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A Comparative Review on the Sustainable Phyto-Fabrication of Silver Nanoparticles using Azadirachta Indica, and Gmelina Arborea

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Abstract:
The rise in bacterial resistance to commonly prescribed antibiotics is a significant issue in public health, which has sparked a growing interest in exploring innovative antibacterial treatments. The primary objective of this scientific pursuit was to discover a potent antibacterial agent derived from unconventional plant sources, to address human health concerns. Azadirachta indica and Gmelina arborea were investigated as alternate sources of antibacterial activities; which were synthesized in silver nanoparticles. Azadirachta indica and Gmelina arborea are both native plants to India so they can be easily used for silver nanoparticle formulations. To identify phytochemicals, present in Azadirachta Indica GC-MS analysis was used and it demonstrated the existence of flavonoids, phenolic compounds, terpenoids, and terpenes. The aqueous extracts derived from the leaves were employed to synthesise silver nanoparticles (AI-AgNPs), which was confirmed through colourimetric analysis with a peak absorbance at 400 nm. By optimizing the reaction parameters, a substantial yield of stable AI-AgNPs was achieved. These nanoparticles were further characterized using UV-Vis spectroscopy, energy-dispersive X-ray spectroscopy, scanning electron microscopy, and transmission electron microscopy. An eco-friendly approach was employed to produce silver nanoparticles (AgNPs) using logging residue from Gmelina arborea (GA) timber trees. The bioactive compounds present in leaves, barks, flowers, fruits, and roots were determined through GC-MS analysis. The synthesis, morphology, and structure of GA-AgNPs were characterized using various techniques including UV-Vis spectroscopy, scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDX), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffractometer (XRD). The antibacterial, antibiofilm, antioxidant, and wound-healing properties of GA-AgNPs were evaluated, and toxicity studies were conducted. The analysis revealed the presence of terpenoids, sterols, aliphatic alcohols, aldehydes, and flavonoids in leaves, indicating that the leaf extract is an ideal choice for the photo-fabrication of silver nanoparticles. Both compounds underwent clinical testing using different methods, which resulted in the discovery of their improved antibacterial, antibiofilm, antioxidant, and wound healing properties. The studies also demonstrated a straightforward and environmentally friendly approach to synthesizing AgNPs, which exhibited enhanced antibacterial effects and the ability to scavenge free radicals. Additionally, AgNPs were found to be a safe and eco-friendly delivery vehicle.

Chapter-1
Introduction

1. INTRODUCTION

By 2050, the global burden will be surpassed by an unprecedented surge in antimicrobial resistance, resulting in an additional 10 million cases annually. Moreover, the economic impact of this crisis is projected to exceed 100 trillion USD in total output. These alarming statistics can be attributed to two primary factors. Firstly, the extensive use of antimicrobials in recent decades has significantly heightened the exposure of microbes to a greater number and concentration of these drugs, consequently elevating the likelihood of resistance development. Secondly, the scarcity of new drugs being developed to replace the ineffectiveness caused by escalating drug resistance, particularly in the realm of antibiotics, is a deeply concerning issue[1,2]. Leading to rise in worldwide need for biocompatible, environmentally friendly, affordable, and reliable natural resources has resulted in a quest to uncover fresh bioactive compounds that are inert, less harmful, and more enduring than the presently prevalent synthetic chemicals, to facilitate their efficient delivery to the intended location. Ancient medicinal practices like Ayurveda, Kampo, Unani, and Chinese medicine have long relied on plants for treating various ailments, and this traditional wisdom is now being harnessed in modern medicine to unearth novel pharmaceuticals. [3,4]

Nanoparticles [NPs] have gained significant attention in the scientific community due to their minute size [1–100 nm], which gives rise to unique physical and chemical characteristics not found in larger particles or fine particles. Consequently, these nanoparticles hold potential for innovative applications in various fields such as healthcare, food and feed, environmental care, cosmetics, optoelectronics, chemical, and biotechnology industries [5]. Metallic nanoparticles [MNPs] possess a wide range of physical and chemical characteristics that have found utility in numerous biomedical fields. The growing need for MNPs has prompted scientists to create efficient, cost-effective, uncomplicated, and environmentally friendly methods to increase their production. Understanding the interactions between MNPs and target cells is essential for uncovering novel biomedical uses [6]. While bacteria, actinomycetes, yeasts, and fungi have been extensively studied for their role in synthesizing metallic nanoparticles, the potential of utilizing different parts of plants for similar biosynthesis methods remains largely unexplored and underutilized [7]. Phyto-nanotechnology has garnered significant interest as a viable option for synthesizing nanoparticles. This approach offers several advantages, including simplicity, speed, scalability, and cost-effectiveness. By utilizing extracts from various sources such as plants, viruses, algae, fungi, and bacteria, nanoparticles can be efficiently produced. The use of plant extracts, in particular, provides the added benefit of biocompatibility. These extracts are abundant in bioactive compounds that can be easily extracted using water as an inert solvent. Furthermore, these compounds serve as both reducing and capping agents during the nanoparticle synthesis process. [8,9]

Neem, scientifically known as Azadirachta indica [AI], is a tropical tree that originated in the Indian sub-continent and has since spread its distribution worldwide. Recognizing its significance, the United Nations has bestowed upon it the title of "Tree of the 21st century." Neem holds a revered position as a traditional home remedy, offering a multitude of uses. Its leaf juice is utilized to combat intestinal worms, while twigs serve as natural tooth-cleaning agents. The bark paste and gum find application in the treatment of leprosy and various skin ailments. Additionally, neem seed oil acts as an effective mosquito repellent, and the leaves possess remarkable properties such as anti-inflammatory, antipyretic, antimalarial, anticancer, and antidiabetic effects[10,11]. Neem contains terpenoids and flavanones, which function as both reducing and capping agents, aiding in the stabilization of nanoparticles. When Neem leaf extract is applied to silver

salt, the silver salt is reduced to AgNPs. Additionally, the nanoparticles synthesized with neem extract as a capping agent demonstrate improved antibacterial properties [12].

Fig. 1. Azadirachta indica [13]

Fig. 2. Gmelina arborea [14]

Gmelina arborea, also known as white teak, is a rapidly growing perennial tree that holds significant economic value due to its high-quality timber. The primary purpose of harvesting these trees is to obtain wood for the production of plywood, packaging, furniture, paper-pulp, vehicle bodies, etc. However, this process leaves behind a substantial amount of biomass, approximately 25-45%, in the form of logging residue such as branches, tops, stumps, barks, leaves, and roots, which is not utilized. In the Indian traditional medicine system, various parts of the Gmelina arborea tree, including the leaves, bark, and roots, are used for their medicinal properties. These extracts have been found to possess antidiabetic, antimicrobial, anticancer, immunomodulatory, cardioprotective, analgesic, anthelmintic, rheumatoid, antipyretic, and blood detoxifying properties.

The current research seeks to evaluate the production of new AgNPs through the use of an environmentally friendly raw material that possesses known antimicrobial characteristics. Additionally, the study aims to investigate the potential of a non-harmful, biocompatible, and thermally responsive hydrogel [PF127] for delivering these nanoparticles. The utilization of this combination, which has demonstrated effectiveness against microbes, biofilms, and oxidative stress, is expected to impede the growth of bacteria, shield against oxidative harm, and expedite the recovery process of wounded areas.

1.1.Materials used in biosynthesis of nanoparticles

1.1.1. Azadirachta indica

The leaves of Azadirachta indica were utilized in the study. They were lyophilized using liquid nitrogen. Subsequently, hexane or ethyl alcohol was employed as solvents, and anhydrous sodium sulfate was added to the mixture.[15]

A commonly used method for grinding frozen leaf tissue is to employ a mortar and pestle that has been cooled with liquid nitrogen. This technique can be carried out by either pre-chilling the mortar and pestle and conducting dry grinding on the frozen leaves, or by immersing the leaves in liquid nitrogen while grinding. The inclusion of liquid nitrogen, which possesses an exceptionally low temperature of -176°C, is crucial in the preparation of leaf extracts for DNA isolation from plant tissues. This extreme coldness facilitates the pulverization of the plant tissue into a fine powder-like consistency and deactivates DNAase enzymes that could potentially degrade the DNA. [16]

Hexane has become the primary solvent used in oil extraction because of its various beneficial characteristics, such as its ability to completely mix with oil, a low boiling point of around 68°C, simple recyclability, and relatively affordable price.[17]

Anhydrous sodium sulfate [Na_2SO_4] is utilized in the organic layer subsequent to aqueous extractions in order to eliminate water. By serving as a desiccant, it absorbs any remaining water and hinders its involvement in subsequent reactions or analyses. This enhances the purity of the organic solution.

1.1.2. Gmelina arborea

Plant samples obtained from Gmelina arborea were utilized in the study. They were lyophilized using liquid nitrogen. Subsequently, hexane or ethyl alcohol was employed as solvents, and anhydrous sodium sulfate was added to the mixture.

For identification of a wider range of phytochemicals the obtained leaf extract can be separated and eluted with distilled water and acetonitrile. [19]

1.2. Identification of Bioactive Compounds [GC-MS Analysis]

The neem leaves underwent lyophilization in liquid nitrogen utilizing a mortar and pestle to achieve a fine powder. Subsequently, 1 g of this powder was measured and dissolved in either 1 mL of hexane or 1 mL of ethyl acetate, together with 1 µL [10 mg/mL] of camphor [internal standard]. Following vortexing, the mixture was placed on a horizontal shaker at 30 rpm for 2 hours. The resultant solution was then centrifuged at 4,200 rpm for 25 minutes at 15°C, and the organic layer that separated was dehydrated using anhydrous sodium sulfate to eliminate any water residues. To conduct phytochemical analysis, the organic extract was transferred into a 2 mL glass vial and introduced into a GC system [Agilent 7890A] equipped with a Mass Selective Detector [MSD, Agilent Technologies 5975C Inert XL] and HP-5MS UI column [30 m × 0.25 mm – 0.25 µm]. The system's experimental parameters were set as follows: injection volume – 2 µL; splitless injection; oven program 50°C [1 min hold] at 8°C min–1 to 300°C [5 min hold].

