

## REVIEW ON GREEN SYNTHESIZED SILVER NANOPARTICLES INCORPORATED NANOHYDROXYAPATITE USING BIOMATERIAL AND ITS APPLICATION

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### ABSTRACT

Nanotechnology has emerged as a rapidly advancing field with significant applications in biomedical and pharmaceutical sciences. Among various nanomaterials, silver nanoparticles (AgNPs) and nanohydroxyapatite (nHA) have gained considerable attention due to their unique antimicrobial and osteoconductive properties. This review focuses on the green synthesis of silver nanoparticle-incorporated nanohydroxyapatite (nHA-AgNP) using plant-based biomaterials, particularly *Ocimum tenuiflorum* (Tulsi) and *Mentha spicata* (Mint). Green synthesis offers an eco-friendly, cost-effective, and sustainable alternative to conventional chemical methods by utilizing natural phytochemicals as reducing and stabilizing agents. The review discusses the synthesis of nanohydroxyapatite from eggshell-derived calcium sources, the preparation of silver nanoparticles using Tulsi and Mint extracts, and the fabrication of the nHA-AgNP

nanocomposite. Various characterization techniques such as X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) are highlighted for confirming the structural and physicochemical properties of the synthesized material. The incorporation of silver nanoparticles significantly enhances the antibacterial activity of nanohydroxyapatite against a broad spectrum of pathogenic microorganisms while maintaining its excellent biocompatibility and bone regenerative potential. The developed nanocomposite shows promising applications in bone tissue

engineering, dental restorations, implant coatings, wound healing, and drug delivery systems. Overall, green-synthesized nHA-AgNP represents a sustainable and innovative biomaterial with substantial potential for future biomedical applications.

**KEYWORDS:** Green synthesis, Silver nanoparticles, Nanohydroxyapatite, nHA-AgNP, Tulsi, Mint, Biomaterials, Antibacterial activity, Bone tissue engineering, Dental implants, Regenerative medicine, Nanotechnology.

## 1. INTRODUCTION

The field of nanotechnology is the most dynamic region of inquiry about in present day materials science. In spite of the fact that there are numerous chemical as well as physical strategies, green union of nanomaterials is the most developing strategy of amalgamation.

The Plant-mediated union of nanoparticles is a green chemical approach that interfaces nanotechnology with plants.

Novel strategies of in a perfect world synthesizing NPs are in this way thought that are shaped at encompassing temperatures, unbiased pH, moo costs and ecologically inviting design. Keeping these objectives in see nanomaterials have been synthesized utilizing different routes. The point of the show think about is to get ready, characterize and assess of silver nanoparticles consolidated nanohydroxyapatite as inserts for bone abandons and distortion.

Hydroxyapatite is a normally happening uncommon mineral, but its most common event is as the primary inorganic constituent of normal bone and teeth mineral.

It has amazing biocompatibility, bioactivity.

Nanohydroxyapatite was disconnected from egg shells and silver nanoparticles were arranged from Tulasi. The silver nanoparticles were consolidated into the arranged nanohydroxyapatite and the amalgamation was affirmed by the characterization such as XRD, FTIR, SEM.

The present study also focus on the application of the in the synthesized nHA-AgNP composite for its antibacterial movement.

### 1.1 NANOCHEMISTRY

Nanochemistry is the combination of Chemistry and Nano science. It is related with

amalgamation of building squares which are subordinate on estimate, surface, shape and imperfection properties.

It is being utilized in chemical, materials and physical, science as well as building, natural and restorative applications. This too includes the ponder of blend and characterization of materials of Nano scale measure.

It is generally a unused department of chemistry that is concerned with interesting properties related with congregations of particles or particles of nano scale measure (1-100nm)so the nanoparticle s lie some place between person iotas or atoms of bulk materials.<sup>[1]</sup>

It has employments in chemical, physical and materials science, building and organic and therapeutic applications. Utilizing single iotas as building pieces offers modern ways to make inventive materials, the opportunity to make the littlest highlights conceivable in coordinates circuits and the chance to investigate quantum computing for case.

