's sides. The presence of sterols in the test samples was indicated by the reddish-brown colour in the lower layer, whereas the presence of triterpenoids was shown by the formation of yellow colour in the lower layer.
- **Libermann-Buchard's test:** It was performed by boiling 5 ml of the test solution with two drops of acetic anhydride, cooling, and then adding sulphuric acid along the edge of the test tube. A reference is the appearance of a brown ring at the intersection of two layers. Sterols are present if the upper layer turns green and triterpenoids are present if the upper layer turns deep red.

4.6.7. Test for tannins

- **$FeCl_3$ test:** A volume of 2 ml of 5% $FeCl_3$ was added to 1 ml of sample. The presence of tannins in the test sample was established by the emergence of a greenish-black or dark blue colour.
- **Alkaline reagent test:** 2 ml of 1N NaOH was added to 2 ml of material for an alkaline reagent test. The presence of tannins was shown by the appearance of a yellow to red colour.

4.7. Antioxidant activity

4.7.1 DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed to assess the extract's free radical scavenging activity based on its ability to donate hydrogen atoms or electrons to neutralise the stable radical DPPH, which was accompanied by colour changes. The experiment was carried out in a microplate format. 100 l DPPH working solution (0.2 mM) was added to 100 l extract at a 1:1 (v/v) ratio for the test procedure. The reaction mixture was incubated for 30 minutes at room temperature in the dark, and the absorbance was measured at 517 nm in a microplate reader. The % inhibition of DPPH was estimated using the following equation to compute radical scavenging activity

% Inhibition = 100 x [Abs of control - (Abs of sample - Abs of blank) / Abs of control]

To determine the extract's effective concentration (EC50), ascorbic acid (vitamin C) and EGCG were utilized as controls.

4.8. Development of a model for evaluation of anti-ageing potential of MA-AgNPs by using Caenorhabditis elegans as a model organism

4.8.1. Collection of *C. elegans* strain

The nematode *C. elegans* strains (Wild type) was provided by the nematology lab, Department of Zoology, Bharathiyar university, Coimbatore, India.

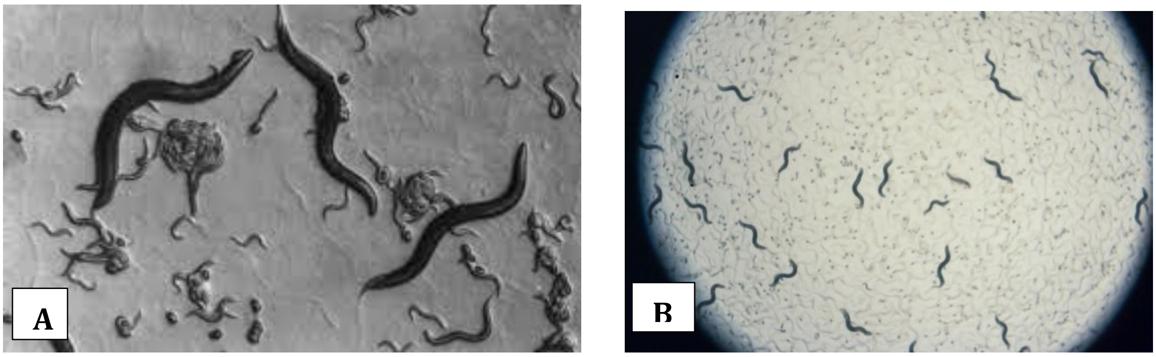


Plate: 5. Microscopic view of C elegans: (A) 1.5 mm view of adult wild type worm,
(B) A Population of *C. elegans* on nematode growth medium plate.

4.8.2. Preparation of growth media

4.8.2.1. Preparation of bacterial food source as *E. coli* strain

C. elegans is usually grown in the laboratory using *E. coli* strain OP50 as a food source (Brenner, 1974). *E. coli* OP50 is the uracil auxotroph whose growth is limited on Nematode growth medium (NGM) plates. A bacterial lawn with a limited number of bacteria was preferred because it allows for simpler observation and better worm mating. 100ml autoclaved nutrient broth solution was aseptically inoculated with a single colony from the streak plate. Inoculated cultures were allowed to develop in an incubator overnight at 37°C. After that, the *E. coli* OP50 solution could be used to seed NGM plates.

4.8.2.2. Preparation of NGM plates

C. elegans was cultivated and kept on nematode growth media (NGM) plates in the laboratory. The NGM plates were prepared with 3 g of NaCl, 17 g of agar, and 2.5 g of peptone mixed in the conical flask. Makeup with 975 ml of water and the mouth of the flask was covered with aluminium foil. This solution was autoclaved for 50 min. The media was allowed to cool to 55°C. After which it was supplemented with 1 ml of 1 molar calcium chloride, 5 mg/ml cholesterol in ethanol, 1 ml of 1 molar magnesium sulphate and 25 ml of 1 molar potassium phosphate buffer. Using sterile procedures, the NGM solution was dispensed into Petri plates using a peristaltic pump and the plates were filled with 2/3 full of agar. Aseptically, the prepared medium plates were poured into 35mm, 60mm, or 90mm plates, as required. The agar plates were left at room temperature for 2-3 days before use to allow for the detection of contaminants, and to allow excess moisture to evaporate. It was further stored in an air-tight container at room temperature and would be using up to 4 weeks.

4.8.2.3. Seeding NGM plates

Aseptically, 0.05 ml of *E. coli* OP50 liquid culture was applied to NGM plates using a micropipette and a drop was spread in the centre of the plate by a glass rod aseptically. Since the worm stays most of the time in the bacterial lawn, it was avoided to extend the lawn up to the edges of the plate. Spreading developed a larger *E. coli* OP50 lawn, which can aid in visualizing the worm. The *E. coli* OP50 lawn was let to develop at 37°C overnight. Before introducing the worms, the plates were brought to room temperature. Seeded plates that were kept in an airtight container for 2-3 weeks for further use.

4.8.3. Culturing of *C. elegans*

4.8.3.1. Passaging of *C. elegans* on Petri plates

C. elegans are transparent and can be seen through 10X eyepieces in a dissecting stereomicroscope with a transmitted light source. Worms were transferred to NGM plates using chunking methods, which involved moving a chunk of agar from an old plate to a new plate with a sterile knife or spatula. The agar chunk would generally contain hundreds of worms. The worms would crawl out of the piece and disseminate throughout the new plate of bacterial lawn. *C. elegans* stocks were occasionally contaminated with other bacteria, yeast, or mould. Moulds were eliminated by chunking and serially transferring them away from the pollutant, allowing the worms to crawl away. Bacterial contaminants and yeast were easily eliminated by using a hypochlorite solution, which killed the contaminants.

4.8.3.2. Age synchronisation of worms

This procedure was used to get synchronous LI larvae as well as to clean the plates of any bacterium, yeast, or mould contamination. Bleaching plates with a lot of gravid hermaphrodites were ideal. All the worms and eggs were collected in a falcon tube after plates were rinsed with sterile distilled water. The pellets were resuspended in double-distilled water after being spun at high speed for one minute. This procedure was repeated until all bacteria had been eliminated. A 1:1 mixture of 10N NaOH and 5% Sodium Hypochlorite was added to the resuspended particle. The tubes were gently inverted for 5-7 minutes to ensure that all of the worms were lysed. All pollutants, including worms, were eliminated in this stage, leaving only the embryos in solution. The supernatant was discarded after the tubes were spun. To remove the bleach from the solution, the embryos were repeatedly rinsed with buffer. Finally, the embryo pellet was suspended in a little amount of buffer and placed on a fresh NGM plate. The LI larvae were transferred to a new NGM plate the next day after the embryos had hatched.