The plant samples from Gmelina arborea, which had been thoroughly washed, were freeze-dried using liquid nitrogen. Then, they were extracted in either 1 mL of hexane or ethyl acetate, along with 10 mg/mL of camphor as an internal standard. This mixture was placed on a horizontal shaker and incubated at 50 rpm for a duration of 2 hours. Following this, the mixture was centrifuged at 4500 rpm for 25 minutes, resulting in the separation of the organic layer. To eliminate any remaining moisture, the organic layer was dried using anhydrous sodium sulphate. After another round of centrifugation, the filtrate was transferred to a glass vial for analysis. The presence of bioactive compounds was determined using GC-

MS, For the analysis, 2 µL of the sample was injected and separated on a 30 m HP-5 MS column [also from Agilent Technologies, USA]. The separation process began at a temperature of 50 °C for 1 minute, and then the temperature was gradually increased at a rate of 8 °C per minute until it reached 300 °C. The temperature was held at 300 °C for 5 minutes.

1.3.Formulation of nanoparticles

1.3.1. Preparation of extract

To eliminate any dust, pests, and spores, if present, the leaves from Azadirachta indica and plant parts of Gmelina arborea were washed under a continuous flow of water. Subsequently, they were air-dried and blended into a fine powder. For the preparation of the aqueous extract, 10 g of the AI and GA powders was added to 100 mL of distilled water in a 1:10 ratio respectively. The mixture was then heated in a water bath at 50°C for 30 minutes. Afterward, the solution was allowed to cool down to room temperature [25°C] and filtered using Whatman filter paper [No. 1]. The resulting filtrate, known as AI-extract, and GA-extract, will be utilized for the synthesis of nanoparticles. [18,37]

1.3.2. Biosynthesis of nanoparticles

5 mL of AI-extract was added dropwise to 45 mL of silver nitrate [AgNO3, 1 mM] in an Erlenmeyer flask. The resulting solution was then adjusted to pH 7 and placed on a rotary shaker at 200 rpm in the dark at room temperature for 24 hours. Throughout this incubation period, visual inspections were conducted to monitor any changes in color. Samples should be withdrawn at regular 6-hour intervals for absorbance measurements using a UV-Visible spectrophotometer. Subsequently, the nanoparticle suspension was centrifuged at 4,500 rpm for 20 minutes to collect AI-AgNPs in the form of a pellet. To eliminate any unreacted silver ions and unbound phyto-constituents, the pellet was washed three times with distilled water, air-dried, and stored at room temperature for future use. The stability of AI-AgNPs in five different solutions, including distilled water, PBS buffer, NaCl [0.9%], was assessed by measuring absorbance in the wavelength range of 100–900 nm. Additionally, the on-shelf stability of AI-AgNPs in distilled water as a storage medium was evaluated over an extended period of 28 days by measuring absorbance in the wavelength range of 100–900 nm. [20]

GA-AgNPs are produced using leaf extract as a precursor. Changes in color and absorbance peak were observed to confirm the phyto-fabrication process. Following the completion of the reaction, the nanoparticle suspension underwent centrifugation at 4500 rpm for 20 minutes to separate the purified pellet of AgNPs from the discarded supernatant. The pellet should be washed three times with distilled water, air dried, and stored in sealed, opaque vials at room temperature for further analysis and application. Various key parameters, including the volume of GA-extract [ranging from 1 to 10 mL], the corresponding volume of 1 mM $AgNO_3$ [ranging from 49 to 40 mL], the concentration of $AgNO_3$ [ranging from 0.5 to 2.0 mM], pH levels [ranging from 3 to 10], and reaction duration [ranging from 6 to 24 hours] were optimized at room temperature in the absence of light. Additionally, the long-term stability of GA-AgNPs was evaluated over a 28-day period. Throughout the experiments, the absorbance peak of GA-AgNPs was determined using a UV-Spectrophotometer [UV1601, Shimadzu] with a wavelength range of 100 to 900 nm.

1.3.3. Phyto-fabrication of AI-AgNPs, and GA-AgNPs

Phyto-fabrication is a method of using biological reducing agents to produce nanoparticles or biosynthesis of noble metal nanoparticles by plants [22]. Various biomolecules found in plant parts, including leaves, roots, stem, bark, flowers, and fruits, have been identified in the literature as phyto-compounds that

possess the ability to act as reducing agents for the conversion of Ag^{+} to Ag^{o}. Polysaccharides, proteins, polyphenols, alkaloids, and flavonoids are among the significant phyto-compounds that exhibit this reducing activity. [21]

In the process of phyto fabrication of AgNPs, plant-derived substances can serve multiple purposes such as acting as a reducing agent, a stabilizing agent, and a functionalizing agent. The biosynthesis of AgNPs involves the use of Azadirachta indica for capping and stabilizing, following a method previously described with some adjustments. To summarize, equal volumes of PE and ionic silver nitrate [200 ml each] were mixed, observing initial color changes and pH variations, and left to incubate in darkness overnight. Subsequently, the purified NPs were obtained through centrifugation, followed by washing with dH2O and ethanol. [23,24]

GA-AgNPs were produced using a leaf extract. The introduction of aqueous GA-extract into a clear silver nitrate [AgNO3] solution caused a modification in the color of the reaction mixture, progressing from a light to a dark brown hue. The alteration in color and the peak absorbance were observed to ascertain the phyto-fabrication process. The conversion of silver ion from Ag+ to Ag° was additionally verified by the peak absorbance at 420 nm in UV–Vis spectroscopy. A substantial yield of GA-AgNPs was achieved when the GA-extract and AgNO3 were combined in a 1:9 ratio. The yield was enhanced with an increase in the concentration of AgNO3 up to 1 mM.

1.3.4. Characterization of synthesized AI-AgNPs, and GA-AgNPs [37,38]

The characterization of AgNPs involves various parameters that offer a detailed analysis of factors such as particle size, shape, arrangement, stability, and potential functional groups that play a role in the bio reduction process and determine the nature of the synthesized nanoparticles.

The AI-AgNPs were analyzed using various techniques to characterize their shape, size morphology, elemental composition, and functional groups. UV-Vis spectroscopy are employed to study the absorption of light in the ultraviolet and visible regions. Scanning electron microscopy allows for high-resolution imaging of the nanoparticles. Energy-dispersive X-ray spectroscopy is used to determine the elemental composition of the AI-AgNPs. Transmission electron microscopy is used to get an insight into detailed information about the shape and size of the nanoparticles. Fourier transform infrared spectroscopy is utilized to analyze the absorption spectra in the range of 4,000–400 cm–1. This allowed for the identification of functional groups involved in the bio-reduction process. X-ray diffraction is used to determine the crystalline nature of the AI-AgNPs. Overall, these characterization techniques provided valuable insights into the properties and composition of the AI-AgNPs.

1.4. Characterization of Nanoparticles

The characterization of nanoparticles involves the examination of various parameters, with size and shape being two of the primary factors under study. Additionally, the size distribution, degree of aggregation, surface charge, surface area, and to a certain extent, the surface chemistry can be measured. The size, size distribution, and the presence of organic ligands on the particle's surface can potentially influence other properties and potential applications of the nanoparticles.[25]

AI-AgNPs underwent analysis using UV-Vis spectroscopy, scanning electron microscopy, energy-dispersive X-ray spectroscopy, and transmission electron microscopy in order to investigate their shape, size morphology, and elemental composition.

Analysis of GA-AgNPs was conducted through Scanning Electron Microscopy [SEM], revealing that the majority of GA-AgNPs exhibited a spherical shape, while some displayed cuboidal structures. Energy-

dispersive spectroscopy [EDX] analysis confirmed the presence of elements, with peaks corresponding to the atomic weight of each component. Transmission Electron Microscopy [TEM] images showed that GA-AgNPs were spherical and evenly distributed.

Table 1 = Characterization of extracted nanoparticles

Plant extract	Nanoparticles	Result
Neem extract, lemon juice	Ag/Au, spherical, 29-92nm	NPs are very effective against Gram-negative and Gram-positive bacteria. [26]
Neem gum	Ag, spherical, 30-60nm Au, spherical, 50-250nm.	Gold and silver nanoparticles have a wide range of antimicrobial activity against animal and human pathogens.[27]
Neem aqueous leaf extract	Ag, spherical, 34nm	The silver nanoparticles showed antibacterial activities against both Gram-positive and Gram-negative microorganisms. [28]
Gmelina arborea ethanol extract	Ag, cuboidal, 36.38nm	Silver nanoparticles using Gmelina arborea exhibit antimicrobial and biofilm inhibition activity [19]

1.5.Evaluation of Nanoparticles

The nanoparticles and hydrogels were visually inspected for attributes like color, uniformity, and texture. The pH level was determined using a standard pH meter after diluting the hydrogels to a 1% concentration with distilled water. The viscosity of the hydrogels was assessed at 4°C using an Ostwald viscometer. Analysis of antibacterial activity, wound healing activity, skin irritation, biofilm inhibition, antioxidant activity, will be done for both plant extract nanoparticles.

First of all we will **identify bioactive compounds** present in both the plant extracts by employing Gas chromatography mass spectroscopy and Liquid chromatography mass spectroscopy. [15,19,25]

For the **analysis of antibacterial activity**, we will use disk diffusion assay being a qualitative method that relies on the diffusion of antimicrobial agents within agar. The extent of this diffusion is measured by determining the diameter of the corresponding inhibition zone, which is used to ascertain the antimicrobial efficacy of the tested material [30]. With this we can also determine Minimum inhibitory concentrations [MICs] which refer to the lowest concentration of an antimicrobial agent that can effectively hinder the observable growth of a microorganism following an incubation period. On the other hand, the minimum bactericidal concentrations [MBCs] denote the lowest concentration of an antimicrobial agent that can impede the growth of an organism after being transferred to a medium devoid of antibiotics[31], The Minimum Bactericidal Concentration [MBC] assay is utilized to ascertain the lowest concentration of antibacterial agents required to eradicate a specific bacterium within a specified timeframe. Following the

determination of the Minimum Inhibitory Concentration [MIC] in antibacterial testing, the MBC test can be conducted[32].