It might appear generally unused, but nanochemistry has been utilized for numerous a long time, for illustration in sunscreens that retain UV light, in clear coatings for cars which secure the shinning paint colours underneath, or in carbon nanotubes for lightweight car parts or wearing hardware.

It has been utilized to think about the wellbeing and security impacts of airborne and waterborne nanosized particulates, and nanoparticles have been utilized to clear up or neutralizes toxins.

### **1.2.1 Applications of Nanochemistry**

#### **(i) Medicine**

Nanochemistry has found particularly significant applications in the pharmaceutical and biomedical fields. A widely recognized example is the incorporation of zinc oxide (ZnO) and titanium dioxide (TiO<sub>2</sub>) nanoparticles in sunscreen formulations. These nanoparticles protect the skin by absorbing or reflecting ultraviolet radiation, thereby preventing photoexcitation-induced DNA damage to dermal cells. The ability to engineer nanoparticles with targeted photoreactive properties represents a major advancement in dermal photoprotection.<sup>[2]</sup>

#### **(ii) Catalysis and Nanoenzymes**

Nanostructured materials engineered to mimic enzymatic activity—commonly referred to as

nanozymes—have attracted increasing scientific attention owing to their unique catalytic properties. Nanoparticles within the 1–100 nm size range exhibit distinctive optical, magnetic, electronic, and catalytic characteristics that are highly favorable for enzyme-like functions. The precisely controllable surface chemistry and structural regularity of these nanomaterials enable the construction of complex active-site analogs, making them versatile platforms for a range of specific catalytic applications.<sup>[3]</sup>

### **(iii) Wound Healing**

Nanochemistry has demonstrated promising applications in the management of wounds and abrasions. Electrospinning, a technique originally employed in tissue engineering, has been adapted for the fabrication of nanofiber-based wound dressings. These nanofibers facilitate cell proliferation, impart antibacterial activity, and create a controlled microenvironment conducive to wound healing. Due to nanotopographical features at the interface, nanofiber dressings exhibit enhanced surface-area interactions with wound tissue compared to their macroscale equivalents, potentially yielding superior *in vivo* therapeutic outcomes.<sup>[4,5]</sup>

### **1.3 Nanohydroxyapatite (nHA)**

Hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] is among the most extensively studied bioceramics in the field of regenerative medicine, primarily due to its established biocompatibility and its compositional similarity to the mineral phase of natural bone and dental tissues. In the context of preventive and restorative dentistry, nanohydroxyapatite has demonstrated significant remineralizing effects on early enamel lesions, with efficacy reportedly superior to that of conventional fluoride-based treatments. Its incorporation as an additive into dental materials such as glass ionomer cements has further resulted in notable improvements in their mechanical properties.<sup>[6]</sup>

The unique biophysical characteristics of nHA—including its capacity to form direct chemical bonds with osseous tissue, its non-toxic and non-inflammatory nature, and its ability to stimulate osteoblast activity—have established it as a preferred material in periodontology, oral and maxillofacial surgery, and dental implantology. Its osteoinductive properties and ability to enhance bone-to-implant integration are particularly valued in clinical settings.<sup>[6,7]</sup>

A persistent challenge in the clinical application of implant-grade biomaterials, including synthetic hydroxyapatite, is the risk of peri-implant bacterial infections. Pathogenic microorganisms can colonize implant surfaces, forming biofilms that are resistant to

conventional antibiotic therapy. Such infections often result in chronic pain and progressive loss of surrounding bone tissue. To mitigate this risk, significant research interest has been directed toward the controlled incorporation of antimicrobial agents—particularly metals such as silver, copper, and zinc—into hydroxyapatite-based matrices. These agents exhibit broad-spectrum antibacterial activity with a comparatively low propensity to induce bacterial resistance.<sup>[8-10]</sup>

#### 1.4 Green Synthesis of Nanoparticles

Green chemistry—also referred to as sustainable chemistry—encompasses the design of chemical products and processes that minimize or eliminate the use and generation of hazardous substances. It advocates for pollution prevention and zero-waste principles at both laboratory and industrial scales, promoting the use of prudent, ecologically compatible methodologies that improve process yields while reducing waste disposal costs.