4.8.4. Lifespan assay

The green synthesized nanoparticles were added to NGM agar plates during the preparatory process of the NGM plates. Different concentrations of MA-AgNPs were used such as 50 µg/ml, 150 µg/ml, 250 µg/ml, 350 µg/ml and 500 µg/ml. Any ionic silver dissolution from AgNPs would disrupt the bacterial lawn and cause secondary adverse effects in Caenorhabditis elegans, ranging from developmental abnormalities to starving death (Boyd *et al.,* 2003). The AgNPs with a Thiolate methoxy poly ethylene glycol (mPEG-SH) coating utilized had no effect on the food sources of *E. coli* and *C. elegans* (Contreras *et al.,* 2014). Around 30-40 worms or embryos were transplanted to the seeded NGM plates for the lifespan assays. When the worms reached adulthood, they were scored for their lifespan, and the experiment was repeated until all of the worms died. Every day, the worms were passaged to new NGM plates to avoid mixing with their offspring. The worms were relocated every two days once they stopped laying eggs. The L4 stage of worms was counted as day 0 for scoring the age of the worms. Percentage survival was calculated based on daily observations of the number of the nematodes that survived.

4.8.5. Stress assay

In stress resistance assay, Heat shock is given at 35°C for 3 hours daily up to 4 days, The MA-AgNPs were tested, showing a maximum increase in lifespan at different concentrations. In stress survival assays, the most effective concentrations were chosen for treatment. The effects of the selected concentration of MA-AgNPs on the survival of *C. elegans* under stress conditions were determined by means of day-to-day evaluation of the number of surviving animals.

RESULTS

5. RESULTS

5.1. Characterization of Bio synthesized silver nanoparticles

The bio synthesized silver nanoparticles of aqueous plant extract of *M. azedarach* were characterized based on their shape, size, and surface area following the standard techniques.

5.1.1. UV-Visible spectroscopy

The aqueous silver nitrate solution turned black within a few seconds and the concentration increased in direct proportion to the incubation period due to the reduction of silver ions. UV–Visible spectroscopy was used to measure the biosynthesis of silver nanoparticles. The absorption spectrum of fluorescent surface plasma resonance observed at 450 nm confirms the formation of AgNPs and thus increased over time through the incubation of silver nitrate with the *M. azedarach* leaf extract.

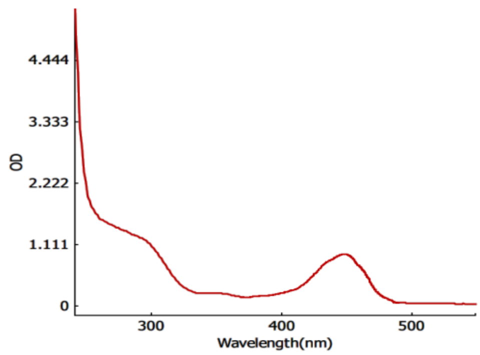


Figure: 1. UV–visible absorption spectrum of biosynthesized Ag nanoparticles.

5.1.2. X-ray diffraction (XRD) analysis

The samples of silver ions exposed to the extract were investigated by XRD to verify the results of the UV–vis spectral analysis. Silver particles formed in the present study were in the form of nanocrystals as evidenced by the peaks at 2θ values of 22.80°, 28.33°, 29.43°, 30.20°, 33.34°, 40.65°, 46.75°, 50.32°, and 55.62°. The reflection at 28.33, 33.34, 40.65, and 55.62 represents the planes of (101), (110), (200), and (211). The interplanar spacing values are 3.150, 2.686, 2.219 and 1.652 for (101), (110), (200), and (211) respectively (Labanni *et al.*, 2020). The sharpening of the peaks clarified the particles were in the nanoregions. The width of Bragg's reflection was used to calculate the mean size of AgNPs using the Debye– Scherrer's equation. For AgNPs generated at normal temperature, the size of the nanoparticles was determined to be around 40nm.

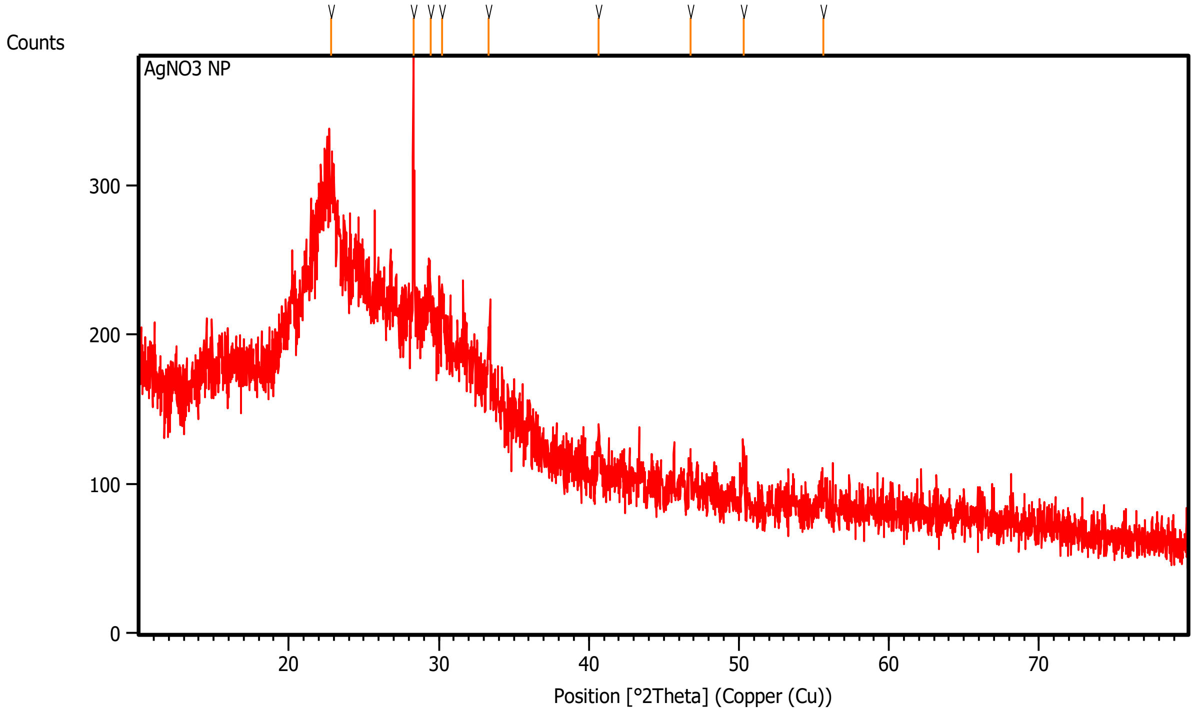


Figure: 2. X-ray diffraction pattern of synthesized silver nanoparticles.

5.1.3. Fourier transform infrared (FTIR) analysis

FTIR spectroscopic analysis was carried out to identify the biomolecules responsible for capping the bio-reduced MA-AgNPs synthesized using a bioactive compound of fluorescence. The significant peaks were identified such as alcohols and phenols, amides, nitro compounds, alkyls and aryl halides. The aqueous extract showed prominent peaks after the synthesis of Ag nanoparticles. The above bands were distinct and indicated the active compounds present in the produced AgNPs.