For evaluation of AI-AgNPs and GA-AgNPs **wound healing activity**, healthy adult male albino mice weighing approximately 25-30 g, free from pathogens, were housed in standard laboratory conditions with a 12-hour light-dark cycle. The mice were provided with a standard rodent diet and distilled water. Following a one-week acclimatization period, the mice were anesthetized using a mixture of 5% isoflurane in air, which was then maintained at 2.5% isoflurane in air. Full-thickness excision wounds of 6 mm in diameter were created on the dorsal skin after shaving and disinfecting with 70% ethyl alcohol. On the first day, 20 µL of the respective hydrogel samples were applied to the wound site, which was then covered with Tegaderm and Opsite Flexifix transparent wound dressing. The wound areas were assessed on the 3rd, 5th, 7th, and 10th days post-surgery to determine the percentage of wound contraction.

$$\text{Percentage of wound contraction} = \frac{\text{Wound area day 1} - \text{Wound area day } n}{\text{Wound area day 1}} \times 100$$

For **dermal irritation test**, a 5cm x 5cm dorsal area was shaved with an electric razor. After 24hours, hydrogels were placed on the backs of the rats in the experimental groups, samples were taken at different durations consecutively for two days to evaluate erythema, and edema.[33]

Biofilms, which consist of microorganisms adhering to surfaces and surrounded by an extracellular matrix, significantly augment the ability of microbes to withstand the effects of antibiotics, thereby complicating the treatment of infections. Pathogens commonly encountered in medical settings, including bacteria, fungi, and viruses, form biofilms on various substrates such as medical equipment, water systems, and tissues, effectively trapping particles and host components. Infections associated with biofilms encompass a range of conditions, such as periodontitis, dental caries, and chronic ailments like cystic fibrosis. The presence of biofilm-producing bacteria that are resistant to treatment, such as Pseudomonas aeruginosa and Staphylococcus aureus, presents substantial challenges in hospital settings. Notably, Candida biofilms exhibit remarkable resistance to antifungal therapies and immune responses, leading to increased mortality rates. Consequently, the development of strategies to combat biofilms is of utmost importance in order to overcome the problem of antimicrobial resistance[34,35,36,39]. Biofilm inhibition was assessed in 96-well plates using ethanolic extracts of Azadirachta indica and Gmelina arborea. Microbial strains were incubated at 37°C for 20 hours, followed by dilution. Microbial cultures [150 µL] were then incubated for 42 hours to allow biofilm formation. Plates were rinsed twice with sterile phosphate-buffered saline [PBS] to remove planktonic cells, and the lids were transferred to another 96-well plate and incubated at 37°C for 4 hours. The pegs were rinsed with PBS and moved to a new 96-well plate containing MTT in PBS, then incubated at 37°C for 4 hours. Formazan crystals, formed by MTT reduction, were dissolved using 50 µL of 25% sodium dodecyl sulfate. The inhibition capability was evaluated by measuring absorbance at 595 nm using a Microplate reader to determine biofilm cell viability. The minimum biofilm inhibitory concentration [MBIC] was defined as the lowest concentration required to inhibit biofilm formation after treatment. [40]

For antioxidant activity, Azadirachta indica, and Gmelina arborea extract nanoparticles at concentrations ranging from 100 to 500 µg/mL was evaluated using DPPH and ABTS radical scavenging assays. The DPPH assay followed a modified standard protocol, wherein 100 µL of 0.1 mM DPPH was added to 100 µL of the sample, incubated at room temperature for 30 minutes in the dark, and absorbance was recorded

at 517 nm. The ABTS assay was similarly conducted with modifications: 10 mL of 7.4 mM ABTS was combined with 10 mL of 2.45 mM ammonium persulfate, and the mixture was left at room temperature for 16 hours in the dark. Subsequently, 100 µL of the ABTS solution was added to 100 µL of the sample, incubated at room temperature for 20 minutes in the dark, and absorbance was measured at 734 nm. Butylated hydroxytoluene [BHT] served as the reference standard, and a reaction mixture without the sample acted as the control. The percentage inhibition was calculated using the specified equation.

Chapter-2
Aim and Objectives
AIM
A comparative review on the sustainable phyto-fabrication of silver nanoparticles using *azadirachta indica*, and *gmelina arborea.*

OBJECTIVES
1. **Synthesis Efficiency:** Compare the efficiency of silver nanoparticle synthesis using Azadirachta indica and Gmelina arborea in terms of nanoparticle yield, size distribution, and stability.
2. **Antibacterial Activity:** Investigate and compare the antibacterial properties of the silver nanoparticles produced from Azadirachta indica and Gmelina arborea against different pathogens, assessing their potential as antibacterial agents.
3. **Characterization of Nanoparticles:** Characterize the physical, chemical, and structural properties of the silver nanoparticles synthesized from Azadirachta indica and Gmelina arborea using techniques like UV-Vis spectroscopy, TEM.
4. **Optimization of Synthesis Conditions:** Optimize the synthesis parameters [temperature, pH, concentration ratios] for both plant extracts to enhance the efficiency and quality of silver nanoparticle production.
5. **Comparison of Phytochemicals:** Identify and compare the phytochemicals present in Azadirachta indica and Gmelina arborea responsible for reducing and stabilizing silver nanoparticles, correlating them with nanoparticle characteristics.
6. **Antioxidant activity:** Investigation and comparison between antioxidant activity of AI-AgNPs, and GA-AgNPs in assay of DPPH and ABTS to confirm a gradual increase in the antioxidant activity in a concentration-dependent manner
7. **Application Potential:** Explore potential applications of silver nanoparticles synthesized from Azadirachta indica and Gmelina arborea in fields such as medicine, agriculture, catalysis, and water treatment, highlighting advantages and limitations.

Chapter-3
Review of Literature
REVIEW OF LITERATURE
3.1.Gandhimathi Chinnaasamy, Smitha Chandrasekharan, and Somika Bhatnagar [2022]
The increasing bacterial resistance to conventional antibiotics necessitates the exploration of novel antibacterial agents. This study aimed to identify an efficient antibacterial alternative derived from the non-conventional plant source Gmelina arborea (GA), utilizing an eco-friendly approach for the phyto-fabrication of silver nanoparticles (AgNPs). Comprehensive GC-MS analysis of GA leaves, bark, flowers,

fruits, and roots identified bioactive compounds, with leaves selected for AgNP synthesis due to their high content of terpenoids, sterols, aliphatic alcohols, aldehydes, and flavonoids.

The biosynthesis of GA-AgNPs was confirmed by the formation of a dark brown colloidal solution and a characteristic absorption peak at 420 nm. Morphological and structural characterization via UV-Vis spectroscopy, SEM, EDX, TEM, FTIR, and XRD revealed that the GA-AgNPs were spherical, uniformly dispersed, crystalline, with a diameter of 34–40 nm, and stable at room temperature. FTIR analysis indicated that flavonoids, terpenoids, and phenols in the leaf extract functioned as reducing and capping agents.

The antibacterial efficacy of GA-AgNPs was validated against Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus through disc diffusion assays, MIC and MBC assays, biofilm inhibition assays, electron microscopy, cell staining, and colony counting techniques. GA-AgNPs exhibited superior antibacterial activity compared to GA extract, with enhanced effects when incorporated into a hydrogel (GA-AgNPs-PF127).

Antioxidant activity was confirmed via DPPH and ABTS free radical scavenging assays. The wound healing potential was demonstrated by cell scratch assays in human dermal fibroblast cell lines. Toxicity studies, including cell proliferation in human Chang liver cell lines and optical microscopy, confirmed the non-toxicity of GA-AgNPs at low doses.

In conclusion, the biosynthesized GA-AgNPs exhibited significant antibacterial, antibiofilm, antioxidant, and wound healing properties, presenting a promising alternative to conventional antibiotics with potential applications in human health.

3.2.Gandhimathi Chinnaasamy, Smitha Chandrasekharan, Tony Wey Koh and Somika Bhatnagar [2021]

The study explores the potential of Azadirachta indica (AI) as an alternative source of antibiotic compounds. Phytochemical and GC-MS analyses identified the presence of flavonoids, phenolic compounds, terpenoids, and terpenes in AI. Aqueous leaf extracts were utilized to synthesize silver nanoparticles (AI-AgNPs), confirmed by a characteristic absorbance peak at 400 nm. Optimal reaction parameters yielded stable AI-AgNPs, characterized through UV-Vis spectroscopy, energy-dispersive X-ray spectroscopy, scanning electron microscopy, and transmission electron microscopy, revealing spherical nanoparticles with a diameter of 33 nm. Fourier transform infrared spectroscopy indicated functional groups in bioactive constituents responsible for reducing silver ions to elemental silver, acting as capping agents during AI-AgNPs formation. X-ray diffraction confirmed their crystalline nature.

Toxicity assessments using Drosophila indicated that AI-AgNPs (100 µg/mL) did not affect egg laying capacity or eclosion of the F1 generation. Antioxidant assays (DPPH and ABTS) demonstrated significant radical scavenging activity of AI-AgNPs at 500 µg/mL, with inhibition rates of 65.17% and 66.20%, respectively. Antibacterial efficacy of AI-AgNPs (1,000 µg/mL) was validated through disc diffusion assays, showing inhibition zones against Bacillus cereus (17.7 mm), Escherichia coli (18.7 mm), Pseudomonas aeruginosa (10.3 mm), and Staphylococcus aureus (17.7 mm). Minimum inhibitory and bactericidal concentrations ranged from 390 to 780 µg/mL. SEM images highlighted significant bacterial cell wall damage and membrane disintegration, confirming higher antibacterial activity of AI-AgNPs compared to AI extract.