In the context of materials science, green synthesis has emerged as a reliable, sustainable, and eco-friendly framework for the fabrication of a broad spectrum of nanomaterials, including metal and metal oxide nanoparticles and hybrid nanostructures. This approach has gained recognition as a viable alternative to conventional synthetic methods, which often involve hazardous reducing agents and generate toxic by-products. Green synthesis protocols aim to eliminate such undesirable outcomes by utilizing clean solvent systems and naturally occurring biological resources.<sup>[11]</sup>

Biological platforms suitable for green nanoparticle synthesis include bacteria, fungi, algae, and plant extracts. Among these, plant-extract-mediated synthesis is particularly attractive due to its non-pathogenic nature, operational simplicity, and amenability to large-scale production. A wide variety of metal nanoparticles have been successfully synthesized via plant-mediated routes, with the resulting materials demonstrating unique optical, thermal, magnetic, and electrical properties.<sup>[11]</sup>

#### 1.5 Green Synthesis of Silver Nanoparticles

The plant-extract-mediated synthesis of silver nanoparticles proceeds extracellularly, offering notably faster reaction kinetics compared to microbial synthesis methods. This approach is also highly scalable, making it practically feasible for large-scale production of AgNPs.

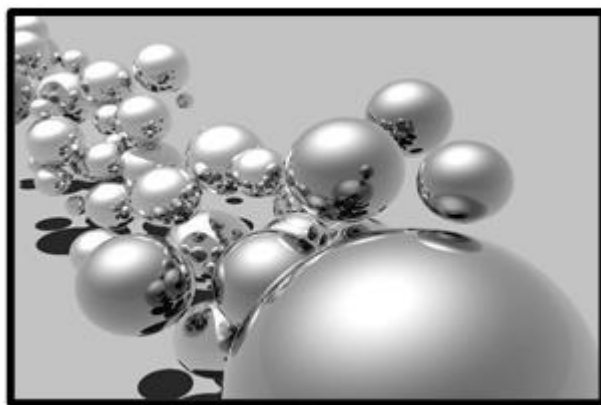
Numerous plant species have demonstrated the capacity to reduce silver nitrate ( $\text{AgNO}_3$ ) and facilitate the formation of stable AgNPs in solution. Documented examples include

Pelargonium graveolens, Medicago sativa, Azadirachta indica, Lemongrass, Aloe vera, Cinnamomum camphora, Emblica officinalis, Capsicum annum, Diospyros kaki, Carica papaya, Coriandrum sp., Boswellia ovalifoliolata, Tridax procumbens, Jatropha curcas, Solanum melongena, Datura metel, and Citrus aurantium, among others.<sup>[12-20]</sup>

Ocimum tenuiflorum (Tulsi or Holy Basil) is a medicinal herb widely distributed and cultivated across India, Malaysia, Australia, West Africa, and parts of the Arabian Peninsula. Its leaves have traditionally been employed in the treatment of various infectious conditions. The antibacterial activity of Tulsi has been attributed primarily to the essential oil constituents present in the leaves, particularly eugenol.

Similarly, Mentha species (Mint) are aromatic medicinal plants widely cultivated throughout India and many parts of the world. Mint leaves are traditionally used for the treatment of microbial and respiratory disorders due to the presence of bioactive compounds such as menthol and menthone, which exhibit significant antibacterial activity.

