SYNTHESIS OF SILVER NANOPARTICLES USING HEMIGRAPHIS COLORATA

Dissertation submitted to

MAHATMA GANDHI UNIVERSITY KOTTAYAM

In partial fulfillment of the

requirements for the award of the

MASTER OF SCIENCE IN PHARMACEUTICAL CHEMISTRY

BY

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(Affiliated to Mahatma Gandhi University, Kottayam)

CERTIFICATE

This is to certify that the project report entitled "**SYNTHESIS OF SILVER NANOPARTICLES USING HEMIGRAPHIS COLORATA**" is an authentic record of the project work carried out by Ms. **SHERIN K. MATHEW** (Reg.no:220011013452) in partial fulfillment of the award of the Degree of Master Of Science in Pharmaceutical Chemistry at Bharata Mata College, Thrikkakara affiliated to Mahatma Gandhi University, Kottayam under my guidance and supervision.

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DECLARATION

I, SHERIN K.MATHEW do hereby declare that the project report entitled **"SYNTHESIS OF SILVER NANOPARTICLES USING HEMIGRAPHIS COLORATA"** is a bona-fide record of the work submitted to Mahatma Gandhi University in partial fulfillment of the requirement for the award of the degree of MSc in PHARMACEUTICAL CHEMISTRY carried out by me under the guidance of Dr. ANU GEORGE, Professor, Department of Chemistry, Bharata Mata College, Thrikkakara.

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ABSTRACT

Green synthesis is preferred over conventional techniques due to its environmental friendliness, low cost, and minimal side effects. Various plant parts like leaves, roots, fruits, and seeds can be utilized in biosynthesis due to their antioxidant content. In this work, silver nanoparticles were synthesized using Hemigraphis colorata leaf extract. This plant, known for its medicinal properties and phytochemical constituents, acts as a natural reducing agent for silver ions.

The bio-reduction of Ag^+ ions was periodically monitored by sampling aliquots of the aqueous component and measuring the UV-Vis spectra of the solution. The chemical composition and structural properties of the synthesized silver nanoparticles were confirmed using FT-IR spectroscopy.

CHAPTER I

INTRODUCTION

1.1 Nanotechnology

Nanotechnology is the scientific and engineering discipline focused on designing, manufacturing, and utilizing structures, devices, and systems by manipulating atoms and molecules at the nanoscale—typically 100 nanometers or smaller. In nanotechnology, particles are classified based on their size as fine particles (100-2500 nanometers) and ultrafine particles(1-100 nanometers), with nanoparticles falling within the ultrafine range. These particles exhibit size-dependent properties distinct from bulk materials and fine particles, making them crucial for advancements in optical, electronic, and biomedical applications. It involves synthesizing and characterizing materials at this tiny scale. Nanotechnology plays a crucial role in medicine, enabling early disease detection, precise diagnosis, and targeted drug therapies. Physicist Richard Feynman introduced the concept in his lecture "There's Plenty of Room at the Bottom," envisioning advancements in working on a minute scale. This field not only enhances the functionality of traditional materials but also contributes to reducing carbon dioxide emissions and energy consumption. Nanotechnology is rapidly expanding across various industries, enabling the transformation of larger materials into nanoparticles (NPs) with unique properties at atomic scales. NPs, ranging from 1 to 100 nm, exhibit distinctive characteristics such as small size, high surface area to volume ratio, significant carrier capacity, heightened reactivity, and adjustable surface properties. Overall, nanoparticles represent a promising frontier in scientific research due to their unique size-related properties and diverse material compositions, driving innovation across multiple fields.

1.2 Nanoparticles

Nanoparticles form the core of nanotechnology, a field that has garnered significant attention over time. Ranging in size from 1 to 100 nanometers, nanoparticles can be composed of biological materials, metals, carbon, or metal oxides. At the nanoscale, they exhibit unique physical, chemical, and biological properties compared to their larger counterparts, thanks to their higher surface area-to-volume ratio, enhanced reactivity, stability in chemical processes, and improved mechanical strength. These characteristics make nanoparticles versatile and valuable across various applications. Nanoparticles vary in shape, size, and structure, with surfaces that can be uniform or irregular, and compositions that may be crystalline or amorphous. They can exist as single crystals, aggregates, or agglomerates. Numerous synthesis methods have been developed or refined to enhance nanoparticle properties and reduce production costs, catering to specific needs in optics, mechanics, and chemistry. Whether nanoparticles exhibit size-dependent properties seen in fine particles can vary. Nanoparticles are scientific materials that have important applications in biotechnology and pharmacology. Nanoparticles bridge the gap between bulk materials and molecular structures, with their physical and chemical properties changing as size decreases towards the nanoscale, impacting applications in biotechnology and pharmacology. The BHproperties of a material change as its size reaches nanoscale and the percentage of surface atoms increases.

Nanoparticles, have wide-ranging applications across sectors like environment, agriculture, food, biotechnology, and medicine, have revolutionized various fields. They're employed for waste water treatment, environmental monitoring, as functional food additives, and as antimicrobial agents. The unique properties of nanoparticles, including their nature, biocompatibility, anti-inflammatory and antibacterial activities, efficient drug delivery, bioactivity, and tumor targeting, have spurred their adoption in biotechnological and applied microbiological applications. Nanoparticles, typically ranging from one to one hundred nanometers in diameter, exhibit size-dependent characteristics owing to their minuscule size and expansive surface area. As the size approaches the nano-scale, the crystalline particles' periodic boundary conditions are disrupted. This alteration results in significant deviations in the physical properties of nanoparticles compared to bulk materials, paving the way for a plethora of novel applications. Other changes that are dependent on the size of nanoparticles are superparamagnetism exhibited by magnetic materials, quantum confinement by semiconductor Q-particles, and surface plasmon resonance in some metal particles.