AI-AgNPs were incorporated into a biocompatible and biodegradable polymer, PF127, forming a viscous hydrogel (AI-AgNPs-PF127). This hydrogel exhibited enhanced antibacterial properties, with inhibition

zones of 13–18.7 mm in disc diffusion assays. Topical application on mice showed no skin irritation and significantly increased wound contraction rates. The study presents a green synthesis route for AI-AgNPs with potent antibacterial and antioxidant activities, and introduces AI-AgNPs-PF127 hydrogel as a promising, low-toxicity, eco-friendly delivery system for wound healing applications.

3.3.Rekha R. Warrier, S. Mohana Priya, R. Kalaiselvi [2020]

Gmelina arborea Roxb. ex Smith is a fast-growing deciduous tree in the Lamiaceae family, commonly found in tropical regions, especially Southeast Asia. It is extensively used in traditional Indian Systems of Medicine for its medicinal properties. The plant possesses a wide range of medicinal properties such as astringent, bitter, digestive, cardiotonic, diuretic, laxative, pulmonary, and nervine tonic qualities. Different parts of the plant are utilized for various therapeutic purposes, including aiding digestion, enhancing memory, treating fever, heart diseases, nervous disorders, and piles.

Phytochemical analyses have revealed the presence of numerous bioactive compounds in G. arborea, including arboreal, verbascoside, tyrosol, iridoids, phenylpropanoid glycoside, premnazole, martynoside, balanophonin, gmelinol, isoarboreol, apigenin, and umbelliferone. These compounds have pharmacological activities and contribute to the plant's medicinal efficacy. Empirical studies have demonstrated the effectiveness of G. arborea extracts in wound healing and treating diarrhea, indicating its potential in both traditional and modern medicinal practices. The plant's diverse phytochemical composition plays a crucial role in its therapeutic benefits.

3.4.Rekha R.Warrier, S.Mohana Priya, R.Kalaiselvi [2020]

Gmelina arborea Roxb. ex Smith, a fast-growing deciduous tree in the Lamiaceae family, is a significant plantation species in tropical regions worldwide, particularly in Southeast Asia. It holds substantial medicinal value in the Indian Systems of Medicine, with various parts of the plant used for their therapeutic properties. The tree exhibits astringent, bitter, digestive, cardiotonic, diuretic, laxative, and nervine tonic qualities, benefiting conditions such as fever, heart diseases, and nervous disorders. The roots, flowers, fruits, and leaves possess distinct medicinal attributes, including antihelmintic, galactagogue, refrigerant, aphrodisiac, and anti-leprosy properties. The plant's phytochemical profile includes 69 compounds, such as lignans, iridoid glycosides, flavonoids, and flavone glycosides, many of which exhibit pharmacological activities. Extracts from G. arborea have demonstrated wound-healing, antidiarrheal, antioxidant, antidiabetic, anti-inflammatory, antiulcer, analgesic, anticancer, and antinociceptive effects, highlighting its therapeutic potential.

3.5. Gandhimathi Chinnaasamy, Smitha Chandrasekharan, and Somika Bhatnagar [2019]

The increasing need for natural, environmentally friendly, and biocompatible resources to explore new bioactive compounds underscores the significance of nanoparticles (NPs) in various industries. Silver nanoparticles (AgNPs) stand out for their antimicrobial and catalytic properties. Conventional physical and chemical NP production methods are costly and harmful to the environment, while biological approaches using plant extracts present a more sustainable and economical option.The research centers on the production of AgNPs from the leaves of Melia azedarach (MA), a rapidly growing tropical tree resistant to diseases and known for its medicinal properties. Water serves as the solvent in the synthesis process, with bioactive compounds from the leaves acting as reducing, capping, and stabilizing agents. The resulting MA-AgNPs are characterized using different techniques, demonstrating improved biological

activities such as antibacterial, wound healing, antidiabetic, antioxidant, and cytotoxic effects compared to raw extracts. This eco-friendly synthe.sis technique provides a viable and efficient solution for the biomedical applications of Melia azedarach.

3.6. Nafeesa Khatoon, Jahirul Ahmed Mazumder and Meryam Sardar [2017]

Silver and its compounds have been employed since ancient times for treating bacterial infections and wounds, particularly in burn patients. The emergence of novel therapeutic agents led to a decline in the use of silver compounds. However, the past decade has witnessed significant advancements in nanotechnology, enabling the transformation of metal ions into the nano range, thereby altering their chemical, physical, and optical properties. Silver nanoparticles (AgNPs) have demonstrated potent antimicrobial activity, revitalizing their use, particularly in response to the increasing antibiotic resistance among pathogenic bacteria.

AgNPs are extensively utilized in the biomedical industry for applications such as coatings on dressings, medicinal devices, and in the form of nanogels in cosmetics and lotions. Established protocols for AgNP synthesis are broadly categorized into physical, chemical, and biological methods. Physical and chemical processes typically involve high temperatures, pressure, and hazardous chemicals, which has shifted research focus towards biological methods. Plant extracts are increasingly recognized as cost-effective, environmentally friendly, and efficient alternatives for large-scale nanoparticle synthesis.

This review critically examines the role of plants in the synthesis of AgNPs and their biomedical applications. The mechanism of AgNP synthesis using plants involves the reduction of silver ions into nanoparticles by plant bio-molecules. These bio-molecules not only facilitate the reduction process but also impart unique properties to the nanoparticles. Due to their broad-spectrum antimicrobial properties, AgNPs present an attractive alternative to conventional antibiotics, paving the way for the development of new-generation antibiotics.

The plant-mediated synthesis of AgNPs is economical, eco-friendly, and scalable, making it suitable for a wide range of applications, including industrial appliances such as bandages, food and water storage, biomedical fields, pharmaceuticals, and wastewater treatment. Further research aimed at elucidating the exact mechanisms of synthesis and controlling the shape and size of AgNPs will enhance their applicability and broaden the scope of their use in various fields.

3.7.S Shiv Shankar, Akhilesh Rai, Absar Ahmad, Murali Sastry [2004]

The study investigates the use of Neem leaf broth for the extracellular synthesis of metallic silver, gold, and bimetallic Au/Ag nanoparticles. Neem leaf extract rapidly produces stable nanoparticles when treated with silver nitrate and chloroauric acid solutions. Gold nanoparticles display a flat, platelike shape, while bimetallic Au core–Ag shell structures are formed due to competitive reduction of Au3+ and Ag+ ions. Transmission electron microscopy confirms the core–shell configuration, with silver nanoparticles attached to gold cores. The reduction rates using Neem leaf extract are superior to those achieved with fungi, suggesting potential for biological synthesis at rates comparable to chemical methods. The presence of flavanones, terpenoids, and reducing sugars in Neem leaf broth likely aids in the reduction and stabilization of nanoparticles. This eco-friendly method provides a rapid alternative for nanoparticle synthesis. Future research will concentrate on achieving monodispersity and shape selectivity by identifying the active compounds responsible for nanoparticle reduction and capping.

Chapter-4
Methodology
METHODOLOGY
4.1.Literature review
A comprehensive review of articles from various academic databases including PubMed, NCBI, Taylor and Francis, Elsevier, Hindawi, MDPI, ScienceDirect, and Springer, spanning from 2000 to 2024, focused on several key topics related to Azadirachta indica [Neem], Gmelina arborea, silver-based nanoparticles, and novel approaches to wound healing. With the following findings:-

4.1.1. Azadirachta indica and Gmelina arborea:Both plants were extensively studied for their antimicrobial, antioxidant, and antibacterial activities, highlighting their medicinal potential.

4.1.2. Biosynthesis of Silver-Based Nanoparticles: Studies explored the use of plant extracts from Azadirachta indica and Gmelina arborea for eco-friendly synthesis of silver nanoparticles [AgNPs], emphasizing their efficacy and sustainability.

4.1.3. Phyto-fabrication of Nanoparticles: The review focused on the green synthesis of nanoparticles, particularly AgNPs, using plant extracts, underscoring their economic viability and environmental benefits over conventional methods.

4.1.4. Antioxidant and Antibacterial Activities:Significant attention was given to evaluating the antioxidant and antibacterial properties of Azadirachta indica and Gmelina arborea, particularly in the context of nanoparticle formulations.

4.1.5. References and Citations: Additional relevant articles were identified through references in comprehensive reviews on sustainable phyto-fabrication of AgNPs using Azadirachta indica and Gmelina arborea, further enriching the scope of the study.

Overall, the research underscores the promising applications of plant-based synthesis of AgNPs from Azadirachta indica and Gmelina arborea in biomedical and environmental fields, advocating for continued exploration into their mechanisms and potential therapeutic benefits.

4.2.Year wise partitioning and study of abstracts
The research utilized specific keywords and date filters across various databases to explore sustainable phyto-fabrication of silver nanoparticles using Azadirachta indica and Gmelina arborea. It identified an evolution in research from initial associations in the 1990s to niche developments in the 2000s, culminating in substantial growth and application from 2010 to 2024. Abstract analysis uncovered 45 pertinent articles focusing on the antibacterial and antioxidant properties of these plants in nanoparticle biosynthesis. These articles provide crucial data to support the study's conclusions, highlighting their efficacy and potential contributions to advancing biomedical applications of plant-based nanoparticle synthesis.

4.3.Collection of paper
The published papers were systematically categorized into distinct groups, eachaddressing a particular area of research. These groups included the phyto-fabrication of silver-based nanoparticles, the investigation of their antimicrobial activity, comprehensive in vivo and in vitro studies conducted to evaluate the effectiveness of phyto-fabricated silver-based nanoparticles, the utilization of silver-based nanoparticles in wound therapy, and there ability to inhibit biofilm.