The intensive peaks at 3356.14, 1635.64, 1350.17, 686.66, 601.79, 563.21, 439.77 depict the interaction of Nanoparticles with biomolecules (*M. azedarach*). Strong absorption peaks at 3356.14 cm^{-1} result from stretching of the -OH bond of the hydroxyl group. The peak at 1635.64 cm^{-1} indicates the presence of C=O stretching vibrations for the carboxylic group. The peak at 1350.17 cm^{-1} was assigned to be the N-O bond of the nitro compounds. The absorption bands at 686.66 $cm^{-1}$and 563.21 cm^{-1} was assigned to be C-Br bond. 439.77 cm^{-1} and 601.79 cm-1 showed strong C-L (halo Compound) bond.

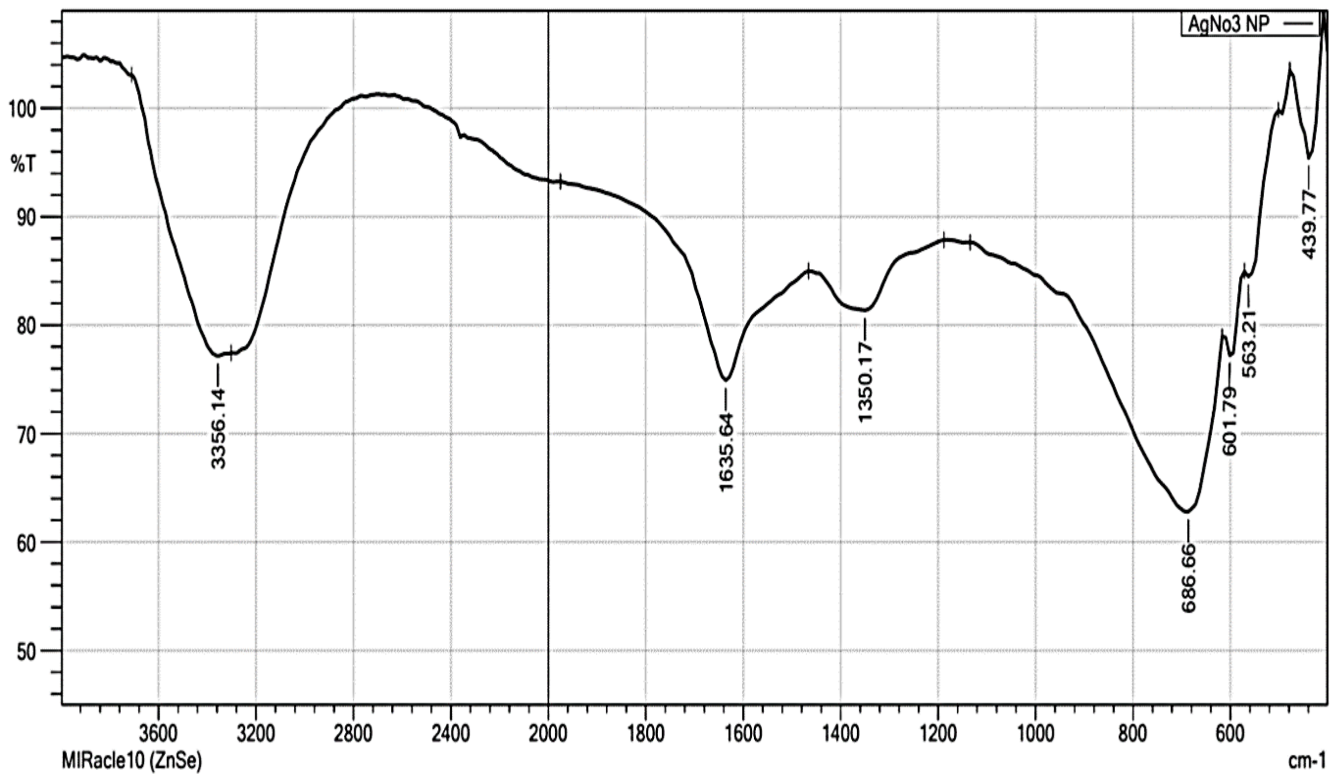


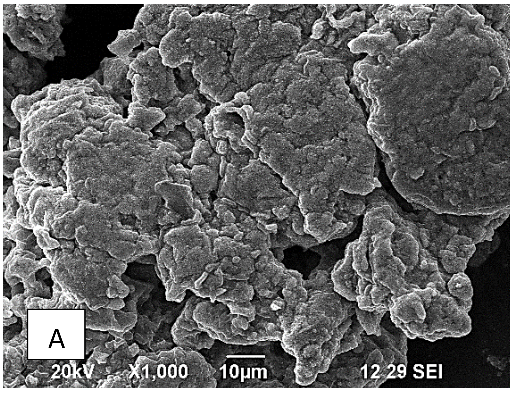
Figure: 3. FTIR spectra of synthesized Ag NPs

Table: 1. Functional group identified from green synthesized Ag NP by FTIR analysis

Absorption peaks (cm^{-1})	Vibration	Functional group
3356.14	-OH stretch	Alcohols and Phenols
1635.64	C=O stretch	Amides
1350.17	N-O stretch	Nitro compounds
686.66	C-Br stretch	Alkyl and Aryl halides
601.79	C-L stretch	Alkyl and Aryl halides
563.21	C-Br stretch	Alkyl and Aryl halides
439.77	C-L stretch	Alkyl and Aryl halides

5.1.4. Scanning Electron Microscopy and EDX analysis

The size and shape of synthesized AgNPs were examined using Scanning electron microscopy (SEM) analysis. SEM image analysis showed a clear shape and size of the nanoparticles of AgNPs for *M. azedarach.* The analysis of silver nanoparticles revealed well-crystallized particles with plate-like morphology. The particle sizes are slightly larger and particles were mostly found to be poly scattered spherical in shape and agglomerated.



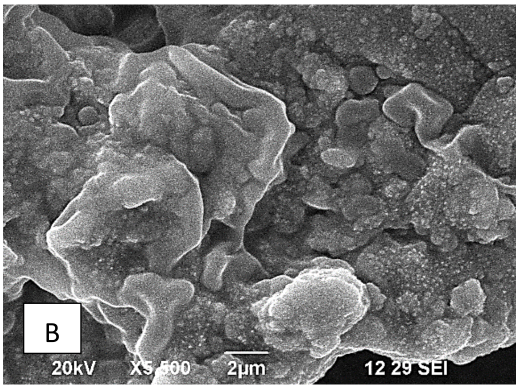


Figure: 4. SEM micrographs of synthesized *Melia azedarach* silver nanoparticles: (A) SEM image at 10µm, (B) SEM image at 2µm

According to the EDS examination, silver nanoparticles synthesized from *M. azedarach* extract had the largest weight percentage. From EDAX spectra analysis clearly show that the silver Nanoparticles reduced the weight in *M. azedarach* equivalent to that of silver (Figure 5). It is indicated that the synthesized Ag NPs were found to be pure and other were noticeable as purities.