1.2.1 Classification Of Nanoparticles

Nanoparticles are typically classified based on several characteristics including dimension, size, shape, uniformity, composition, and agglomeration. Based on dimension:

- Zero-dimensional nanomaterial: All dimensions are measured in nanometers, such as nanodots.
- One-dimensional nanoparticles: Only one dimension is in the nanoscale, like nanowires and nanofibers.
- Two-dimensional nanoparticles: These include thin films and nanosheets.
- Three-dimensional nanoparticles: Represent materials in bulk

Additionally, nanomaterials can be classified based on their chemical nature

Inorganic: Inorganic nanoparticles are synthesized from materials devoid of carbon and are typically categorized into metal and metal oxide nanoparticles. A wide range of metallic elements spanning s-block, p-block, and transition metals are utilized for their synthesis. Metal nanoparticles have been employed since ancient times in colored windows and glass. They exhibit unique optoelectronic properties attributed to surface Plasmon resonance. Examples include Ag, Au, Cu, Al, Pt, Zn, among others. Metal oxide nanoparticles possess distinct chemical and physical properties due to their high-density edge surface sites and confined size. They find applications in fuel cells, piezoelectric devices, catalysis, and as pigments in paints (e.g., TiO_2), sunscreens, and cosmetics (e.g., TiO_2, ZnO). Examples include WO_x, SnO_2, Fe_2O_3, known for their photo-catalytic characteristics.

Organic: Organic nanoparticles are solid particles ranging in diameter from 10 nm to 1um, primarily composed of organic compounds such as lipids or polymers [18]. Examples include liposomes, dendrimers, carbon nanomaterials, and polymeric micelles. Dendrimers are highly branched, nano-sized polymeric molecules extensively utilized in pharmaceuticals and medical applications like gene delivery and boron neutron capture therapy. Liposomes are spherical particles composed of one or more phospholipids, exhibiting amphiphilic properties due to their structure, which makes them both hydrophilic and lipophilic. Phospholipids within liposomes have a distinctive property known as liquid crystalline transition temperature. Recently, liposomes have been employed to enrich dairy products with vitamins.

Carbon- based Nanoparticles : Carbon-based nanoparticles are currently highly popular in the fields of nanoscience, materials science, engineering, and technology. These nanostructures consist of various low-dimensional allotropes of carbon, such as fullerene, carbon nanotubes, and graphene. They exhibit unique hybridization properties and are sensitive to perturbations during synthesis, allowing for precise manipulation of their mechanical properties. Carbon nanostructures possess diverse characteristics including electrical, mechanical, and structural diversity, making them valuable in numerous biological applications such as drug delivery, tissue engineering, biosensing, imaging, diagnostics, and cancer treatment.

1.2.2 Silver Based Nanoparticles

Silver has been extensively utilized for over 5000 years due to its indigenous properties. Silver nanoparticles (AgNPs) are widely used in biotechnology for their antibacterial, antiviral, and antifungal properties. Silver nanoparticles (AgNPs) are valued for their efficacy in addressing multiple biomedical needs including antibacterial, antiviral, anticancer effects, bone healing, bone cement, dental applications, and wound healing. They have gained significant traction in the biomedical field in recent years due to these properties. Their antimicrobial properties make them suitable for a wide range of household products like textiles, food storage containers, home appliances, and medical devices [23]. Silver is known for its effective antimicrobial action with low toxicity [24]. Silver nanoparticles (AgNPs) are favored for their ability to combat bacteria without harming human cells. This phenomenon, where bacteria are either killed or their growth is inhibited while surrounding cells remain unaffected, is termed antibacterial activity. The demand for AgNPs is rapidly increasing across medical, pharmaceutical, healthcare, food, consumer goods, and cosmetics industries. In the medical industry, silver nanoparticles are extensively used in topical ointments to prevent infections in burns and wounds [25]. Due to their appealing physicochemical properties, silver nanoparticles play a crucial role in biology and medicine. Silver nanoparticles find applications in antibacterial treatments, household products, medical devices, food processing, wound dressings, diagnostics, orthopedics, and even as anticancer agents. These nanoparticles possess unique properties and can alter their physical, chemical, and biological characteristics, making them versatile for diverse applications. Multiple synthesis methods are employed to meet the growing demand for AgNPs. Various materials such as leaf extract, bark, root, stem, leaf, fungi, etc., are utilized in the synthesis of nanoparticles.

1.2.2.1 Silver Nanoparticles Using Leaf Extract

Nanoparticles can be synthesized using various methods: chemical, physical, and biology. Chemical synthesis, while efficient for producing large quantities quickly, requires capping agents to stabilize nanoparticle size. However, the chemicals involved are often toxic and produce non-eco-friendly byproducts. The demand for environmentally friendly nanoparticle synthesis has spurred interest in biological approaches, which avoid toxic chemicals and their byproducts. This shift towards "green nanotechnology" highlights the use of biological entities such as bacteria [6], fungi [7, 8], and plants for both extracellular and intracellular nanoparticle synthesis.

Plants offer an advantageous platform for nanoparticle synthesis due to their lack of toxic chemicals and natural provision of capping agents. Additionally, using plant extracts reduces costs associated with isolating microorganisms and preparing culture media, thereby enhancing the cost competitiveness of nanoparticle synthesis compared to methods involving microorganisms. Nanobiotechnology is the most active area of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution, and morphology.

Green synthesis is superior to conventional techniques due to its environmental friendliness, cost-effectiveness, and minimal side effects. Various parts of plants such as leaves, roots, fruits, and seeds contain antioxidants, making them suitable for biosynthesized nanoparticles. Silver nanoparticles can be easily synthesized using leaf extracts and detected using UV-Vis spectroscopy. The reduction of $Ag+$ ions in solution can be monitored by sampling aliquots and analyzing their UV-Vis spectra over time. Plant-based nanoparticle production is eco-friendly and falls under green synthesis practices, contributing to lower production costs. These nanoparticles can be synthesized on a large scale without the need for energy, specific temperatures, or toxic chemicals, eliminating the requirement for elaborate isolation techniques. Numerous studies have reported the successful synthesis of silver nanoparticles using various leaf extracts.