4.4.Data analysis

In order to analyse a compilation of data obtained from a set of published papers,I adhered to a series of fundamental procedures. Firstly, I identified the specificdata that had the potential to address my research objectives. Subsequently, I systematically arranged the data in an organized manner. Following this, I engaged in the process of synthesizing, analyzing, and ultimately presenting thedata in a comprehensive final report. Through this meticulous analysis, I was able to gain fresh insights that could potentially contribute to the formulation ofa new theory

4.5.Combined revision and compilation of results

The data obtained from the published papers was thoroughly examined, and a variety of tools and techniques were employed to synthesize the information, such as textual descriptions, tabulation, grouping, and thematic/content analysis, as deemed suitable. Subsequently, revisions and compilation of research were conducted to evaluate the caliber of the published papers and the data pertaining to the research topic.

4.6.Drafting of report

By collating information from multiple sources, I successfully compiled a comprehensive report that delineates the sustainable Phyto-fabrication of Silver Nanoparticles using Azadirachta indica and Gmelina arborea. This report encompasses a wide range of important activities that are exhibited by both.

Chapter-5

Results

Results

5.1. Synthesis efficiency [15,19]

In comparing the effective yield and distribution of nanoparticles post-administration, it was observed that Gmelina arborea-derived nanoparticles (GA-AgNPs) exhibited a size range of 34-40 nm, whereas Azadirachta indica-derived nanoparticles (AI-AgNPs) ranged from 44.6 nm to 66 nm, indicating a significant size disparity between the two types.

Both AI-AgNPs and GA-AgNPs demonstrated the capability to evade immune surveillance, prolong systemic retention, achieve enhanced distribution, and attain higher concentrations in target tissues. Notably, nanoparticles possess the ability to mitigate diffusion into adjacent tissues and facilitate controlled release of therapeutic agents or drugs in response to specific stimuli, thereby extending their therapeutic duration. Furthermore, nanoparticles can elicit tailored biological effects conducive to applications in imaging modalities.

This characterization underscores the potential of nanoparticles, derived from natural sources such as Gmelina arborea and Azadirachta indica, to serve as versatile platforms in biomedical applications, offering precise targeting, sustained therapeutic efficacy, and enhanced imaging capabilities.

5.2. Antibacterial activity[19,15]

Studies on the determination of diameter of Zone of Inhibition, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of AI-AgNPs, and GA-AgNPs tested against bacterial species resulted in the following findings :-

Table 2 = Antibacterial activity of Azadirachta indica and Gmelina arborea

Name of species	Azadirachta indica			Gmelina Arborea		
	ZOI of AI-AgNPs	MIC of AI-AgNPs	MBC of AgNPs	ZOI of GA-AgNPs	MIC of GA-AgNPs	MBC of GA-AgNPS
Bacillus cereus	17.7±1.24	390	390	15.6±1.15	20	20
Escherichia coli	18.7±1.15	780	780	23.0±1.73	20	40
Pseudomonas aeruginosa	10.3±0.50	780	780	13.3±0.50	90	90
Staphylococcus aureus	17.7±0.47	390	390	18.3±0.57	90	90

5.3. Characterization of nanoparticles

5.3.1 Wound healing activity

Neem [Azadirachta indica] leaf extracts enhance wound healing by boosting inflammatory responses and promoting neovascularization in excision and incision wound models. Gmelina arborea leaves exhibit anti-inflammatory properties and significantly enhance wound contraction, epithelization, hydroxyproline content, collagen levels, and granuloma breaking strength. Cell scratch assays on human dermal fibroblast cell lines demonstrate that both Neem and Gmelina arborea silver nanoparticles [AI-AgNPs and GA-AgNPs] accelerate wound closure, highlighting their therapeutic potential in wound healing applications. [45]

5.3.2. Skin irritation [50,51]

The application of the treatment on the skin of mice did not result in any undesirable side effects, such as erythema [redness], xerosis [dryness], or desquamation [flakiness]. Observations indicated that the skin condition of both the control and treated groups remained normal and unaffected throughout the study period. This suggests that the treatment is well-tolerated and does not compromise skin integrity or cause visible dermatological issues.

5.3.3. Biofilm inhibition [49,19]

The impact of GA-extract, GA-AgNPs, and GA-AgNPs-PF127 (20–1000 µg/mL) on biofilm inhibition was assessed using crystal violet staining. The effect was more pronounced at lower concentrations and reached a consistent inhibition level at higher concentrations. Biofilm inhibition by GA-extract varied from 6% to 46%, while the inhibition significantly increased to a range of 24% to 62% for GA-AgNPs.

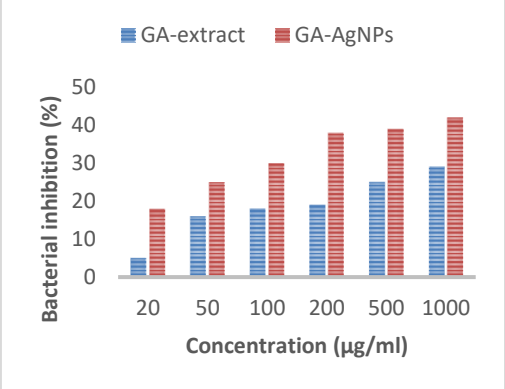

Figure 3 = Biofilm inhibition effect of GA-extract, GA-AgNPs on E.coli

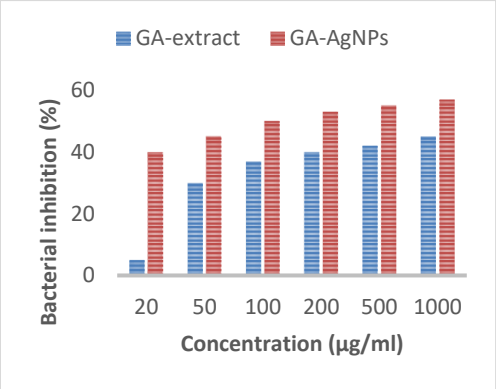

Figure 4 = Biofilm inhibition effect of GA-extract, GA-AgNPs on S.aureus

For AI-AgNPs biofilm inhibition was checked in a control condition, the biofilm cells of E. coli exhibited an absorbance of 0.38 ± 0.0001. As the concentration of nanoparticles increased, the biofilm formation demonstrated a significant reduction, with absorbance values ranging from 0.30 ± 0.01 to 0.02 ± 0.01 nm. Similarly, for *Staphylococcus aureus* biofilm cells, the control condition showed an absorbance of 0.029 ± 0.01. Upon treatment with increasing concentrations of nanoparticles, a corresponding decrease in biofilm formation was observed.

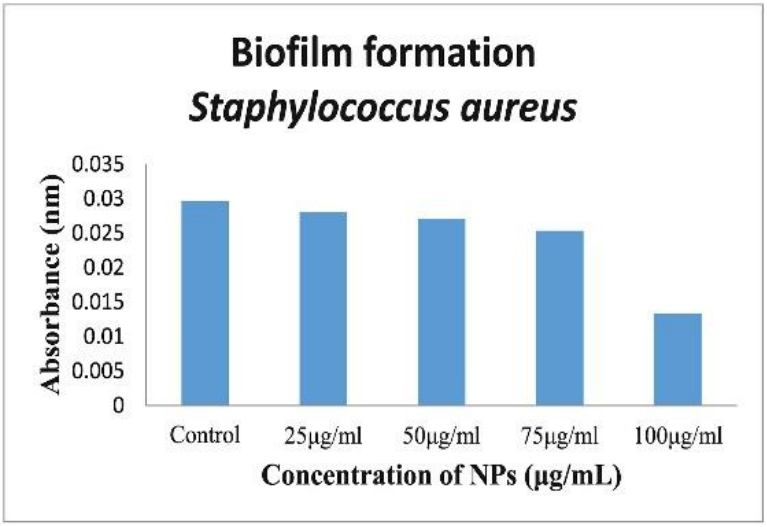

Figure 5 = Biofilm inhibition effect of AI-AgNPs on S.Auerus.

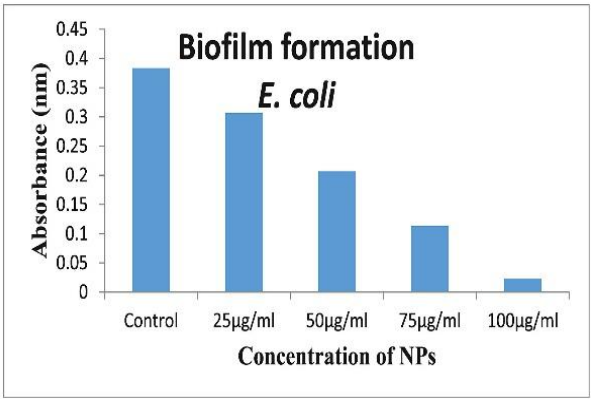

Figure 6 = Biofilm inhibition effect of AI-AgNPs on E.co

5.4. Optimization of synthesis conditions [52,53]

The optimal conditions for synthesizing stable Azadirachta indica silver nanoparticles (AI-AgNPs) involved mixing 5 mL of AI-extract with 45 mL of 1 mM AgNO3 at pH 7. The reaction was conducted at 25°C in darkness with agitation at 200 rpm for 18 hours. AI-AgNPs demonstrated stability across various test solutions and remained stable in distilled water at room temperature over a 28-day period.

For Gmelina arborea silver nanoparticles (GA-AgNPs), a high yield was achieved by mixing GA-extract and AgNO3 in a 1:9 ratio. Increasing the concentration of AgNO3 up to 1 mM improved yield. The pH of the reaction mixture significantly influenced synthesis: negligible GA-AgNPs formed at pH 3, while pH 7 yielded smaller-sized nanoparticles with enhanced yield. At pH 10, particles tended to agglomerate.

Temperature also affected GA-AgNP synthesis, with minimal yield at 4°C, optimal production at room temperature (25°C), and constant yield at higher temperatures (37°C). Absorbance measurements confirmed a time-dependent increase in GA-AgNP yield, reaching maximum levels after 18 hours.