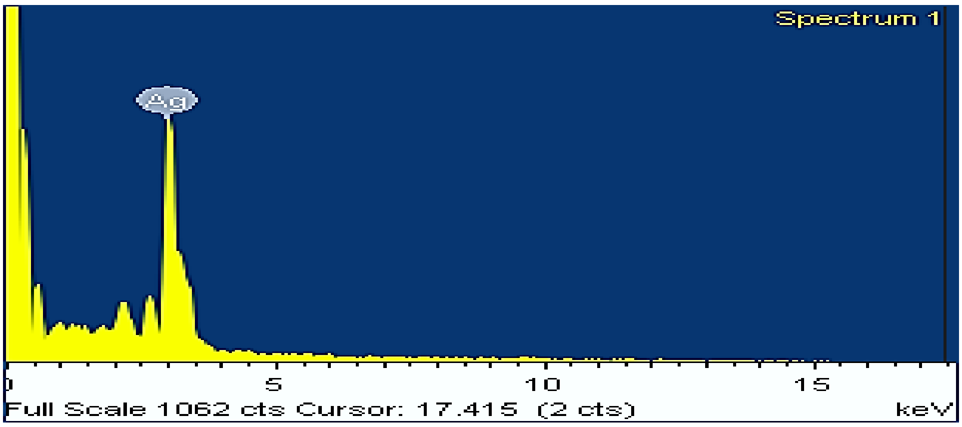


Figure: 5. EADX analysis of *Melia azedarach* mediated AgNPs

5.2. Phytochemical analysis

The qualitative phytochemical screening of MA-AgNPs extracts revealed the presence of bioactive compounds such as alkaloids, glycosides, proteins, and triterpenoids while carbohydrates, flavonoids, sterols, and tannins are absent.

Table: 2. Phytochemical analysis of AgNPs from *M. azedarach*

S.No	Phytochemicals	Name of the test	Result
1	Alkaloids	Mayer's test	+
		Hager's test	+
		Dragendroff's test	+
2	Carbohydrates	Benedict's test	-
		Fehling's test	-
3	Flavonoids	$FeCl_3$ test	-
		Alkaline reagent test	-
4	Glycosides	Keller Killani test	+
5	Proteins	Biuret test	+
		Warming test	+
6	Sterols	Salkowski's test	-
		Libermann-Buchard's test	-
7	Triterpenoids	Salkowski's test	+
		Libermann-Buchard's test	+
8	Tannins	$FeCl_3$ test	-
		Alkaline reagent test	-

5.3. Antioxidant activity

5.3.1. DPPH assay

The organic chemical complex 2, 2-diphenyl-1-picrylhydrazyl is a dark-colored crystalline powder composed of stable free-radical scavenging activity. The percentage inhibition (% inhibition) at MA-AgNPs as well as standard ascorbic acid were calculated. This assay is based on the scavenging capacity of antioxidants towards a stable free radical α, α-diphenyl- β- picrylhydrazyl (DPPH). The high percentage was recorded at the concentrations 50µl and 500µl compared to that of others with the absorbance value of ($Y = 0.263x + 58.203$), $R^2 = 0.9708$

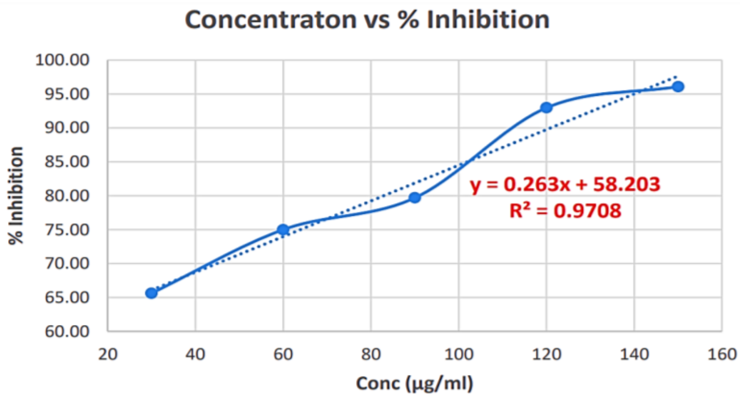


Figure: 6. Concentration vs Inhibition

Table: 3. Antioxidant activity of AgNPs from *Melia azedarach*

Samples	Concentration (µl)	% Inhibition
Standard (Ascorbic acid)	30	65.63
	60	75.00
	90	79.69
	120	92.97
	150	96.09
Sample	50	66.4
	150	67.1
	250	69.5
	350	73.4
	500	75.7

5.4. Effects of Melia azedarach synthesized silver nanoparticle on the lifespan of *C. elegans*

The green synthesized nanoparticles were added to NGM agar plates during the preparatory process of the NGM plates. Before nematodes were treated, bacterial lawns were dosed with freshly prepared synthesized silver nanoparticles to test particle stability. Different concentrations were used such as 50 µg/ml, 150 µg/ml, 250 µg/ml, 350 µg/ml and 500 µg/ml. Per experiment, a minimum of 100 worms were divided over the dishes and were observed to evaluate the effect of each concentration of MA-AgNPs on the survival of worms. Percentage survival was derived on the basis of day-to-day assessment of the number of surviving animals. Percentage survival of *C. elegans* culture has been depicted in table 4 to table 9

Table: 4. Survival of *C. elegans* culture in lifespan assay (control)

Days	No. of dead worms					% Growth inhibition						% Survival					
	Plate					Plate						Plate					
	1	2	3	4	T	1	2	3	4	M	SD	1	2	3	4	M	SD
0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	100	100	0
1	0	1	1	1	3	0	3	4	3	3	2	100	97	96	97	97	1.7
2	2	2	3	3	10	6	3	15	10	9	5	94	97	85	90	91	5.2
3	4	4	7	6	21	16	14	22	17	17	3	84	86	78	83	83	3.5
4	10	10	8	7	35	31	29	30	24	28	3	69	71	70	76	72	3.0
5	12	12	11	11	46	41	37	37	34	37	3	59	63	63	66	63	2.5
6	16	15	14	13	58	50	46	48	45	47	2	50	54	52	55	53	2.4
7	19	19	15	15	68	59	54	56	52	55	3	41	46	44	48	45	3.2

Table: 5. Survival of *C. elegans* culture treated with MA-AgNPs 50 µg/ml in lifespan assay

Days	No. of dead worms				% Growth inhibition					% Survival				
	Plate				Plate					Plate				
	1	2	3	T	1	2	3	M	SD	1	2	3	M	SD
0	0	0	0	0	0	0	0	0	0	100	100	100	100	0
1	0	0	0	0	0	0	0	0	0	100	100	100	100	0
2	2	1	2	5	6	3	6	5	2	94	97	94	95	2
3	4	2	4	10	14	9	15	12	3	84	89	83	85	3
4	6	6	6	18	18	17	23	19	3	82	83	77	81	3
5	8	8	9	25	24	27	29	27	2	76	73	71	73	2
6	11	10	12	33	33	33	38	33	3	67	66	60	64	3
7	14	12	14	40	39	43	45	43	3	61	57	55	57	3

Table: 6. Survival of *C. elegans* culture treated with MA-AgNPs 150 µg/ml in lifespan assay

Days	No. of dead worms				% Growth inhibition					% Survival				
	Plate				Plate					Plate				
	1	2	3	T	1	2	3	M	SD	1	2	3	M	SD
0	0	0	0	0	0	0	0	0	0	100	100	100	100	0
1	0	0	0	0	0	0	0	0	0	100	100	100	100	0
2	0	0	0	0	0	0	0	0	0	100	100	100	100	0
3	0	1	0	1	0	3.13	0	1.04	2	100	96.9	100	99	1.8
4	1	1	0	2	3.23	3.13	0	2.12	2	96.8	96.9	100	97.9	1.8
5	2	3	1	6	6.45	9.38	3.3	6.39	3	93.5	90.6	96.7	93.6	3
6	4	2	2	8	15.2	8	9	10.3	4	84.5	91	91.6	88.9	3.9
7	7	5	5	17	22.6	17	15.6	18.3	4	77.4	84.4	83.3	81.7	3.8

Table: 7. Survival of *C. elegans* culture treated with MA-AgNPs 250 µg/ml in lifespan assay