1. Leaf Extracts Of Clitoria ternatea and Solanum nigrum

Fresh leaves of two different plants, Clitoria ternatea and Solanum nigrum, were collected from Madurai, ensured to be disease-free, and washed thoroughly with tap water followed by sterile water. Each sample, weighing 20 g, was finely chopped and added to 100 mL of distilled water, then stirred at 60°C for 1 hour. After boiling, the mixture was allowed to cool and filtered using Whatman paper number 1 to obtain the filtrate. For the synthesis of silver nanoparticles, a 0.1M aqueous solution of silver nitrate ($AgNO3$) was prepared. Subsequently, 5 mL of the leaf extract from Clitoria ternatea and Solanum nigrum was separately added to 45 mL of the 0.1 M $AgNO_3$ solution at room temperature for the bioreduction process.

2. Leaf Extract of Green Tea

To synthesize green tea (GT) silver nanoparticles (AgNPs), various concentrations of aqueous GT extracts were tested to determine the optimal stability and yield. The chosen concentration was mixed dropwise with 10 ml of 0.01 M silver nitrate solution, followed by the addition of 2.5 ml of 0.1 M NaOH. The resulting mixture was heated at 50°C for 30 minutes to enhance AgNP yield. Afterward, the GT AgNPs were separated via centrifugation at 6000 rpm, washed with 50% acetone, and dried at room temperature.

3. Leaf Extract of Cucumis prophetarum

The pale yellow color of the silver nitrate solution turned reddish-brown within 3 hours upon addition of the aqueous leaf extract of C. prophetarum, indicating the reduction of Ag^+ ions to Ag° nanoparticles). This transformation confirmed the biosynthesis of Cp-AgNPs. UV–vis spectra of both Cp-AgNPs and the leaf extract exhibited a prominent peak at 420 nm after the 3-hour incubation (Figure 1B). UV-visible spectroscopy is widely used for nanoparticle structural characterization. The presence of this absorbance peak at approximately 420 nm clearly signifies the formation of AgNPs, attributed to the surface plasmon resonance (SPR) phenomenon, where electrons on the nanoparticle surface oscillate collectively. The intensity of the SPR band increased over time, indicating progressive synthesis of AgNPs. The SPR pattern is influenced by factors such as nanoparticle size, shape, dielectric properties of the synthesis medium, and interactions between nanoparticles.

4. Leaf Extract Of Catharanthus Roseus Linn. G. Don

To prepare the broth solution from fresh C. roseus leaves, 10 g of thoroughly washed and finely cut leaves were placed in a 300 mL Erlenmeyer flask with 100 mL of sterilized double distilled water. The mixture was boiled for 5 minutes and then decanted. The resulting extract was filtered through Whatman filter paper no. 1 and stored at -15°C for up to 1 week. Next, the filtrate was combined with an aqueous 1 mM $AgNO_3$ solution in another Erlenmeyer flask and left at room temperature. This led to the formation of a brown-yellow solution, indicating the successful synthesis of silver nanoparticles. This demonstrates that the plant extract can effectively reduce aqueous silver ions, resulting in the production of highly stable silver nanoparticles in water.

5. Leaf Extract Of Azadirachta Indica

Silver nitrate was used without further modification. A 100 mL solution of 1 mM silver nitrate was prepared in an Erlenmeyer flask. Subsequently, 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL of plant extract were separately added to 10 mL portions of the 1 mM silver nitrate solution. The concentration of silver nitrate remained constant throughout these additions. Silver nanoparticles were also synthesized by varying the concentration of silver nitrate (ranging from 1 mM to 5 mM) while keeping the concentration of the plant extract constant at 1 mL. The reaction mixtures were incubated in a dark chamber at room temperature to prevent photo-activation of silver nitrate. The reduction of Ag^+ ions to Ag^0 was confirmed by observing a color change in the solution from colorless to brown. Additionally, confirmation of silver nanoparticle formation was carried out using UV-Visible spectroscopy.

6. Leaf Extract Of Artocarpus Heterophyllus

An aqueous solution of 0.05 M silver nitrate ($AgNO_3$) was prepared using double-distilled water for the synthesis of silver nanoparticles. Artocarpus heterophyllus leaf extract was added to the freshly prepared 0.05 M $AgNO_3$ solution in a conical flask. The mixture was heated at 50°C continuously for 15 minutes. Initially dark brown or black in color, the mixture gradually turned brown, indicating the formation of silver nanoparticles. After synthesis, the nanoparticles were separated by centrifugation at 10,000 rpm for 10 minutes to remove the supernatant. The collected nanoparticles, referred to as sample S-I, were stored in dark glass bottles with diethylene glycol to prevent nanoparticle coagulation. A similar procedure was followed to synthesize silver nanoparticles using Azadirachta indica plant extract, resulting in sample. For further analysis, the collected nanoparticles were dried on a watch glass.

7. Leaf Extract Of Uvaria Narum

Silver nanoparticles (AgNPs) were synthesized by heating a solution of.silver nitrate ($AgNO_3$) and leaf extract from U. narum in a household microwave oven. In a typical process, 10 mL of the leaf extract was mixed with 50 mL of 0.001 M silver nitrate solution, and the mixture was microwaved at 2.45 GHz and 350 W for approximately 5 minutes. The color of the solution changed from colorless to light yellow and eventually to dark brown, indicating the formation of AgNPs. The progress of the reaction was monitored by recording the UV-Vis absorption spectrum of the reaction mixture at regular intervals.

8. Leaf Extract Of Saraca Indica

10 ml of S. indica leaf extract was combined with 90 ml of 1 mM silver nitrate and heated on a hot plate at 60°C for 30 minutes. The initial light greenish-yellow color of the mixture turned dark brown, indicating the

formation of silver nanoparticles (Fig. 1). Reduction of Ag^+ ions was monitored by measuring UV–Vis spectra every 5 minutes within the range of 200–800 nm."

1.2.2.2 Application Of Silver Nanoparticles

Silver no are widely utilized for sterilization in consumer and medical products like textiles, grocery bags, refrigerator surfaces, and personal care items. Compared to their bulk counterparts, nanoparticles of precious metals such as silver exhibit distinct physical, chemical, and biological properties. Their size (typically below 100 nm) grants them unique attributes such as a large surface area, quantum effects, and enhanced reactivity.