In summary, AI-AgNPs were synthesized under specific conditions to ensure stability and consistent performance, while GA-AgNPs production was optimized based on reactant ratio, pH, temperature, and reaction duration to achieve high yields and desired particle characteristics.

5.5. Comparison of phytochemicals [15,19,25]

Gas chromatography mass spectroscopy [GC–MS] GC–MS identified terpenes, sterols, aliphatic alcohols, esters, fatty acids, and aldehydes in GA plant parts. LC–MS of GA leaf extract detected flavonoids: luteolin, kaempferol, quercetin, isoquercitin, rutin, and astragalin. Azadirachta indica leaf broth contains flavanones and terpenoids stabilizing nanoparticles.

Table 3 = Identified bioactive compounds in AI-AgNPs, and GA-AgNPs

No.	Name of compound	Presence in Azadirachta indica	Presence in Gmelina arborea	Activity
1.	Ethyl propionate	Present	Absent	Antimicrobial
2.	Caryophyllene	Present	Present	Antimicrobial
3.	1-Octen-3-ol	Absent	Present	Antimicrobial
4.	Elemene	Present	Present	Anti-inflammatory
5.	α-Tocopherol	Present	Absent	Antioxidant, wound healing
6.	Trimethylsilyl laurate	Absent	Present	Wound healing
7.	Alloaromandedrene	Present	Present	Antioxidant, antiaging
8.	α-Selinene	Present	Present	Antioxidant, analgesic
9.	Caryophyllene oxide	Present	Present	Anti-inflammatory, analgesic

10.	[3,7,11,15-tetramethyl-2-hexadecenyl]oxy	Present	Present	Antimicrobial
11.	Oleic acid	Present	Present	Antioxidant

5.6. Antioxidant activity[50,19]

2,2-Diphenyl-1-picrylhdrazyl [DPPH] and 2, 2′-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] [ABTS] free radical scavenging assays confirmed a gradual increase in the antioxidant activity in a concentration-dependent manner.

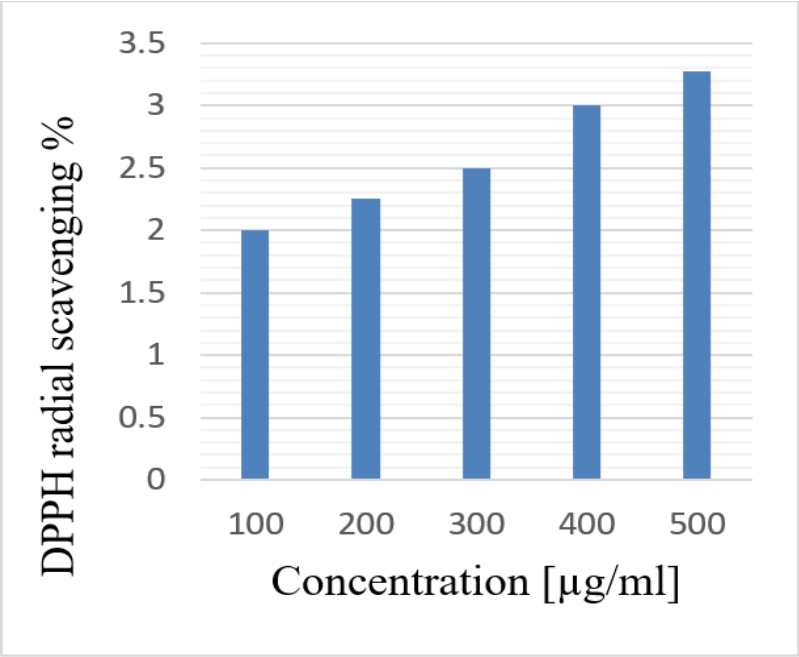

Figure 7 = Antioxidant effect of GA-AgNPs DPHH radial scavenging

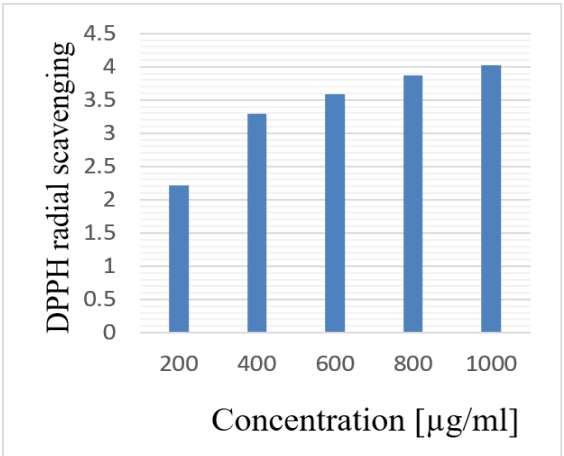

Figure 8 = Antioxidant effect of AI-AgNPs DPHH radial scavenging

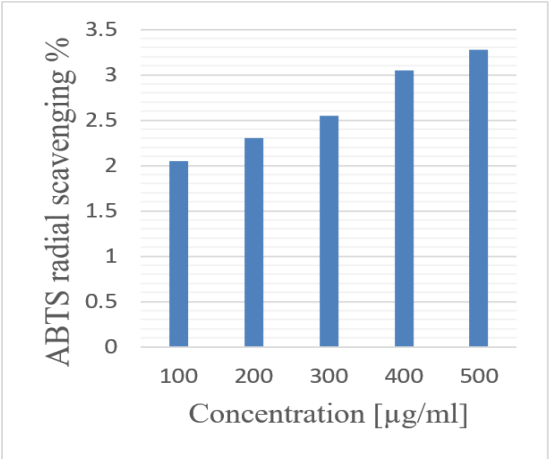

Figure 9 = Antioxidant effect of GA-AgNPs ABTS radial scavenging

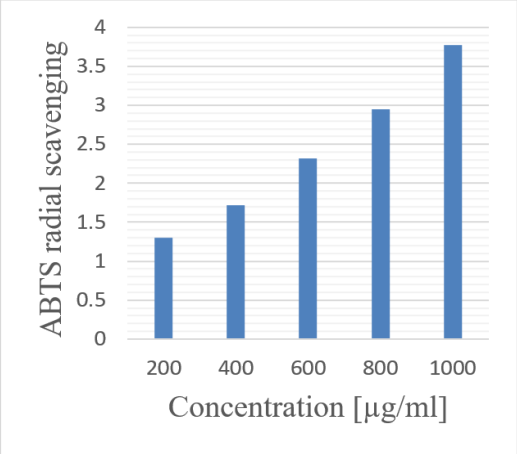

Figure 10 = Antioxidant effect of GA-AgNPs ABTS radial scavenging

5.7. Application potential

Gmelina arborea silver nanoparticles (AgNPs) and Azadirachta indica silver nanoparticles have garnered attention due to their potential applications in various industries, including pharmaceuticals and health.

5.7.1 Antimicrobial properties

Silver nanoparticles are well-known for their antimicrobial activity against a broad spectrum of microorganisms, including bacteria, viruses, and fungi. Incorporating Gmelina arborea and neem silver nanoparticles into pharmaceutical formulations can enhance their effectiveness in combating infectious diseases.

5.7.2.Wound Healing

Silver nanoparticles have been studied for their wound healing properties. They can promote faster healing, reduce inflammation, and prevent infection in wounds. Gmelina arborea and neem silver nanoparticles could be incorporated into wound dressings or topical creams for better wound management.

5.7.3.Anti-inflammatory Effects

AgNPs derived from Gmelina arborea and neem may possess anti-inflammatory properties, which can be beneficial in treating inflammatory conditions such as arthritis, dermatitis, and other inflammatory diseases.

5.7.4. Drug Delivery Systems

Nanoparticles can serve as carriers for drugs, enhancing their delivery to specific targets in the body. Gmelina arborea and neem silver nanoparticles can potentially be used to encapsulate drugs and deliver them to targeted tissues or cells, improving the efficacy and reducing side effects of pharmaceutical treatments.

5.7.5. Diagnostic Applications

Silver nanoparticles have unique optical properties that can be exploited in diagnostic tests. They can be used in biosensors and assays for detecting biomolecules, pathogens, or disease markers with high sensitivity.

5.7.6.Biocompatibility and Safety

Gmelina arborea and neem silver nanoparticles are derived from a natural source, which may confer better biocompatibility compared to synthetic nanoparticles. This can be advantageous for their use in biomedical applications without causing significant toxicity or adverse effects.

5.7.7. Environmental Applications

Silver nanoparticles from natural sources like Gmelina arborea and neem are also of interest in environmental health. They can be used in water purification systems or disinfectants to combat microbial contamination.

Chapter-6

Discussion

The rise in antibiotic resistance and the emergence of new pathogenic strains have resulted in significant economic losses attributed to diseases and illnesses. This situation underscores the importance of exploring alternative sources of antibacterial compounds and novel treatment methods. The screening of plants for bioactive compounds that can be developed into potent drugs represents a contemporary scientific pursuit aimed at leveraging the advantages of traditional medicine systems. Plants are rich sources of secondary metabolites that are well-suited for nanoparticle synthesis. Various plant parts from a wide range of plant species have been investigated for the production of nanoparticles, which are extensively utilized for their antimicrobial and biofilm inhibitory properties. In our study focusing on the medicinal properties of the Azadirachta indica and Gmelina tree, we have identified multiple potential applications of AI-AgNPs and GA-AgNPs derived from its leaf waste.

GC-MS analysis of neem extracts revealed various phytochemicals with medicinal properties, including elemene, caryophyllene, tocopherol, 2-hexanal, and phytol in hexane extract, and ethyl propionate, hexadecanoic acid, trimethylsilyl ester, and Silane in ethyl acetate extract. These compounds are known for analgesic, anti-inflammatory, antimicrobial, antioxidant, and wound healing activities. GC–MS and LC–MS analysis of Gmelina arborea leaves revealed bioactive compounds such as flavonoids, lignans, iridoid glycosides, and sterols. These compounds contribute to the plant's antimicrobial, antioxidant, and wound healing properties. Secondary metabolites in plants play a crucial role in plant defense and have beneficial medicinal properties with low human toxicity and environmental safety.