Days	No. of dead worms				% Growth inhibition					% Survival				
	Plate				Plate					Plate				
	1	2	3	T	1	2	3	M	SD	1	2	3	M	SD
0	0	0	0	0	0	0	0	0	0	100	100	100	100	0
1	0	0	0	0	0	0	0	0	0	100	100	100	100	0
2	0	0	0	0	0	0	0	0	0	100	100	100	100	0
3	0	1	2	3	3.03	0	6.7	3.23	3	97	100	93.3	96.8	3
4	2	2	4	8	6.06	7.14	13	8.85	4	93.9	92.9	86.7	91.2	4
5	5	3	5	13	12.1	10.7	17	13.2	3	87.9	89.3	83.3	86.8	3
6	7	6	4	17	21.2	20	14.3	18.5	4	78.8	85.7	80	81.5	4
7	9	6	8	23	27.3	21.4	27	25.1	3	72.7	78.6	73.3	74.9	3

Table: 8. Survival of *C. elegans* culture treated with MA-AgNPs 350 µg/ml in lifespan assay

Days	No. of dead worms				% Growth inhibition					% Survival				
	Plate				Plate					Plate				
	1	2	3	T	1	2	3	M	SD	1	2	3	M	SD
0	0	0	0	0	0	0	0	0	0	100	100	100	100	0
1	0	0	1	1	0	3.13	0	1.04	2	100	96.9	100	99	1.8
2	2	1	3	6	5.88	9.38	3.2	6.16	3	94.1	90.6	96.8	93.8	3.1
3	4	3	4	11	11.8	9.46	13	11.3	1.8	88.2	90	87.1	88.4	1.4
4	6	8	7	21	20	25	23	22.7	2	79.4	75	77.4	77.3	2.2
5	10	10	9	29	29.4	33.4	32	31.4	2	77.4	73	75.2	75.2	2.2
6	14	13	12	39	41.2	42.8	41	49.3	1	75.4	73	74	74.1	1.2
7	16	16	15	46	46.2	52.5	51	50	3	73.6	72	73.6	73	1

Table: 9. Survival of *C. elegans* culture treated with MA-AgNPs 500 µg/ml in lifespan assay

Days	No. of dead worms				% Growth inhibition					% Survival				
	Plate				Plate					Plate				
	1	2	3	T	1	2	3	M	SD	1	2	3	M	SD
0	0	0	0	0	0	0	0	0	0	100	100	100	100	0
1	0	0	0	0	0	0	0	0	0	100	100	100	100	0
2	0	1	0	1	0	3.13	0	1.04	2	100	96.9	100	99	1.8
3	1	2	2	5	3.23	6.25	7.1	5.54	2	96.8	93.8	92.9	94.5	2.1
4	4	3	4	11	12.5	9.68	14	12.2	2	87.5	90.3	85.7	87.8	2.3
5	4	5	5	14	12.9	15.6	18	15.5	2	87.1	84.4	82.1	84.5	2.5
6	7	8	8	23	22.6	25	29	25.4	3	77.4	75	71.4	74.6	3
7	10	9	10	29	31.3	29	36	32	3	68.8	71	64.3	68	3.4

This transfer day was recorded as Day 0 and continuously observed until day 7. MA-AgNPs cause a concentration-dependent increase in the lifespan of C. elegans. There was a highly substantial change in longevity was observed.

Table: 10. Prolongation of the mean life span of *C. elegans* by MA-AgNPs in life span assay

Mean life span of *C. elegans* in life span assay	
Treatment	**% Survival on the final day**
Control	45
50 µg/ml	57
150 µg/ml	81.7
250 µg/ml	74.9
350 µg/ml	70
500 µg/ml	68

In the study of lifespan assay, the *Melia azedarach* silver nanoparticle (MA-AgNPs) was able to increase the lifespan of the C. elegans worms. It is more efficient at 150 µg/ml concentration, increasing the mean lifespan of C. elegans (Table 10).

5.5. Heat stress-resistant assay

The effect of heat stress conditions on *C. elegans* survival in the presence and absence of MA-AgNPs was compared to survival values in stressed control cultures. Tables 11–14 show the effect of different MA-AgNPs concentrations on the survival of C. elegans under heat stress.

Table: 11. Survival of *C. elegans* culture in heat stress (control)

Days	No. of dead worms					% Growth inhibition						% Survival					
	Plate					Plate						Plate					
	1	2	3	4	T	1	2	3	4	M	SD	1	2	3	4	M	SD
0	0	0	1	0	1	0	0	3	0	1	2	100	100	97	100	99	2
1	3	4	6	4	17	11	11	19	12	13	4	89	89	81	88	87	4
2	7	10	10	7	34	26	29	31	21	27	4	74	71	69	79	73	4
3	12	15	14	12	53	44	43	44	36	42	4	56	57	56	64	58	4
4	17	21	19	17	74	63	60	59	52	58	5	37	40	41	48	42	5
5	22	26	24	23	95	81	74	75	70	75	5	19	26	25	30	25	5
6	26	32	31	29	118	96	91	97	88	93	4	4	9	3	12	7	4
7	27	35	32	33	147	100	100	100	100	100	0	0	0	0	0	0	0

Table: 12. Survival of *C. elegans* culture treated with MA-AgNPs 350 μg/ml in heat stress

Days	No. of dead worms Plate 1	2	3	T	% Growth inhibition Plate 1	2	3	M	SD	% Survival Plate 1	2	3	M	SD
0	0	0	0	0	0	0	0	0	0	100	100	100	100	0
1	2	1	1	4	5	3	3	4	1	95	97	97	96	1
2	7	5	4	16	18	14	11	14	4	82	86	89	86	4
3	12	9	10	31	32	24	29	28	4	68	76	71	72	4
4	17	14	16	47	45	38	46	43	4	55	62	54	57	4
5	21	20	20	61	54	55	57	55	2	45	46	43	45	2
6	25	25	23	73	66	68	66	66	1	34	32	34	34	1
7	30	27	31	88	79	77	84	80	3	21	23	60	20	3

Table: 13. Survival of *C. elegans* culture treated with MA-AgNPs 500 μg/ml in heat stress

Days	No. of dead worms Plate 1	2	3	T	% Growth inhibition Plate 1	2	3	M	SD	% Survival Plate 1	2	3	M	SD
0	0	0	0	0	0	0	0	0	0	100	100	100	100	0
1	2	1	2	5	6	3	5	4	2	94	97	95	96	2
2	5	6	5	16	14	16	13	14	2	86	84	88	86	2
3	8	10	10	28	23	26	25	25	2	77	74	75	75	2
4	13	16	14	43	37	42	35	38	4	63	58	65	62	4
5	18	21	18	57	51	55	45	51	5	49	45	55	49	5
6	23	24	23	70	66	63	58	62	4	34	37	43	38	4
7	26	27	26	79	74	71	65	70	5	26	29	35	30	5

Table: 14. Survival of *C. elegans* culture treated with MA-AgNPs 1 mg/ml in heat stress

Days	No. of dead worms				% Growth inhibition					% Survival				
	Plate				Plate					Plate				
	1	2	3	T	1	2	3	M	SD	1	2	3	M	SD
0	1	1	0	2	3	3	0	2	2	97	97	100	98	2
1	6	4	2	12	17	11	6	11	5	83	89	94	89	5
2	11	9	7	27	21	26	20	25	5	69	74	80	75	5
3	15	13	14	42	42	37	40	40	2	60	58	63	60	2
4	20	19	17	56	56	54	49	53	4	44	46	51	47	4
5	24	22	23	69	67	63	66	65	2	33	37	34	35	2
6	27	24	25	76	75	69	71	72	3	25	31	29	28	3
7	34	32	33	99	94	91	94	93	2	6	9	6	7	2

Table: 15. Mean value of MA-AgNPs on survival of *C. elegans* under heat stress

Mean value of *C. elegans* in Heat stress-resistant assay	
Treatment	**% Survival on the final day**
Control	0
350 μg/ml	20
500 μg/ml	30
1 mg/ml	7

In the study of Heat stress-resistant assay, the *Melia azedarach* silver nanoparticle (MA-AgNPs) was able to give the resistance against heat stress in C. elegans worms. It is more efficient at 500 μg/ml concentration, increasing the mean resistance of C. elegans (Table 15).