The present study employs Tulsi and Mint leaf extracts as bioreductants for the synthesis of silver nanoparticles, with the aim of synergistically combining the inherent antimicrobial activities of both silver and the plant extracts to achieve enhanced antibacterial efficacy.<sup>[21]</sup>



**Figure 1: Silver nanoparticles.**

## **1.6 Applications of Silver Nanoparticles**

### **1.6.1 Dental and Biomedical Applications**

Silver nanoparticles have found extensive utility in dentistry and biomedical engineering owing to their potent and broad-spectrum antimicrobial properties. The oral cavity serves as a gateway to the systemic circulation, and protection against oral biofilm formation is a central

concern in dental practice. AgNPs have been successfully integrated into a range of dental biomaterials— including endodontic sealers, periodontal dressings, restorative composites, and orthodontic adhesives—to reduce biofilm formation without compromising the mechanical performance of the host material. The large surface-to-volume ratio and small particle size of AgNPs confer superior antimicrobial activity, making them fillers of choice in advanced dental biomaterial formulations.<sup>[22]</sup>

### 1.6.2 Antibacterial Textile Applications

Silver nanoparticles have been incorporated into cotton textile fibers through impregnation techniques to confer durable antibacterial properties. In one representative study, a nano-silver colloidal suspension was prepared by combining silver nitrate with an amino-terminated hyperbranched polymer (HBP-NH<sub>2</sub>) under dynamic mixing conditions at ambient temperature. Cotton fabrics treated with this nano-silver colloid demonstrated a bacterial reduction of 99.01% against *Staphylococcus aureus* and 99.26% against *Escherichia coli*, with a residual silver content of approximately 88 mg/kg on the fabric surface. Crucially, the antibacterial efficacy was maintained above a 98.77% reduction threshold even after 20 consecutive domestic wash cycles. Scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) analyses confirmed that the silver nanoparticles were uniformly distributed and firmly anchored on the cotton fiber surface, predominantly in the Ag<sup>+</sup> state.<sup>[23]</sup>

### 1.6.3 Antiviral Activity

The remarkably high surface area of silver nanoparticles relative to their mass enables superior contact with microbial targets, underpinning their potent antimicrobial and antiviral effects. Upon interaction with bacterial cells, AgNPs become adsorbed onto the cell membrane and may traverse into the intracellular milieu. Within the cell, silver nanoparticles interact with sulfur- containing proteins embedded in the cell membrane and with phosphorus-containing biomolecules such as DNA. Intracellular silver ions form low-molecular-weight complexes that impair DNA replication and disrupt the respiratory electron transport chain, ultimately halting cell division and leading to cell death. The release of Ag<sup>+</sup> ions from nanoparticles within bacterial cells further potentiates the antibacterial mechanism.<sup>[24]</sup>

### 1.6.4 Antimicrobial Food Packaging

Silver nanoparticles have been investigated as functional additives in antimicrobial food packaging materials due to their broad-spectrum activity against pathogenic bacteria and food

spoilage organisms. Both biodegradable and non-biodegradable polymer matrices have been explored as carriers for AgNPs, with the aim of extending the shelf life of packaged food products. However, rigorous migration testing is considered mandatory for any new food packaging material incorporating AgNPs, in order to ensure compliance with safety regulations and define acceptable concentration limits for inclusion in packaging substrates.<sup>[25]</sup>

### 1.7 Silver-Incorporated Nanohydroxyapatite (nHA-AgNP)

Hydroxyapatite-based coatings for biomaterial surfaces have been extensively explored as vehicles for localized antibiotic delivery, exploiting the osteoconductive properties of HA. However, the loading efficiency and release kinetics of antibiotics from HA are strongly influenced by the acid–base character of the drug molecule. Acidic antibiotics exhibit higher affinity for HA than their basic counterparts, owing to chelating interactions between carboxylate groups and the calcium ions of the HA lattice. This differential binding behavior imposes limitations in clinical scenarios where basic antibiotics are required.<sup>[26]</sup>

The emergence of antibiotic-resistant bacterial strains has further underscored the need for alternative antimicrobial strategies. Metals such as silver and zinc have attracted attention as viable alternatives, capable of disrupting bacterial adhesion and biofilm formation on implant surfaces.<sup>[27]</sup> The well-documented antibacterial efficacy of silver at low concentrations, its activity against a broad range of pathogens implicated in implant-associated infections, and its negligible toxicity toward mammalian cells make silver an attractive candidate for incorporation into biomaterial scaffolds.<sup>[28-33]</sup>