The properties of silver nanoparticles, crucial for applications, are heavily influenced by factors like shape, size, and distribution, which can be controlled through various synthesis methods, reducing agents, and stabilizers. These nanoparticles are renowned for their antibacterial properties, making them effective in dentistry, surgery, wound care, and biomedical devices. Their ability to inhibit microbial growth stems from their high surface area-to-volume ratio, without compromising mechanical integrity.

In dentistry, the addition of silver nanoparticles to biomaterials depends on specific application requirements. Silver ions and compounds derived from silver are known for their toxicity to microorganisms. They can be integrated into a wide range of surfaces including metals, plastics, and glass, enhancing their antimicrobial efficacy. Medical devices coated with silver nanoparticles have demonstrated reduced bacterial colonization compared to conventional technologies.

Beyond antimicrobial uses, silver nanoparticles are increasingly explored in biomedicine for their physicochemical properties. Recent studies suggest promising anti-cancer effects against various types of cancers, both in vivo and in vitro, including cervical and breast cancers. Their ability to induce necrosis in cancer cells, due to their size and shape, makes them potential candidates for cancer therapy by inhibiting tumor cell proliferation and angiogenesis.

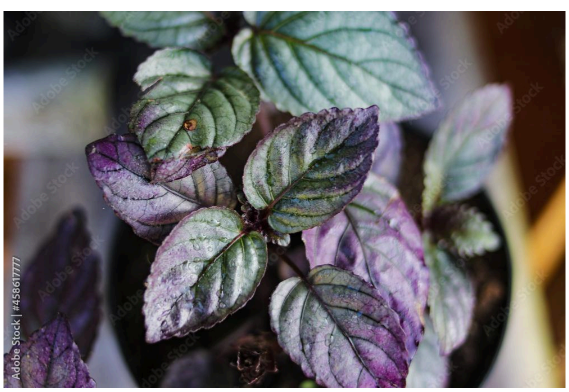
In addition to biomedical applications, silver nanoparticles find utility in bioimaging, vaccine development, wound healing, and as antidiabetic agents. They are also applied in diverse fields such as mosquito control, water treatment, environmental cleanup, food safety, agricultural practices, drug delivery systems, healthcare diagnostics, antiviral treatments, and biosensing technologies.

Overall, silver nanoparticles represent a versatile class of materials with significant potential across various industries, owing to their unique properties and wide-ranging applications.

1.3 Hemigraphis Colorata

The plant Hemigraphis colorata, commonly known as purple waffle plant, belongs to the Acanthaceae family. Hemigraphis colorata is a versatile plant suitable for both indoor and outdoor environments, prized for its vibrant foliage. When grown on the ground, it spreads through rooting stems, while in hanging baskets, it cascades elegantly. It's also popular for decorating aquariums and goldfish bowls, thriving particularly well in tropical climates. Known by various names such as Aluminium plant, Cemetery plant, Metal leaf, Red flame Ivy, Waffle plant, and Java Ivy, in Kerala it's referred to as 'murikootti' or 'murian pacha'. It grows prostrate with Its leaves are slender and lance-shaped, with toothed, scalloped, or lobed edges, colored grayish-green tinged with red-purple on top and darker purple underneath . Small white flowers appear sporadically throughout the year. It is a low-creeping perennial herb, reaching heights of 15 to 30 cm. Its leaves display a metallic purple sheen on the upper surface and are solid dark purple underneath. These leaves, opposite in arrangement, are ovate to cordate, serrate-crenate, typically measuring 2 to 8 cm in length and 4 to 6 cm in width, with well-defined veins. The plant blooms irregularly year-round in tropical regions. Its small flowers (1 to 1.5 cm in diameter) are bell-shaped with five lobes, adorned with faint purple markings on a white background, appearing in terminal spikes measuring 2 to 10 cm long. The capsules are small, slender, oval-linear, and light green, housing small, flat white seeds."

Plant Profile



Scientific Classification kingdom: Plantae

Order: Lamiales

Family: Acanthaceae

Genus:Hemigraphis

Species: colorata(27)

Traditionally, Hemigraphis colorata has been used:

- Externally for treating fresh wounds, cuts, ulcers, and inflammation.
- Internally for conditions such as anemia, hemorrhoids, and gallstones.
- It is also employed as a diuretic and in the management of diabetes mellitus.(28)

Pharmacology and Phytochemistry

"Hemigraphis is valued in traditional medicine for its therapeutic properties, known to treat conditions such as vitiated pitta, fresh wounds, cuts, ulcers, inflammations, and various skin complaints. It is also used to promote urination, stop dysentery, and treat venereal diseases. Additionally, it aids in healing hemorrhoids and is used internally for curing anemia. Traditionally, its leaves are used to address issues like gallstones and excessive menstruation and as contraceptive. In Vanuatu, the sap from leaf buds is consumed in water at dawn for four days as a contraceptive and to induce sterility [4]. In Java, the leaves are employed to manage conditions like bloody dysentery and piles, also possessing diuretic properties. In folk medicine, the leaf juice is applied directly to open wounds to stop bleeding [5, 6] and consumed internally to treat anemia.

Phytochemicals, such as phenolic compounds found in the benzene extract of H. colorata leaves, demonstrate activity against Acinetobacter species and Streptococcus aureus [7]. Phenolic acids like chlorogenate, cinnamate, coumarate, gallate, and ferulate act as pro-oxidants and exhibit free radical scavenging activity [8]. Steroids and coumarins in the extract contribute to its anti-diabetic effects [9]. The crude leaf paste accelerates healing in excision wounds [10, 11], showing faster wound contraction and epithelialization in mice, though oral administration is found ineffective [12].

Studies using excision and incision wound models reveal that the methanolic extract performs comparably to the standard reference Vokadine [13]. Additionally, a herbal scaffold made from chitosan is highly hemostatic and effective for treating infectious wounds."