DISCUSSION

6. DISCUSSION

The current investigation was undertaken to study the lifespan prolonging effects of *Melia azedarach* mediated silver nanoparticles (MA-AgNPs). Silver nanoparticles synthesized from *M. azedarach* seed extract were selected for the study. *Caenorhabditis elegans* was selected as a model organism to study the effect of MA-AgNPs on aging. In recent years, developed countries have turned their attention to traditional medicine, which includes the use of herbal medications and cures. Around 1400 herbal remedies have been used in Belgium, Germany, France, and the Netherlands due to their popularity and importance in primary healthcare (Kamboj, 2000). Secondary metabolites are phytochemicals that are responsible for the majority of the plant's medicinal properties (Kumar *et al.,* 2017). These phytochemicals are non-nutritive compounds used to treat and cure a variety of human diseases. Alkaloids, Flavonoids, Saponins, Phenolic chemicals, Phytosterols, Proteins, Amino Acids, Gums, Mucilage, and Lignin are the key bio components (Mallikaharjuna *et al.,* 2011). Plant biochemical ingredients are the basis for the formation of numerous pharmaceutical industries and are crucial in the discovery of crude medicines. Melia azedarach is used as an antioxidative, analgesic, anti-inflammatory, insecticidal, rodenticidal, antidiarrhoeal, deobstruent, diuretic, antidiabetic, cathartic, emetic, antirheumatic, and antihypertensive in Ayurvedic medicine in India and Unani medicine in Arab nations (Sharma and Paul, 1930).

6.1. Nanomedicine and Bio nanoparticles

Nanotechnology in healthcare shows significant promise for transforming medical treatments and therapies in areas such as imaging, speedier diagnosis, medication delivery, tissue regeneration, and the development of new medical goods. Indeed, nanometric materials and devices are already approved for clinical use, and several products are being assessed in clinical studies (Zhang, 2008). In recent years, Nanoscience, nanobiotechnology, and nanomedicine have resulted in a number of inventions for life science applications. In the near future, the application of nanoscience in the field of medicine could transform the way disease is detected and treated, and several technologies that were only conceived a few years ago are making great progress toward becoming reality.

Nanoparticles have increasingly found practical applications in technology, science, and medicine in recent years. The microscopic particle size, combined with their distinct chemical and physical properties, is expected to highlight their potential biomedical applications. It can take the form of a latex body, polymer, ceramic particle, metal particle, or carbon particle (Germain *et al.,* 2020). Nanoparticles have the potential to transform medical imaging, diagnostics, and treatments, as well as carry out functional biological processes, due to their small size and physical similarities to physiological molecules such as proteins. The nanomedicine field is concretely capable of not only designing products that overcome critical barriers in conventional medicine in a novel way but also of delivering within cells new drug-free therapeutic effects by using pure physical modes of action and thus making a difference in patient lives (Germain *et al.,* 2020). A variety of nanoparticle classes seem promising for biomedical applications. Because such nanoparticles penetrate the body and come into direct touch with tissues and cells in biomedical applications, it is vital to investigate their biocompatibility.

6.2. Silver nanoparticles

Silver nanoparticles are widely beneficial applications to humans there is a need to develop quick and efficient testing methods for the synthesis of biocompatible silver nanoparticles. Different types of nanoparticles, such as Ag, Au, Pt, and Pd, have recently been created using chemical, physical, and biological processes. Photochemical methods (Callegari *et al.,* 2003) and biological procedures are among the various options available for the synthesis of silver Nanoparticles (Singaravelu, et, al., 2016). Chemical reduction, mechanical smashing, a solid-phase reaction, freeze-drying, spread drying, and precipitation are all examples of processes. The use of biological entities in the synthesis of silver nanoparticles is gaining popularity since biological approaches provide nontoxic and environmentally friendly "green chemistry" procedures. Green production of nanoparticles is a new research field these days. Silver nanoparticles are widely beneficial applications to humans there is a need to develop quick and efficient testing methods for the synthesis of biocompatible silver nanoparticles. Different types of nanoparticles, such as Ag, Au, Pt, and Pd, have recently been created using chemical, physical, and biological processes.

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AgNPs synthesized from plant extracts have been reported to have biomedical uses (Burdușel *et al.,* 2018). MA-AgNPs showed improved antibacterial potency in this study. Even at low concentrations, cereus and *E. coli* were inhibited by the leaf extract. This difference in antibacterial action was caused by the nanomaterial's lower particle size, which resulted in a greater surface area for the extract molecules. MA-AgNPs are smaller in size and also aided in their simple entry into the bacterial cell and had a bactericidal impact. MA-AgNPs treatment resulted in increased cell length and decreased cell breadth. Smaller NPs (50nm) have been found to have a significant role in bacterial cell contact and penetration, demonstrating stronger antibacterial properties than larger ones. (Gurunathan *et al.,* 2014).

6.3. Characterization of Green synthesized silver nanoparticles

The reduction of Ag NO_3 was visually evident from the colour change of the reaction mixture after the reaction. The intensity of the black colour increased in direct proportion to the length of the incubation period. It could be a result of the extraction of the Surface Plasmon Resonance (SPR) effect and the decrease of AgNO3. Visual observations revealed that the colour changes occurred when the bioactive compounds were incubated with Ag NO_3 solution. It is widely acknowledged that UV-VIS spectroscopy could be useful. The AgNO3 solution (without active chemicals) exhibited no colour change. UV-spectrum absorption with wavelengths ranging from 200 to 800 nm indicated peaks at 450 nm. This is similar to surface plasmon vibrations with silver particle characteristics peaks generated by chemical reduction.

The typical XRD pattern revealed that the material comprises mixed-phase (cubic and hexagonal) forms the silver nanoparticles. Metal nanoparticles generated in solution must be stabilized against Van der Waals forces of attraction, which could otherwise cause coagulation. Furthermore, numerous other XRD observations of silver nanoparticles were consistent with the current study (Raghunandan *et al.,* 2010). The current study of XRD data also reveals that bioorganic phase crystallization occurs on the surface of silver nanoparticles. FTIR has shown to be an effective method for characterizing and identifying chemicals (chemical bonds) or functional groups present in an unknown combination of plant extracts. The result suggests the attachment of some polyphenolic components onto AgNPs. Field emission scanning electron microscopy measurements of produced AgNPs reveal the images are polydispersed spherical in shape. The particle form of plant-mediated AgNPs is typically spherical, with the exception of neem (*A. indica*), which produces polydispersed particles with spherical and flat plate-like morphologies that range in size from 5 to 35 nm (Shankar *et al.,* 2004). The FTIR spectra of various functional groups of newly synthesized silver nanoparticles show that the Si-O group is attached to the surface of silver nanoparticles.