Neem extract, rich in phytochemicals, acts as hydrogen donors, oxygen quenchers, and redox agents, disrupting oxidation reactions. The radical scavenging activity of the extract was determined using DPPH and ABTS assays. Biosynthesized AI-AgNPs with polyphenol-rich extract showed up to a two-fold increase in free radical scavenging ability in a dose-dependent manner. Overproduction of free radicals can cause cell damage and diseases, making it crucial to deactivate them. GA-extract, rich in thiols,

polyphenols, ascorbic acid, and flavonoids, forms GA-AgNPs with AgNO3, significantly boosting antioxidant efficiency in DPPH and ABTS assays compared to GA-extract alone. Similarly, Teucrium polium AgNPs showed increased free radical scavenging in a concentration-dependent manner, unlike chemically synthesized AgNPs. Antioxidant activity in plant extracts is mainly due to polyphenols and carotenoids, with tannins having more antioxidant potential than flavonoids, supporting similar findings in Gmelina.

The synergistic interactions between Ag+ ions and phytochemicals in the plant extract led to the formation and stability of bioactive AI-AgNPs, which exhibited superior antibacterial efficacy compared to the AI-extract alone. The AI-AgNPs' small size and larger surface area facilitate penetration into bacterial cell walls. Treatment with GA-extract caused slight cell distortion, whereas GA-AgNPs induced significant cell wall disintegration and cytoplasmic leakage. AgNO3 treatment had negligible effects, while Rifampicin caused extensive damage. Confocal microscopy and live/dead cell staining assays confirmed a higher red fluorescent dead cell population in GA-AgNPs-PF127 and GA-AgNPs groups, indicating their high bacterial killing efficiency.

Wound healing involves four phases: homeostasis, inflammation, proliferation, and remodeling. The proliferation and migration of keratinocytes and fibroblasts close the wound, but untreated wounds risk bacterial infections like S. aureus. AgNPs have broad-spectrum antimicrobial activity, prompting their use in wound dressings. AgNPs reduce inflammation through cytokine modulation and promote wound healing with less scar formation. AgNPs decrease the release of growth factors and inflammatory cytokines in human dermal keratinocytes. AI-AgNPs demonstrated bacterial disruption and free-radical scavenging, suggesting their potential in wound dressings. In vitro cell scratch assays showed enhanced cell migration with GA-AgNPs, attributed to phytoconstituents, indicating their promise for further evaluation in animal models for wound healing efficacy.

Chapter-7
Conclusion

This study presents an innovative and environmentally sustainable approach to waste utilization through the conversion of residual botanical material, specifically leaves, into green nanoparticles. Utilizing a cost-effective phyto-fabrication method at ambient temperature, this technique circumvents the need for high-temperature processes or the use of toxic chemicals, aligning with contemporary green chemistry principles.

The phyto-fabricated silver nanoparticles (AgNPs) demonstrated remarkable stability, uniformity, and monodispersity, with a crystalline structure characterized by an average particle size ranging from 34 to 40 nm. The intrinsic bioactivity of the leaf extract was significantly enhanced in the resulting AgNP formulations, specifically those synthesized using plant extracts designated as AI-AgNPs and GA-AgNPs. Notably, both AI-AgNPs and GA-AgNPs exhibited potent antibacterial properties against a range of pathogenic microorganisms, including both Gram-negative and Gram-positive bacteria. This antibacterial efficacy positions these nanoparticles as promising candidates for the development of broad-spectrum antibacterial agents, particularly for applications in the healing of infected wounds.

In addition to their antibacterial activities, the AI-AgNPs and GA-AgNPs were found to possess significant antioxidant properties, which play a crucial role in mitigating oxidative stress at wound sites. The ability of these nanoparticles to enhance cellular migration further underscores their potential in promoting effective wound healing while concurrently reducing the formation of reactive oxygen species (ROS).

In conclusion, the phyto-fabrication method employed in this study is not only straightforward and rapid but also demonstrates stability and repeatability, suggesting its suitability for scaling up to large-scale production of AgNPs in an eco-friendly manner. The non-toxic nature of the AI-AgNPs and GA-AgNPs, combined with their notable antibacterial, biofilm-inhibiting, antioxidant, and wound-healing properties, underscores their potential for future therapeutic applications in the biomedical field. This research contributes to the ongoing exploration of sustainable practices in nanoparticle synthesis and their application in advanced medical technologies.

Chapter-8

Bibliography

1. O'Neil, J. Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. *Rev. Antimicrobe.* https://amr-review.org/background.html Publications (2014)
2. WHO (2020). *Antimicrobial resistance.* Geneva: World Health Organization.
3. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol. 2006 Jun 15;106(1):1-28. doi: 10.1016/j.jep.2006.03.021. Epub 2006 Apr 3. PMID: 16678368.
4. Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules. 2016 Apr 29;21(5):559. doi: 10.3390/molecules21050559. PMID: 27136524; PMCID: PMC6273146.
5. Khatoon N, Mazumder JA, Sardar M. Biotechnological applications of green synthesized silver nanoparticles. *J Nanosci Curr Res*. 2017;2(1):107. doi: 10.4172/2572-0813.1000107.
6. Ahmad F, Ashraf N, Ashraf T, Zhou RB, Yin DC. Biological synthesis of metallic nanoparticles (MNPs) by plants and microbes: their cellular uptake, biocompatibility, and biomedical applications. Appl Microbiol Biotechnol. 2019 Apr;103(7):2913-2935. doi: 10.1007/s00253-019-09675-5. Epub 2019 Feb 18. PMID: 30778643.
7. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci 2004;275:496-502.
8. Noruzi M. Biosynthesis of gold nanoparticles using plant extracts. Bioprocess Biosyst Eng. 2015 Jan;38(1):1-14. doi: 10.1007/s00449-014-1251-0. Epub 2014 Aug 5. PMID: 25090979.
9. Ahmad F, Ashraf N, Ashraf T, Zhou RB, Yin DC. Biological synthesis of metallic nanoparticles (MNPs) by plants and microbes: their cellular uptake, biocompatibility, and biomedical applications. Appl Microbiol Biotechnol. 2019 Apr;103(7):2913-2935. doi: 10.1007/s00253-019-09675-5. Epub 2019 Feb 18. PMID: 30778643.
10. Kumar VS, Navaratnam V. Neem (Azadirachta indica): prehistory to contemporary medicinal uses to humankind. Asian Pac J Trop Biomed. 2013 Jul;3(7):505-14. doi: 10.1016/S2221-1691(13)60105-7. PMID: 23835719; PMCID: PMC3695574.
11. Sarah R., Tabassum B., Idrees N., Hussain M. K. (2019). "Bio-active compounds isolated from neem tree and their applications," in *Natural bio-active compounds.* Singapore: Springer Nature, 509–528. 10.1007/978-981-13-7154-7_17
12. Aparajita Verma, Mohan Singh Mehata, Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity, Journal of Radiation Research and Applied Sciences, Volume 9, Issue 1, 2016, Pages 109-115, ISSN 1687-8507, https://doi.org/10.1016/j.jrras.2015.11.001.
13. By J.M.Garg - Own work, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=7069304

14. By Obsidian Soul - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=22581734
15. Chinnasamy G, Chandrasekharan S, Koh TW, Bhatnagar S. Synthesis, Characterization, Antibacterial and Wound Healing Efficacy of Silver Nanoparticles From *Azadirachta indica*. Front Microbiol. 2021 Feb 19;12:611560. doi: 10.3389/fmicb.2021.611560. PMID: 33679635; PMCID: PMC7932996.
16. Hasan S, Prakash J, Vashishtha A, Sharma A, Srivastava K, Sagar F, Khan N, Dwivedi K, Jain P, Shukla S, Gupta SP, Mishra S. Optimization of DNA extraction from seeds and leaf tissues of Chrysanthemum (Chrysanthemum indicum) for polymerase chain reaction. Bioinformation. 2012;8(5):225-8. doi: 10.6026/97320630008225. Epub 2012 Mar 17. PMID: 22493524; PMCID: PMC3314876.
17. Cravotto C, Fabiano-Tixier AS, Claux O, Abert-Vian M, Tabasso S, Cravotto G, Chemat F. Towards Substitution of Hexane as Extraction Solvent of Food Products and Ingredients with No Regrets. Foods. 2022 Oct 28;11(21):3412. doi: 10.3390/foods11213412. PMID: 36360023; PMCID: PMC9655691.
18. Chinnasamy G, Chandrasekharan S, Bhatnagar S. Biosynthesis of Silver Nanoparticles from *Melia azedarach*: Enhancement of Antibacterial, Wound Healing, Antidiabetic and Antioxidant Activities. Int J Nanomedicine. 2019 Dec 11;14:9823-9836. doi: 10.2147/IJN.S231340. PMID: 31849471; PMCID: PMC6913292.
19. Chandrasekharan S, Chinnasamy G, Bhatnagar S. fable phyto-fabrication of silver nanoparticles using Gmelina arborea exhibit antimicrobial and biofilm inhibition activity. Sci Rep. 2022 Jan 7;12(1):156. doi: 10.1038/s41598-021-04025-w. PMID: 34997051; PMCID: PMC8742086.
20. Aparajita Verma, Mohan Singh Mehata, Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity, Journal of Radiation Research and Applied Sciences, Volume 9, Issue 1, 2016, Pages 109-115, ISSN 1687-8507, https://doi.org/10.1016/j.jrras.2015.11.001.
21. Ahmad B, Shireen F, Rauf A, Shariati MA, Bashir S, Patel S, Khan A, Rebezov M, Khan MU, Mubarak MS, Zhang H. Phyto-fabrication, purification, characterisation, optimisation, and biological competence of nano-silver. IET Nanobiotechnol. 2021 Feb;15(1):1-18. doi: 10.1049/nbt2.12007. Epub 2021 Feb 2. PMID: 34694726; PMCID: PMC8675842.
22. Phytofabrication of nanoparticles through plant as nanofactories, Advances in Natural Sciences: Nanoscience and Nanotechnology, Mittal Jitendra, Batra Amla, Singh Abhijeet, Sharma Madan Mohan, 2014, https://dx.doi.org/10.1088/2043-6262/5/4/043002.
23. Dutt Y, Dhiman R, Singh T, Vibhuti A, Gupta A, Pandey RP, Raj VS, Chang CM, Priyadarshini A. The Association between Biofilm Formation and Antimicrobial Resistance with Possible Ingenious Bio-Remedial Approaches. Antibiotics (Basel). 2022 Jul 11;11(7):930. doi: 10.3390/antibiotics11070930. PMID: 35884186; PMCID: PMC9312340.
24. Pragyan Roy and Bhagyalaxmi Das and Abhipsa Mohanty and Sujata Mohapatra,Green synthesis of silver nanoparticles using Azadirachta indica leaf extract and its antimicrobial study, Applied Nanoscience (Switzerland),2017, volume = {7}, Springer Nature,https://doi.org/10.1007%2Fs13204-017-0621-8.
25. C. Minelli , talk on ‘Measuring nanoparticle properties: are we high and dry or all at sea?’ at ‘Nanoparticle Characterisation – Challenges for the Community’ event – IOP (Institute of Physics), book of abstracts, July 2016, London.
26. S. S. Shankar , A. Rai , A. Ahmad and M. Sastry , J. Colloid Interface Sci., 2004, 275 , 496 —502.