Medicinal Uses

- **Phytoremediation/Indoor Air Purifying Plant:** Phytoremediation involves using plants to purify indoor air contaminated with volatile organic compounds (VOCs) such as benzene, xylene, hexane, heptane, octane, decane, trichloroethylene, and methylene chloride. Studies have demonstrated that Hoya colorata exhibits the highest removal rates of these VOCs through phytoremediation, contributing significantly to improving indoor air quality. (30)
- **Antibacterial activity**: During antibacterial testing, the benzene extract showed the largest area where bacterial growth was inhibited when tested against two pathogens: Acinobacter species and S. aureus. This effect was attributed to the presence of phenolic compounds in the extract, which are known for their antibacterial properties. In essence, the extract was effective in stopping the growth of these bacteria due to its phenolic content.(31)
- **Wound Healing properties:** In traditional medicine, extracts from Hemigraphis colorata leaves are topically applied to enhance wound healing. The plant is reputed for its anti-inflammatory and antimicrobial properties, which are thought to prevent infections and support the healing process. A study investigated the wound healing properties of a methanolic extract ointment derived from dried leaves of H. colorata in albino rats, utilizing excision and incision models. In the excision model, significant enhancement in wound closure compared to

the control was observed. In the incision model, treated wounds exhibited higher tensile strength. These findings suggest the potential of H. colorata for topical wound management. Additionally, crude leaf paste accelerated wound contraction and epithelialisation in mice. A chitosan-based herbal scaffold demonstrated strong hemostatic properties, suitable for treating infectious wounds. Comparative studies using excision and incision models indicated that the methanolic extract's efficacy is similar to the standard reference Vokadine.(32)

- **Anti-oxidant/Anti-inflammatory/Cytotoxicity:** "The antioxidant effect of the ethanolic extract from the entire plant is attributed to its glycosides. Phenolic compounds in the extract act as effective hydrogen donors, making them potent antioxidants. Specifically, phenolic acids like chlorogenate, cinnamate, coumarate, gallate, and ferulate can act as pro-oxidants while also exhibiting free radical scavenging properties. The presence of these phenolic compounds is primarily responsible for the antioxidant activity observed. Compared to chloroform and acetone extracts, ethanolic extracts from the leaves of H. colorata demonstrate higher levels of antioxidant and anti-inflammatory activities. Additionally, the plant shows cytotoxic effects against DLA cell lines at concentrations up to 200μg/mL in short-term bioassays." (17,18)
- **Anti-diabetic Activity**: Preliminary studies have indicated that Hemigraphis colorata extracts may have potential antidiabetic effects by helping to regulate blood sugar levels. This suggests that the plant could be explored further as a natural treatment for diabetes, although more research is necessary to confirm its effectiveness and safety". The n-hexane and ethanol extracts from the entire plant demonstrated a reduction in blood glucose levels in rats fed with glucose. This effect is

attributed to the presence of steroids and coumarins within the extract, which contribute to its hypoglycemic and anti-diabetic properties."(19)

- **Miscellaneous activity:** This plant is utilized in green technology for its ability to significantly reduce sediment transport, as evidenced by its minimal sediment value [18]. Moreover, H. colorata has been identified as an ornamental plant capable of effectively removing harmful volatile organic compounds found in indoor environments, which can otherwise cause various health issues when people are exposed to them.
- **Digestive health:** In traditional medicine systems, Hemigraphis colorata is sometimes used to improve digestion and alleviate gastrointestinal issues such as indigestion, bloating, and stomach discomfort. It is believed to have carminative properties that help soothe the digestive system.
- **Antiasthmatic properties:**In some traditional medicine practices, Hemigraphis colorata is used to alleviate symptoms of respiratory conditions such as asthma and bronchitis. It is believed to have bronchodilator properties that help relax the airways and improve breath.

1.4 Objective And Scope Of The Work

The primary objective is to synthesize silver nanoparticles using a green and eco-friendly method. Hemigraphis colorata, a plant with medicinal properties and phytochemical constituents, serves as a natural source for reducing silver ions to nanoparticles. Another goal is to characterize the synthesized silver nanoparticles using various analytical techniques such as UV-Vis spectroscopy and Fourier-transform infrared spectroscopy (FTIR). These analyses are crucial for understanding the chemical composition and structural properties of the nanoparticles. Additionally, the nanoparticles will be evaluated for their potential applications in wound healing and antibacterial activity. These assessments aim to determine their effectiveness in biomedical applications.