The chemical may interact with the silver surface via the (Si-O) group, resulting in very stable silver nanoparticles. Strong absorption peaks at 3356.14 cm-1 result from stretching of the hydroxyl group's -OH bond in the current investigation. The existence of C=O stretching vibrations for the carboxylic group is indicated by the peak at 1635.64 cm-1. The N-O bond of the nitro compounds was ascribed to the peak at 1350.17 cm-1. The absorption bands at 686.66 cm-1 and 563.21 cm-1 were ascribed to the C-Br bond, while the absorption bands at 439.77 cm-1 and 601.79 cm-1 showed a strong C-L (halo Compound) bond. *Calophyllum tomentosum* silver nanoparticles showed significant antioxidant activity (DPPH, H2O2 scavenging, nitric oxide scavenging power, and reducing power). In comparison to -amylase, the *Calophyllum tomentosum* silver nanoparticles strongly inhibited -glucosidase and DPPIV. Silver nanoparticles from *Calophyllum tomentosum* displayed potent anti-inflammatory action (albumin denaturation, membrane stability, heat hemolysis, protein inhibitory, lipoxygenase, xanthine oxidase) and tyrosinase inhibitory activity (Govindappa *et al.,* 2018).

6.4. Phytochemical screening

Phytochemicals are chemical substances found naturally in plants that have either beneficial or harmful health effects. The richest bio reservoirs of diverse phytochemicals are medicinal plants used to treat various diseases and disorders. The phytochemical elements of plants determine their therapeutic qualities. Nature is a unique supply of structures with significant phytochemical diversity, including phenolics (45%), terpenoids and steroids (27%), and alkaloids (18%) as key groupings of phytochemicals (Saxena *et al.,* 2013). Although these compounds appear to be non-essential to the plant that produces them, they serve an important role in survival by mediating ecological interactions with rivals, protecting them from illnesses, pollution, stress, UV radiation, and contributing to the plant's colour, scent, and flavour. Plant metabolites used to protect themselves from biotic and abiotic challenges have been transformed into medications that people can employ to treat a variety of ailments (Kocabas, 2017).

The presence of metabolites are namely carbohydrates and secondary metabolites such as alkaloids, flavonoids, glycosides, tannin, steroids and phenol. The phenolic compounds are one of the most numerous and widespread groups of plant metabolites (Singh *et al.,* 2007). Natural antioxidants are mostly found in plants in the form of phenolic chemicals such as flavonoids, phenolic acids, tocopherols, etc (Ali *et al.,* 2008). The phytochemical screening for Datura metal leaves crude extracts from both dry and fresh leaves was tested and showed positive results for alkaloid, flavonoid, saponin and tannin compounds (Alabri *et al.,* 2014). Timor Island garlic ethanolic extract included secondary metabolites including flavonoids, phenols, and terpenoids. It was also very effective at inhibiting free radicals, with IC50 values of 50 ppm, which equalled 9.729 ppm (Priska *et al.,* 2019). According to Mishra *et al.,* 2017, the ethanolic extract of *Leptadenia pyrotechnica* contains phytoconstituents such as alkaloids, phenols, terpenoids, flavonoids, glycosides, saponin, steroids, and tannins. Our results of the phytochemical screening showed the presence of alkaloids, glycosides, proteins and triterpenoids.

6.5. Antioxidant assay

The free radical theory of ageing posits that oxidative stress plays an essential driving role in ageing (Harman 1956). According to this theory, antioxidants should be effective anti-ageing agents. Despite more than 50 years of research, experimental evidence that antioxidants do really protect against ageing is, at best, ambiguous (Gruber et al. 2008) The most abundant source of antioxidants is found in plants, and they protect the human body from injury caused by free radicals induced by oxidative pressure (Phaniendra *et al.,* 2015). In plants and animals, these free radicals are deactivated by cell reinforcements. Those cell reinforcements act as an oxidation cycle inhibitor, even at low concentrations, and so play a variety of physiological roles in the body. In general, up to 5% of inhaled oxygen may be converted to Reactive Oxygen Species (ROS). These ROS have been implicated in a variety of obsessive cycles in humans, including maturation, aggravation, reoxygenation of ischemic tissues, atherosclerosis, malignant growth, and, interestingly, Parkinson's infection (Setiadi *et al.,* 2016). The percentage inhibition of DPPH, ABTS, Hydroxyl, and Nitric Oxide antioxidant activity at methanolic extract and standard ascorbic acid were determined and displayed. Mondal *et al.,* 2019 discovered that the maximum capabilities in DPPH were found in methanolic extracts of *Colocasia affins* leaves.

The reactive oxygen species (ROS) level and antioxidant enzyme activity revealed that polyphenols were the predominant component. In vitro, olive leaves could effectively scavenge DPPH and superoxide anion radicals. The activity of interior antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX) were increased, which may have been related to the induced-turned-nuclear DAF-16 transcription factor. As a result, EOL demonstrated high antioxidant activity both in vitro and in vivo (Luo *et al.,* 2019). According to Mohamed *et al.,* 2019, the methanol extract of Loranthus europaeus stalks with the best capabilities in FRAP, DPPH, and phosphomolybdenum assays. It was also stated that Plinia peruviana leaf extract was tested against the MCF-7 and Hela cell lines. Hasmila *et al.,* 2019 revealed that the DPPH method was used to assess the antioxidant activity of a methanol extract of Moringa and Celery leaves.

6.6. Heat stress abilities in *C. elegans*

Animals are constantly subjected to shifting environmental conditions. *C. elegans* are transitory natural habitat that fluctuates fast, requiring worms at various stages of development to sense and respond to changes in pH, ultraviolet light, breathing gases, temperature, and a variety of other environmental factors. *C. elegans* has evolved a number of stress-protective strategies in response to this environmental stress. The ability of animals to detect and respond to high temperatures is critical for life. The researchers studied the miRNA response to heat stress in *Caenorhabditis elegans* and discovered that a specific group of miRNAs was thermoregulated. Using in-depth phenotypic investigations of miRNA deletion mutant strains, we discovered that certain miRNAs have multiple developmental and post-developmental survival and behavioural functions during heat stress. These discoveries add another degree of complexity to the regulation of stress signalling, allowing animals to respond robustly to changing environments (Nehammer, *et al.,* 2015). Tangeretin is a polymethoxylated flavonoid found naturally in citrus fruits that has a variety of pharmacological characteristics, including anti-inflammatory, antiproliferative, and neuroprotective qualities. *Caenorhabditis elegans* to perform a lifetime test, detect aging-related functional changes in nematodes, the fluorescence changes of stress-related proteins (DAF-16 and HSP-16.2) and their response to stress assays, and monitor the influence of tangeretin on mRNA expression levels. According to the current findings, tangeretin can dramatically lengthen lifespan and improve heat stress tolerance in an insulin/insulin-like growth factor signalling dependent manner (Liu *et al.,* 2022).

6.7. Aging studies

Aging is a universal and basic phenomenon that affects all living organisms in the world. It is the progressive accumulation of changes in the body over time that causes the organism to lose vitality and function. Despite being a natural and unavoidable process, biologists have yet to unravel the process of aging and lifetime determination. The extracts of specific medications were able to improve the average lifetime of *C. elegans*. However, higher concentrations had either no effect or even reduced the mean lifespan of the worms. Furthermore, when worms were cultured in the presence of MA-AgNPs, their stress resistance increased.