27. P. Velusamy , J. Das , R. Pachaiappan , B. Vaseeharan and K. Pandian , Ind. Crops Prod., 2015, 66 , 103 —109.
28. M. J. Ahmed , G. Murtaza , A. Mehmood and T. M. Bhatti , Mater. Lett., 2015, 153 , 10 —13.
29. Rekha R. Warrier, S. Mohana Priya, R. Kalaiselvi, Gmelina arborea– an indigenous timber species of India with high medicinal value: A review on its pharmacology, pharmacognosy and phytochemistry, Journal of Ethnopharmacology, Volume 267, 2021, 113593, ISSN 0378-8741, https://doi.org/10.1016/j.jep.2020.113593.
30. Kongyan Li, Wei Zhong, Penghui Li, Jianpeng Ren, Kangjie Jiang, Wenjuan Wu, Antibacterial mechanism of lignin and lignin-based antimicrobial materials in different fields, International Journal of Biological Macromolecules,Volume 252, 2023, 126281, ISSN 0141-8130, https://doi.org/10.1016/j.ijbiomac.2023.126281.
31. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001 Jul;48 Suppl 1:5-16. doi: 10.1093/jac/48.suppl_1.5. Erratum in: J Antimicrob Chemother 2002 Jun;49(6):1049. PMID: 11420333.
32. https://microbe-investigations.com/minimum-inhibitory-concentration-test.
33. Najwa Mohamad, Mohd Cairul Iqbal Mohd Amin, Manisha Pandey, Naveed Ahmad, Nor Fadilah Rajab, Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: Accelerated burn wound healing in an animal model, Carbohydrate Polymers, Volume 114, 2014, Pages 312-320, ISSN 0144-8617, https://doi.org/10.1016/j.carbpol.2014.08.025.
34. Costerton JW. Introduction to biofilm. Int J Antimicrob Agents. 1999 May;11(3-4):217-21; discussion 237-9. doi: 10.1016/s0924-8579(99)00018-7. PMID: 10394973.
35. Cavalheiro M, Teixeira MC. *Candida* Biofilms: Threats, Challenges, and Promising Strategies. Front Med (Lausanne). 2018 Feb 13;5:28. doi: 10.3389/fmed.2018.00028. PMID: 29487851; PMCID: PMC5816785.
36. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x. Epub 2011 Jul 27. PMID: 21793988.
37. S.Shiv Shankar, Akhilesh Rai, Absar Ahmad, Murali Sastry, Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth, Journal of Colloid and Interface Science, Volume 275, Issue 2, 2004, Pages 496-502, ISSN 0021-9797, https://doi.org/10.1016/j.jcis.2004.03.003.
38. Rekha R. Warrier, S. Mohana Priya, R. Kalaiselvi, Gmelina arborea– an indigenous timber species of India with high medicinal value: A review on its pharmacology, pharmacognosy and phytochemistry, Journal of Ethnopharmacology, Volume 267, 2021,113593,ISSN 0378-8741, https://doi.org/10.1016/j.jep.2020.113593.
39. Alfuraydi RT, Alminderej FM, Mohamed NA. Evaluation of Antimicrobial and Anti-Biofilm Formation Activities of Novel Poly(vinyl alcohol) Hydrogels Reinforced with Crosslinked Chitosan and Silver Nano-Particles. Polymers (Basel). 2022 Apr 16;14(8):1619. doi: 10.3390/polym14081619. PMID: 35458369; PMCID: PMC9026349.

40. Wylie MR, Merrell DS. The Antimicrobial Potential of the Neem Tree Azadirachta indica. Front Pharmacol. 2022 May 30;13:891535. doi: 10.3389/fphar.2022.891535. PMID: 35712721; PMCID: PMC9195866.
41. Alzohairy, Mohammad A., Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment, *Evidence-Based Complementary and Alternative Medicine*, 2016, 7382506, 11 pages, 2016. https://doi.org/10.1155/2016/7382506.
42. Ofusori D. A., Falana B. A., Ofusori A. E., Abayomi T. A., Ajayi S. A., and Ojo G. B., Gastroprotective effect of aqueous extract of neem *Azadirachta indica* on induced gastric lesion in rats, *International Journal of Biological and Medical Research*. (2010) 1, 219–222.
43. Barua C. C., Talukdar A., Barua A. G., Chakraborty A., Sarma R. K., and Bora R. S., Evaluation of the wound healing activity of methanolic extract of *Azadirachta Indica* (Neem) and Tinospora cordifolia (Guduchi) in rats, *Pharmacologyonline*. (2010) 1, 70–77, 2-s2.0-77953188505.
44. Osunwoke Emeka A., Olotu Emamoke J., Allison Theodore A., and Onyekwere Julius C., The wound healing effects of aqueous leave extracts of azadirachta indica on wistar rats, *Journal of Natural Science and Research*. (2013) 3, no. 6.
45. Shirwaikar, A., Ghosh, S. and Padma, G.M., 2003. Effect of Gmelina arborea Roxb. leaves on wound healing in rats. *Journal of Natural Remedies*, pp.45-48.
46. Singh P, Pandit S, Beshay M, Mokkapati VRSS, Garnaes J, Olsson ME, Sultan A, Mackevica A, Mateiu RV, Lütken H, Daugaard AE, Baun A, Mijakovic I. Anti-biofilm effects of gold and silver nanoparticles synthesized by the Rhodiola rosea rhizome extracts. Artif Cells Nanomed Biotechnol. 2018;46(sup3):S886-S899. doi: 10.1080/21691401.2018.1518909. Epub 2018 Nov 13. PMID: 30422688.
47. Soni V, Raizada P, Singh P, Cuong HN, S R, Saini A, Saini RV, Le QV, Nadda AK, Le TT, Nguyen VH. Sustainable and green trends in using plant extracts for the synthesis of biogenic metal nanoparticles toward environmental and pharmaceutical advances: A review. Environ Res. 2021 Nov;202:111622. doi: 10.1016/j.envres.2021.111622. Epub 2021 Jul 7. PMID: 34245729.
48. Mohanta YK, Biswas K, Jena SK, Hashem A, Abd Allah EF, Mohanta TK. Anti-biofilm and Antibacterial Activities of Silver Nanoparticles Synthesized by the Reducing Activity of Phytoconstituents Present in the Indian Medicinal Plants. Front Microbiol. 2020 Jun 23;11:1143. doi: 10.3389/fmicb.2020.01143. Erratum in: Front Microbiol. 2020 Sep 11;11:1784. doi: 10.3389/fmicb.2020.01784. PMID: 32655511; PMCID: PMC7324531.
49. Zaharaddeen Umar, Usman Rabiu Bello, Ankita Mathur, Giriraj Tailor, Jyoti Chaudhary, Saurabh Singh, Modulation of Biofilm with synthesized silver nanoparticles from Azadirachta indica, Current Research in Green and Sustainable Chemistry, Volume 4, 2021, 100203, ISSN 2666-0865,https://doi.org/10.1016/j.crgsc.2021.100203.
50. Ahmed, M.; Marrez, D.A.; Mohamed Abdelmoeen, N.; Abdelmoneem Mahmoud, E.; Ali, M.A.-S.; Decsi, K.; Tóth, Z. Studying the Antioxidant and the Antimicrobial Activities of Leaf Successive Extracts Compared to the Green-Chemically Synthesized Silver Nanoparticles and the Crude Aqueous Extract from *Azadirachta indica*. *Processes* **2023**, *11*, 1644. https://doi.org/10.3390/pr11061644.
51. Shirwaikar, A. & Ghosh, Soumadeep & Rao, P.G.M.. (2003). Effect of Gmelina arborea Roxb. Leaves on Wound Healing in Rats. Journal of Natural Remedies. 3. 45-48. 10.18311/jnr/2003/362.

52. Singh P, Kim YJ, Zhang D, Yang DC. Biological Synthesis of Nanoparticles from Plants and Microorganisms. Trends Biotechnol. 2016 Jul;34(7):588-599. doi: 10.1016/j.tibtech.2016.02.006. Epub 2016 Mar 2. PMID: 26944794.
53. Baig, Nadeem, Irshad Kammakakam, and Wail Falath. "Nanomaterials: A review of synthesis methods, properties, recent progress, and challenges." *Materials advances* 2, no. 6 (2021): 1821-1871.