CHAPTER II

EXPERIMENTAL SECTION

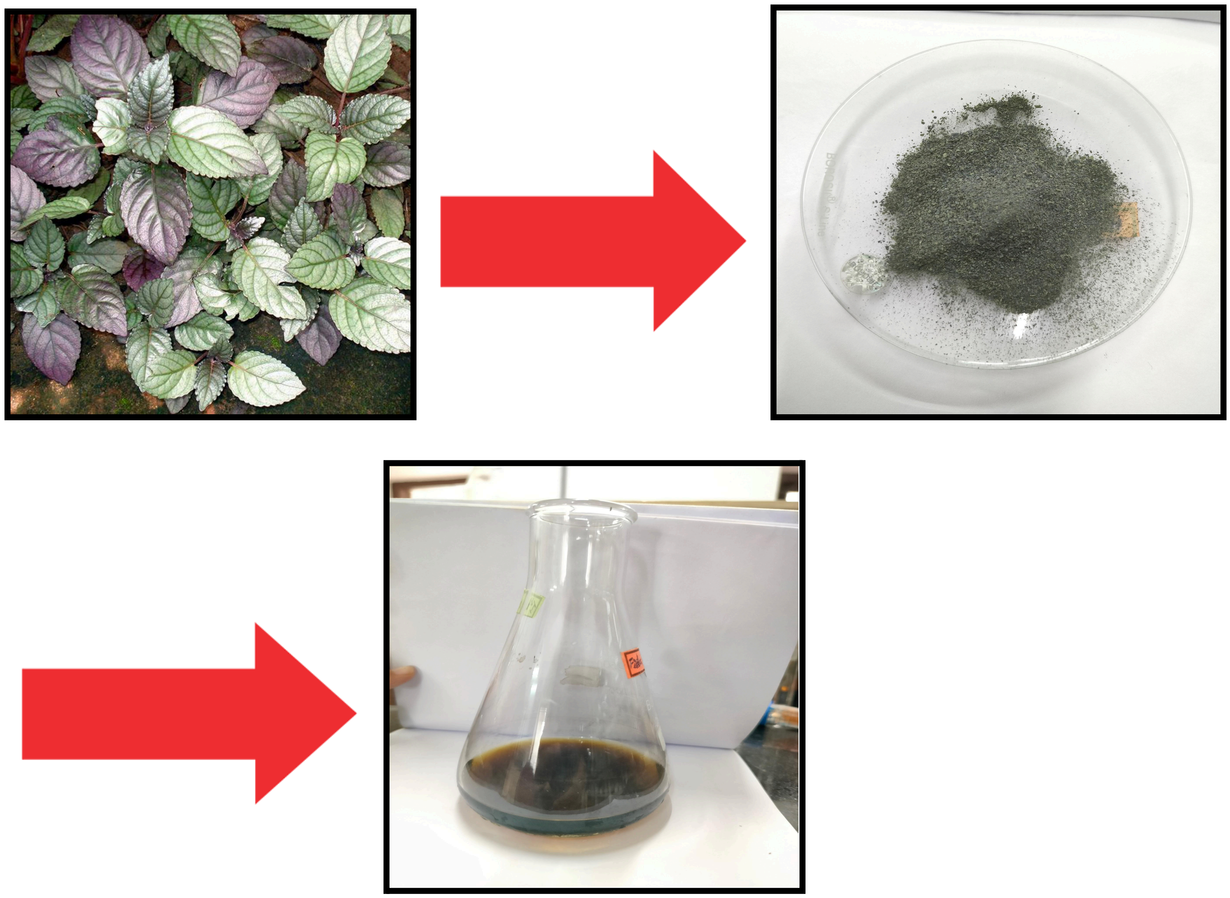
2.1 Materials

- Leaf extract of plant Hemigraphis colorata
- Silver Nitrate
- Distilled Water
- Methanol

2.2 Synthesis of Silver nanoparticle using Hemigraphis Colorata

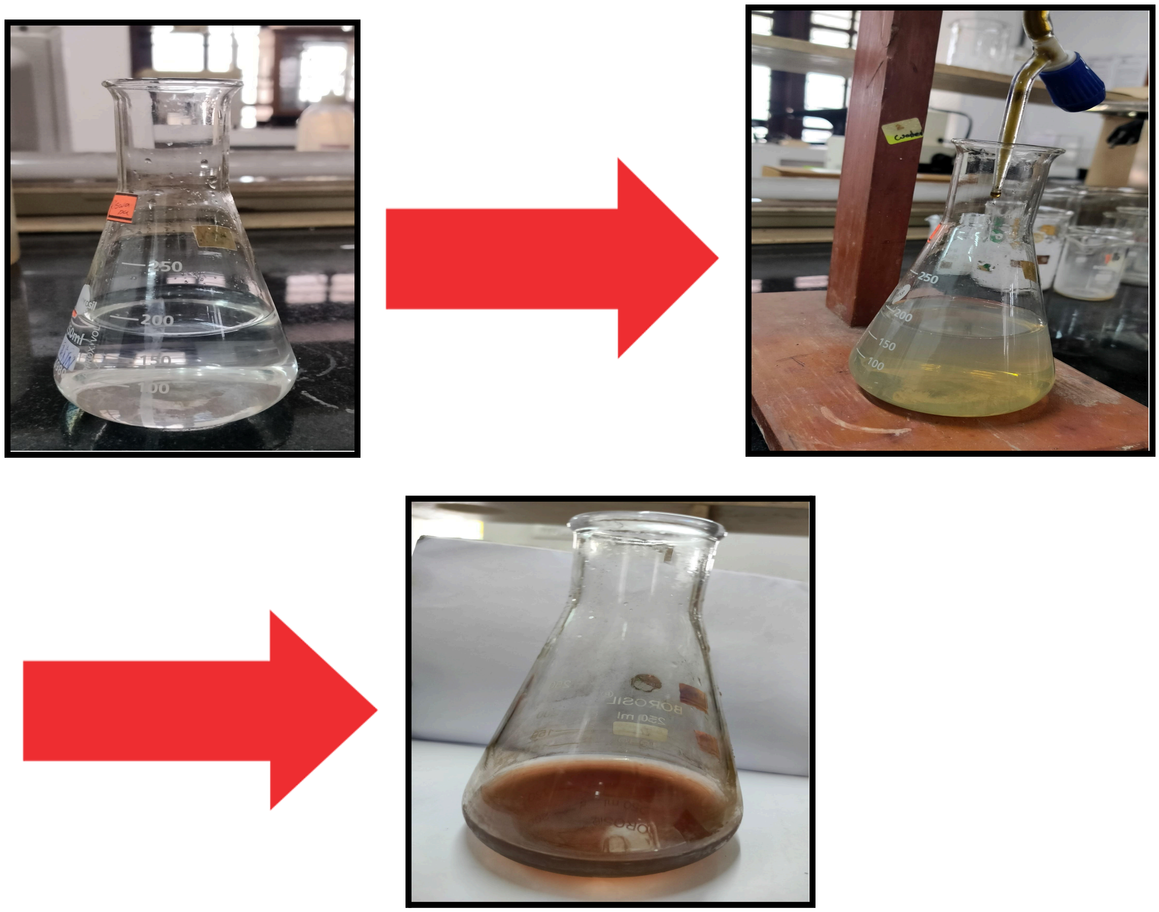
2.2.1 Preparation Of of Leaf Extract

Fresh leaves of Hemigraphis colorata (Blume) were collected from Kunamavu and Angamaly in Ernakulam district, Kerala. The leaves were washed thoroughly with water and then dried in a hot air oven at 50°C for 48 hours. Once dried, the leaves were ground into a fine powder using a grinder. For extraction, 2 grams of the powdered dried leaves were weighed and added to a 250 mL Erlenmeyer flask containing 100 mL of water. The mixture was stirred at 80°C for 45 minutes using a magnetic stirrer. After extraction, the solution was filtered using Whatman No. 40 filter paper, and the filtered supernatant was collected and stored at 4°C.