Caenorhabditis elegans is a useful system in ageing and behavioural investigations, and it may be utilised at both the molecular and organismal levels to evaluate novel therapies for age-related neurodegeneration due to its short life span, relative simplicity, and high degree of experimental tractability, as well as significant conservation of disease genes and signalling pathways with humans (wang *et al.,* 2014). In recent studies, it has been demonstrated that other extracts or isolated compounds from plants like phytochemical compounds in *Alpinia zerumbet* have beneficial effects on the lifespan of *C. elegans* and were also able to increase the normal lifespan in *Caenorhabditis elegans* (Upadhyay *et al.,* 2013). *Anacardium occidentale* extracts exerted both oxidative stress resistance and anti-aging properties in the *Caenorhabditis elegans* model (Duangjan *et al.,* 2019). The leaf extract of *Caesalpinia mimosoides* improved resistance to oxidative stress and reduced intracellular ROS accumulation in *C. elegans* (Rangsinth *et al.,* 2019). In this regard, there is a huge interest in researching chemicals that may slow the aging process, for which *C. elegans* is an excellent model. *Melia azedarach* seeds appear to be an additional group of plant extracts and components deserving of future investigation. Kim *et al.,* 2008 reported that the Platinum nanoparticles prolonged the lifespan of *Caenorhabditis elegans* regardless of thermotolerance or nutritional constraints. Platinum nanoparticles act as an excellent antioxidant in Caenorhabditis elegans, causing a lifespan extension and strong resistance to excessive oxidative stress (Kim *et al.,* 2010).

The anti-oxidation properties of tea water extract at low concentrations differed among four different brands of green tea. The fundamental mechanisms were investigated further using genetically modified mutant worms. Green tea water extract's anti-oxidative stress actions are dependent on dietary restriction and germline signaling pathways, but not on FOXO and mitochondrial respiratory chain signals. As a result, tea water extract has anti-aging, anti-AD, and anti-oxidant properties (Fei *et al.,* 2017).

6.8. MA-AgNPs resemble Hormetic components

Hormesis refers to the phenomena in which low-level stress creates a protective stress response. Low-level exposure to stress can extend the lifespan of worms and some other animals, suggesting that hormesis can delay aging (Rattan, S.I., 2004). Hormesis is thus a potential mode of action for medications that lengthen longevity. The discovery that medicine is harmful at large dosages but extends longevity at low doses may imply that the substance is slightly hazardous at low doses and operates through hormesis. However, all compounds are poisonous at sufficiently large doses, demonstrating that high dose toxicity is not particular proof of a hormesis mechanism of action.

Mild stress-induced hormesis is a hopeful strategy to enhance longevity and healthy ageing. This meta-analysis focused on the effect of hormesis on Caenorhabditis elegans to fully analyze the application of hormesis in ageing intervention. the molecular processes underlying these favourable effects of hormesis This meta-analysis provides significant support for hormesis' anti-aging activity in *Caenorhabditis elegans*, highlighting its lifespan-prolonging, health-span-improving, and resistance-increasing benefits. Given the high conservatism of dauer formation protein-16, hormesis offers the theoretical option of postponing intrinsic ageing in humans via exogenous intervention (sun *et al.,* 2020).

Plant extracts are inert, less poisonous, and stable in nature, combined with easy and efficient manufacturing techniques, making them a feasible alternative to physical and chemical approaches in nanoparticle synthesis (Roy *et al.,* 2019). Thus, traditional therapeutic qualities of Melia azedarach leaf extract were used in the current investigation. In comparison to the bulk of bigger molecules, nanoparticles have a smaller size and thus a higher surface area to volume ratio. This increases the chemical reactivity of nanoparticles. Our results showed the effective conversion of inert or less active Melia azedarach extract to highly active Melia azedarach silver nanoparticles (MA-AgNPs) with increased biological characteristics by bioreduction.

In the present study, low concentrations of MA-AgNPs caused an increase in stress resistance and mean lifespan of *Caenorhabditis elegans*, whereas at higher concentrations there was a decrease in the mean lifespan. Similar results have been described in the field of longevity hormesis (Mattson, 2008). Such an acting mechanism is consistent with current anti-aging techniques in the realm of hormesis. A range of mild stressful stimuli has been identified as attractive options for activating the adaptive response and thereby increasing cellular resistance and maintenance processes with the goal of designing anti-aging therapies (Rattan, 2008). It has been demonstrated that the genes involved in aging are conserved in humans and smaller animals such as worms, the findings of this study can be generalized to humans.

SUMMARY

7. SUMMARY

- The main findings of this current study on green synthesis of AgNPs using *Melia azedarach* and their main characterization of their structure such as size, elemental composition, presence of active functional groups in the silver nanoparticles and the various biological applications such as phytochemical, antioxidant assays and aging studies under laboratory conditions are summarised.
- Secondary metabolites such as alkaloids, glycosides, proteins, and triterpenoids were found in the phytochemical examination. Tannins, sterols, flavonoids, and carbohydrates are among the metabolites that were found to be lacking in this phytochemical analysis.
- The synthesis of silver nanoparticles using UV- Visible spectrometry revealed the absorbance peak at 450nm, which corresponds to silver nanoparticle absorbance. The presence of six primary components with corresponding peaks, as determined by FT-IR analysis, correlates to the O-H bond stretching of alcohol, phenols, alkyl halides, nitro compounds, and amides.
- Antioxidant activity of DPPH assay was assessed by organic chemical complex 2, 2-diphenyl-1-picrylhydrazyl is a dark-colored crystalline powder composed of stable free-radical scavenging activity. The maximum percentage was attained at the concentrations of 50µl and 500µl compared to that of others with the absorbance value of ($Y= 0.263x + 58.203$), $R^2= 0.9708$
- SEM analysis of silver nanoparticles revealed plate-like particles that were well-crystallized and according to the EDS, synthesized silver nanoparticles had the largest weight proportion of elemental silver, as well as other metals. It was concluded that the produced Ag NPs were pure, with additional contaminants present.
- In the analysis of the lifespan assay, the *Melia azedarach* silver nanoparticle was able to increase the lifespan of the *C. elegans* worms. It is more beneficial at a concentration of 150 g/ml, increasing the average lifetime of *C. elegans*. It was also shown to be resistant to heat stress in a study on a heat stress-resistant test. It is more effective at 500 g/ml concentration.

CONCLUSION

8. CONCLUSION

The present research study carried out the lifespan prolonging effects of *Melia azedarach* mediated silver nanoparticles (MA-AgNPs). Based on the findings of this investigation, it was concluded that Melia azedarach mediated silver nanoparticles (MA-AgNPs) boost *Caenorhabditis elegans* lifespan and resistance to many sorts of stressful circumstances. The findings support the anti-aging properties of Melia azedarach mediated silver nanoparticles MA-AgNPs. Thus, the presence of antioxidants during particle treatment normalizes enzymatic activity, reduces macromolecular damage, and has a negative influence on the growth, reproduction, behaviour, and lifespan of exposed organisms. Significant recovery in morphological and behavioural features of particle-exposed worms in the presence of antioxidants is functional confirmation of antioxidants in a protective role against particle effects. As a result, our findings support the protective effect of curcumin and ascorbic acid against nanoparticle toxicity, as well as the possibility of avoiding nanotoxicity by adding these antioxidants into daily diets. Because the aging mechanism is preserved in humans and smaller animals such as worms, the findings of this study can be applied to humans. Further research should be conducted to determine the mechanism of anti-aging action of *Melia azedarach* mediated silver nanoparticles and the molecules responsible for this action.

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APPENDIX

10. APPENDIX

भारतसरकार
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भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2022/Tech. /21

दिनांक/Date: 7th April 2022

पौधे प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen brought by you for authentication is identified as *Melia azedarach* L. - MELIACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

7/4/2022

डॉ. एम. यु. शरीफ़/DR. M. U. SHARIEF
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सेवा में / To

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