Silver-containing biomaterials may incorporate either elemental silver or Ag<sup>+</sup> (in the form of silver salts or complexes) within organic (polymeric) or inorganic (bioglass and HA) matrices. While the antibacterial properties of silver-loaded polymers such as polyamide, polyurethane, and PMMA, as well as silver-doped bioglasses, have been thoroughly examined *in vitro*<sup>[34-37]</sup>, detailed investigations into the bactericidal characteristics of Ag-HA composites remain comparatively limited. Recent investigations have reported the preparation of Ag-loaded HA composites via ion-exchange processes such as sol-gel synthesis and co-precipitation, in which silver substitutes for calcium within the HA crystal lattice, yielding a calcium-deficient hydroxyapatite phase.<sup>[38-41]</sup>

Although such materials exhibit satisfactory antimicrobial performance, this approach carries

two key limitations

- Depletion of calcium from the HA lattice may adversely affect the structural integrity of nHA and diminish its osteoinductive potential.<sup>[40,41]</sup>
- The pH-dependent rapid release of silver from the material may result in suboptimal sustained antimicrobial activity.

These limitations have prompted growing interest in silver nanoparticles as an alternative bactericidal reservoir, given their relatively low solubility in biological fluids. Studies on nanosilver-loaded polymer films indicate that the duration of silver release is strongly correlated with the total silver nanoparticle loading.<sup>[28]</sup> Furthermore, the biocidal efficacy of colloidal silver nanoparticles is inversely related to particle size—smaller particles exhibiting greater antimicrobial potency—though particle aggregation remains a recognized challenge in nanoparticle applications.<sup>[31]</sup> A commonly employed strategy to address aggregation is the immobilization of nanoparticles on solid support substrates.

Despite the considerable clinical promise of nHA-Ag composites—which combine osteoconductive and bactericidal functionalities—reports of silver nanoparticles supported on hydroxyapatite substrates remain limited in the literature. Such materials have been prepared by co-sputtering of silver and HA to form coatings on titanium surfaces,<sup>[42]</sup> and by in situ reduction of silver cations on HA particle surfaces. The present work introduces a facile and economical approach to fabricate a silver-supported hydroxyapatite nano-hybrid composite containing approximately 1 wt % elemental silver, with demonstrable antimicrobial activity.<sup>[43]</sup>

## 2. DRUG PROFILE

### 1. *Ocimum tenuiflorum* (Tulsi)<sup>[44]</sup>



**Figure 2:** *Ocimum tenuiflorum* (Tulsi).

Table No. 1: Taxonomical Classification of *Ocimum tenuiflorum* (Tulsi)

Category	Information
Plant Sample	<i>Ocimum tenuiflorum</i> (Tulsi)
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	<i>Ocimum</i>
Species	<i>O. tenuiflorum</i>
Common Names	Tulsi, Holy Basil, Sacerd Basil
Uses of Tulsi	Used in herbal teas and Ayurvedic medicine; Helps in relieving cough and cold; Boosts immunity; Used as a stress reliever (adaptogen); Used in religious rituals; Helps in digestion
Chemical Constituents	Eugenol, Ursolic acid, Rosmarinic acid, Linalool, Essential oils
Medicinal Properties	Antibacterial; Antiviral; Anti-inflammatory; Antioxidant; Immunomodulatory; Helps in respiratory disorders

## 2. *Mentha spicata* (Mint)<sup>[45]</sup>

Figure 3: *Mentha spicata* (Mint).Table No. 2: Taxonomical Classification of *Mentha spicata* (Mint)

Category	Information
Plant Sample	<i>Mentha spicata</i> (Mint)
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	<i>Mentha</i>

<b>Species</b>	<i>M. spicata</i>
<b>Common Names</b>	Mint, Spearmint, Pudina
<b>Uses of Mint</b>	Used as flavoring agent; Helps in digestion; Used in mouth fresheners; Used in toothpaste and dental products; Possesses antimicrobial activity; Used in herbal medicines and teas
<b>Chemical Constituents</b>	Menthol, Menthone, Flavonoids, Rosmarinic acid, Essential oils
<b>Medicinal Properties</b>	Antibacterial; Antioxidant; Anti-inflammatory; Antifungal; Cooling and soothing effect

### 3. MATERIALS AND METHOD

This chapter provides a concise description of the raw materials and experimental reagents utilized in the present investigation.