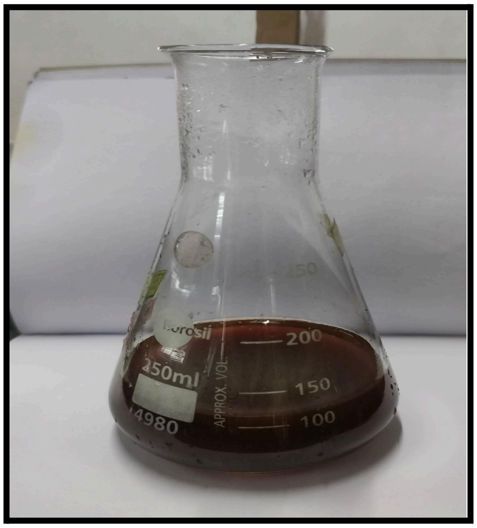
2.2.2 synthesis Of Silver Nanoparticles

In the green synthesis of silver nanoparticles (AgNPs), 1mM silver nitrate ($AgNO_3$, 99.99%) was prepared by dissolving 0.0169g in 100ml of distilled water. 10 mL of Hemigraphis colorata leaf extract was slowly added to 90 mL of a 1mM aqueous solution of silver nitrate and allowed at ambient conditions to react. A visible color change occurred within two minutes, turning the solution from yellowish-brown to reddish-brown, indicating the formation of silver nanoparticles. This color change confirmed that the plant extract effectively reduced silver ions, resulting in stable silver nanoparticles.



2.2.3 Purification of Silver Nanoparticles

The solution was then subjected to separation and concentration processes, involving magnetic stirring for 4 hours. Then the solution containing silver nanoparticles is isolated and precipitated using methanol. After the addition of methanol the solution is refrigeration at 10°C for 24 hours. The nanoparticles are then purified through multiple cycles of washing and centrifugation. High-speed centrifugation (12,000 rpm) is employed for 10 minutes to efficiently separate the nanoparticles from the solution, resulting in their concentration and facilitating their collection for subsequent analysis and experimental applications. This eco-friendly method demonstrates the potential of plant extracts for reducing silver ions and synthesizing nanoparticles. The synthesized AgNPs were confirmed through UV-Vis spectroscopy at a range of 350-500 nm.

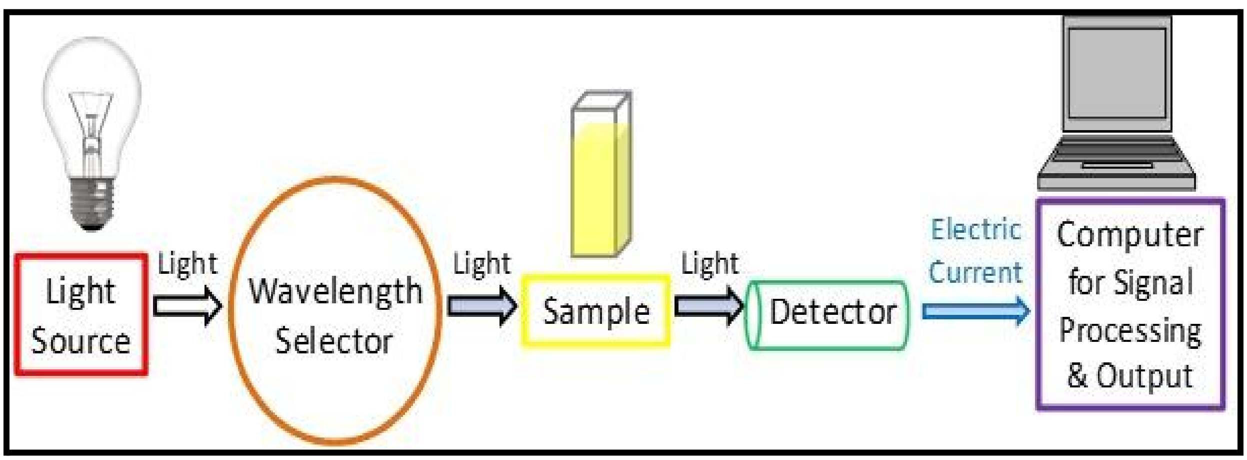


CHAPTER III

CHARACTERIZATION TECHNIQUES

3.1 UV-VISIBLE SPECTROSCOPY

Ultraviolet-visible (UV-Vis) spectroscopy is a highly popular and versatile analytical technique used to detect nearly every molecule. In this method, UV-Vis light is passed through a sample, and the transmittance of light by the sample is measured. This technique helps in understanding the absorption characteristics of the sample, providing valuable insights into its chemical composition and concentration. UV-visible spectroscopy operates on the principle that molecules absorb light in the ultraviolet and visible regions of the electromagnetic spectrum. This absorption occurs due to electronic transitions within the molecules.



Principle

Electronic transition :UV-visible spectroscopy focuses on the electronic transitions within molecules. When a molecule absorbs UV or visible light, it causes electrons to move from their ground state (lower energy level) to an excited state (higher energy level). The energy required for this transition corresponds to the energy difference between these states and typically falls within the UV-visible range of the electromagnetic spectrum. This absorption of light at specific wavelengths allows UV- visible spectroscopy to provide insights into the electronic structure of molecules, aiding in the identification of compounds, determination of concentrations, and understanding of chemical environments.