#### 3.1 Materials Required

- Orthophosphoric acid ( $H_3PO_4$ ) – 0.3 M
- Eggshells – freshly collected, washed, and dried
- Tulsi (*Ocimum tenuiflorum*) leaves – freshly collected
- Mint (*Mentha spicata*) leaves – freshly collected
- Silver nitrate ( $AgNO_3$ ) – 1 M aqueous solution
- Distilled water
- Whatman No. 40 filter paper<sup>[46]</sup>

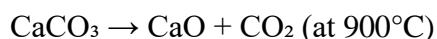
#### 3.2 Equipment Required

- Muffle furnace
- Magnetic stirrer with hot plate
- pH meter
- Hot air oven
- Centrifuge (capable of 5000 rpm)
- Electronic weighing balance
- Conical flasks and beakers
- XRD, FTIR, and SEM instrumentation (for characterization)<sup>[46]</sup>

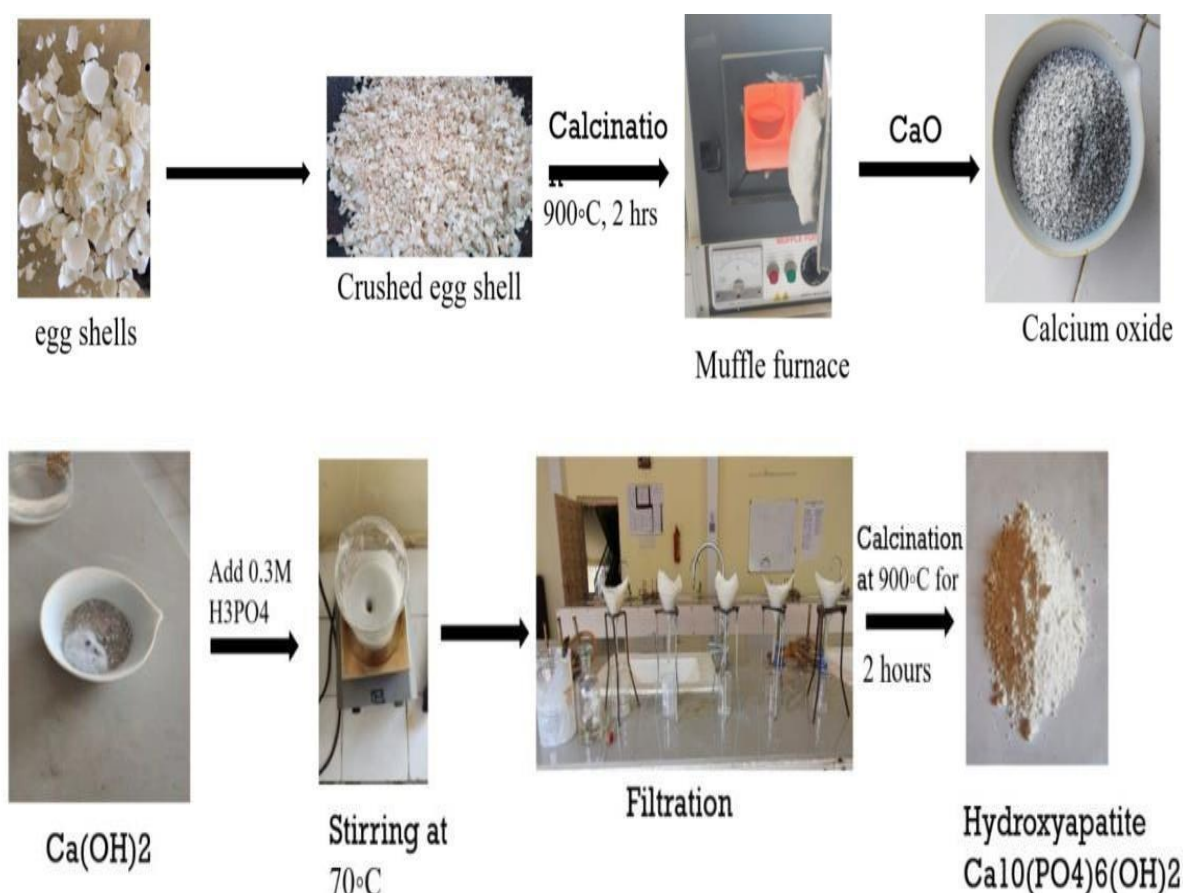
#### 3.3 Synthesis of Nanohydroxyapatite from Eggshells