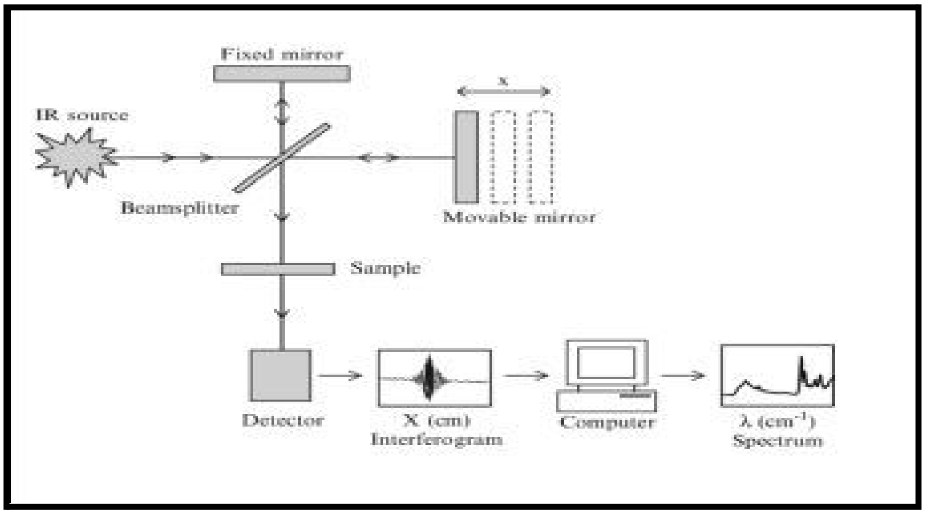
Absorption spectra: Absorption spectra are generated when a molecule absorbs light, producing a plot known as an absorption spectrum. This spectrum depicts absorbance (A) against either wavelength or frequency (ν). It serves as a comprehensive tool for identifying the wavelengths of light that a molecule strongly absorbs and quantifying the absorption intensity at each wavelength. Thus, absorption spectra offer crucial insights into a molecule's light absorption characteristics, aiding in detailed analysis of its composition and behavior in various environments.

Beer-Lambert Law

The Beer-Lambert Law states that the absorbance (A) of a solution is directly proportional to the concentration (c) of the absorbing substance and the path length (l) of the sample cell. The relationship is given by the equation: $A=\varepsilon cl$, ε is the molar absorption coefficient (also known as molar absorptivity or extinction coefficient).

3.2 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a highly effective technique in the realm of infrared spectroscopy. It excels due to its sensitivity, speed, and cost- effectiveness, particularly in the study of polymers. FTIR measures both the intensity and wavelength of infrared radiation absorbed by a sample. In modern times, FTIR has become increasingly essential for analyzing and monitoring the chemical structure and functional groups present in lignocellulosic compounds with great depth and precision.



Principle

Infrared spectroscopy operates on the interaction of infrared radiation with molecules. Infrared radiation comprises electromagnetic waves with frequencies ranging from 4000 to 400 cm^{-1}, corresponding to wavelengths between 2.5 and 25 micrometers. When infrared radiation interacts with a sample, it induces changes in the vibrational and rotational energy levels of the molecules within that sample. The spectrum fingerprints that each molecule or chemical structure produces are unique, making FTIR (Fourier-transform infrared spectroscopy) an excellent tool for chemical identification. With FTIR analysis, one can: Determine the identity and properties of unknown materials, such as liquids, solids, films, or powders. Identify whether a substance is contaminated (e.g., by particles, fibers, powders, or liquids). Identify additives after they have been extracted from a polymer matrix. Determine the presence of oxidation, breakdown products, or uncured monomers in failure analysis inquiries. Vibrational Modes: Molecules exhibit various vibrational motions, such as stretching, bending, and twisting of chemical bonds. Each type of vibrational motion corresponds to specific energy levels, which align with distinct frequencies of infrared (IR) radiation. Functional groups and chemical bonds within molecules absorb IR radiation at characteristic frequencies, facilitating their identification within a sample.This specificity in absorption frequencies forms the basis for using IR spectroscopy to analyze and characterize molecular structures in diverse materials.

Absorption Spectra: When a sample interacts with infrared (IR) radiation, it produces an absorption spectrum. This spectrum is a plot of absorbance (or transmittance) against wavenumber (the inverse of wavelength, expressed in cm^{-1}). It illustrates the frequencies of IR radiation absorbed by the sample, revealing the presence of distinctive functional groups and chemical bonds within the molecule. This data is essential for identifying and characterizing the molecular composition of substances analyzed using infrared spectroscopy, providing valuable insights into their structure and properties.

Fourier Transform: In FTIR spectroscopy, the sample is exposed to a broad range of IR radiation, covering all relevant frequencies. The resulting absorption spectrum is then subjected to a mathematical technique called Fourier transform, which converts the time-domain signal (interferogram) into a frequency-domain spectrum. This process enhances the signal-to-noise ratio and improves the spectral resolution compared to conventional dispersive spectroscopy methods.

FTIR spectroscopy offers several advantages

- Broad range analysis: Simultaneously analyzes a wide range of frequencies.
- High sensitivity: Enhanced signal-to-noise ratio allows for the detection of low concentrations.
- Improved resolution: Provides high spectral resolution for detailed analysis.
- Rapid data collection: Faster data acquisition compared to dispersive methods.

Overall, FTIR spectroscopy is a powerful and versatile tool for identifying chemical structures, analyzing material compositions, and detecting contaminants or degradation products.

3.3 Particle size Analyzer

Particle size analysis is a technical procedure used to characterize the size distribution of particles in a powder or liquid sample. It is widely employed in research and development (R&D) as well as quality control across various industries, including:

- Nanotechnology
- Pharmaceuticals
- Cosmetics
- Food
- Electronic materials
- Sintering materials
- Li-ion battery electrodes

This analysis is crucial for understanding the properties and behavior of materials, ensuring product consistency, optimizing manufacturing processes, and improving the performance and quality of end products.

Principle

A Particle Size Analyzer is a widely used method for characterizing sediment particles based on the diffraction of a laser light source by the samples under analysis. The analyzer determines the size distribution of a powder, suspension, or emulsion based on light diffraction. When a particle is illuminated by a monochromatic light source (laser), a diffraction pattern, known as Airy's pattern, is produced at infinity. This pattern, which consists of concentric rings, provides the light scattering intensity (I) as a function of the diffraction angle. The distance between the rings is dependent on the particle size. Additionally, the analyzer can visualize the shape of particles in suspension.