Nanohydroxyapatite was synthesized through a wet chemical precipitation technique. Eggshells were collected, thoroughly rinsed with distilled water, air-dried, and subsequently crushed into a coarse powder. The crushed eggshells were then subjected to calcination at 900°C for 2 hours in a muffle furnace, resulting in the thermal decomposition of calcium

carbonate ( $\text{CaCO}_3$ ) to calcium oxide ( $\text{CaO}$ ) through the reaction:



The freshly prepared  $\text{CaO}$  was hydrated with a stoichiometric quantity of distilled water to produce calcium hydroxide [ $\text{Ca}(\text{OH})_2$ ]. A 0.5 M  $\text{Ca}(\text{OH})_2$  suspension was prepared and vigorously stirred at  $70^\circ\text{C}$  for one hour. A 0.3 M orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ) solution was subsequently added dropwise under slow continuous stirring, while maintaining the solution pH in the range of 10.5–11.0 using ammonium hydroxide as necessary. After complete addition of the acid, the reaction mixture was stirred for a further one hour at  $70^\circ\text{C}$ . The resulting precipitate was filtered, washed with distilled water, and dried in a hot air oven at  $105^\circ\text{C}$  for 24 hours. The dried powder was then subjected to a second calcination at  $900^\circ\text{C}$  for 2 hours to yield the final nanohydroxyapatite product.<sup>[46]</sup>



**Figure 4: Preparation of hydroxyapatite.**

### 3.4 Preparation of Plant Extracts

#### 3.4.1 Tulsi (*Ocimum tenuiflorum*) Leaf Extract

Healthy, fresh Tulsi leaves were collected and thoroughly washed under running tap water to

remove surface impurities. The cleaned leaves were spread on an absorbent surface and allowed to air-dry at room temperature. Approximately 20 g of dried leaves were weighed, finely chopped, and added to 100 mL of distilled water in a beaker. The mixture was then subjected to boiling for 30 minutes to facilitate the extraction of phytochemical constituents. After cooling to ambient temperature, the extract was filtered through Whatman No. 40 filter paper to obtain a clarified aqueous extract, which was stored at 4°C until further use.<sup>[47]</sup>

### 3.4.2 Mint (*Mentha spicata*) Leaf Extract

The preparation protocol for Mint leaf extract was identical to that described for Tulsi. Fresh Mint leaves were collected, washed, air-dried, and 20 g of the dried material was extracted in 100 mL of distilled water by boiling for 30 minutes. The cooled extract was similarly filtered through Whatman No. 40 filter paper and stored at 4°C.<sup>[48]</sup>

### 3.4.3 Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by combining 100 mL of the aqueous plant extract with 50 mL of a 1 M silver nitrate ( $\text{AgNO}_3$ ) solution. The mixture was allowed to react at ambient temperature under intermittent agitation. The bio reduction of silver ions was monitored visually by the gradual development of a brownish coloration in the reaction mixture, indicative of AgNP formation. The resulting silver nanoparticle suspension was allowed to settle, after which the nanoparticles were separated by filtration through Whatman No. 40 filter paper and subsequently dried.<sup>[49]</sup>



**Figure 5: Preparation of silver nanoparticles.**

### 3.4.4 Preparation of Silver-Incorporated Nanohydroxyapatite (nHA-AgNP)

To prepare the nHA-AgNP nanocomposite, 0.50 g of nanohydroxyapatite powder was dispersed in 25 mL of deionized water under vigorous magnetic stirring at 60°C until completely dissolved. Subsequently, 0.25 g of the synthesized silver nanoparticles was added

to this suspension with continued stirring, and the reaction mixture was allowed to react overnight at ambient temperature. The resulting product exhibited a characteristic greenish-brown coloration. The nHA-AgNP composite was then recovered by centrifugation at 5000 rpm, washed with deionized water, and dried for subsequent characterization.<sup>[50]</sup>



**Figure 6:** solution was stirred to obtain the silver incorporated nanohydroxyapatite.

### 3.4.5 Antimicrobial Activity by Disk Diffusion Method Introduction

Antimicrobial activity means the ability of a substance to stop or kill microorganisms such as bacteria. The disk diffusion method is a simple laboratory technique used to check the antibacterial activity of antibiotics, plant extracts, nanoparticles, and other antimicrobial agents.

#### Principle

In this method, paper disks containing the test sample are placed on a Mueller–Hinton agar plate inoculated with bacteria. The antimicrobial substance diffuses into the agar medium and inhibits bacterial growth around the disk. This forms a clear circular area called the zone of inhibition.

Larger zone of inhibition indicates stronger antibacterial activity, while a smaller zone indicates weaker activity.

#### Procedure

1. Prepare Mueller–Hinton agar plates.
2. Prepare bacterial suspension and spread it evenly on the agar surface using a sterile swab.
3. Place antimicrobial disks or sample disks on the agar plate using sterile forceps.
4. Incubate the plates at 35–37°C for 18–24 hours.
5. Measure the zone of inhibition around the disks in millimeters.

#### Zone of Inhibition

- 0–5 mm → Weak activity

- 6–10 mm → Moderate activity
- More than 10 mm → Strong activity<sup>[51]</sup>

#### 4. CONCLUSION

The present review concludes that the green synthesis of silver nanoparticle-incorporated nanohydroxyapatite (nHA-AgNP) using Mint (*Mentha spicata*) and Tulsi (*Ocimum tenuiflorum*) is an eco-friendly, safe, and effective method for developing advanced biomaterials. The plant extracts act as natural reducing and stabilizing agents, which helps to avoid the use of harmful chemicals during nanoparticle synthesis.

The synthesized nHA-AgNP nanocomposite showed excellent physicochemical properties such as nanosized particles, good crystallinity, high surface area, and uniform morphology. Characterization studies like XRD, FTIR, and UV-Vis spectroscopy confirmed the successful formation of the nanocomposite and its stability.

The incorporation of silver nanoparticles significantly improved the antibacterial activity of hydroxyapatite against both Gram-positive and Gram-negative bacteria. The Mint and Tulsi mediated silver nanoparticles showed strong inhibitory effects against *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli*. The antibacterial action mainly occurs due to the release of silver ions, which damage bacterial cell membranes and inhibit their growth.

In addition, nanohydroxyapatite maintained its biocompatibility and bone regenerative properties, making the nHA-AgNP composite suitable for applications such as bone tissue engineering, dental materials, implant coatings, and drug delivery systems. Future research should focus on *in vivo* studies, toxicity evaluation, and large-scale production for successful clinical applications.

#### 5. FUTURE SCOPE

The green synthesis approach adopted in this study presents several attractive prospects for further development:

- The entire synthesis process relies on natural plant-based reducing and stabilizing agents, rendering the method environmentally benign and free from chemical waste.
- The scalability of plant-extract-mediated nanoparticle synthesis to industrial production volumes offers significant commercial potential.
- The use of renewable, biocompatible, and low-cost precursor materials (eggshells and plant

extracts) supports cost-effective and sustainable manufacturing.

- The nHA-AgNP composite shows promise as a high-yield, multifunctional biomaterial with potential for diverse biomedical applications including bone grafts, implant coatings, and drug delivery.
- Further characterization using UV-Visible spectroscopy may complement XRD, FTIR, and SEM analyses to provide a comprehensive physicochemical profile of the nanocomposite.

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