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Green Synthesis of Eggshell-Derived Calcium Oxide and Silver Nanoparticles: Characterization, Antibacterial Activity and Cosmetic Applications

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Abstract: *Nanoparticles are extremely small particles with sizes less than 100 nm and exhibit unique physical and chemical properties due to their high surface area-to-volume ratio. These properties make them highly useful in various scientific and industrial applications. Nanoparticles can be classified into different types such as carbon-based, ceramic-based, metal-based, semiconductor-based, and polymer-based nanoparticles. Among these, metal and metal oxide nanoparticles are widely used because of their catalytic, electrical, optical, and antimicrobial properties. Calcium oxide nanoparticles derived from eggshells have gained attention as an eco-friendly and sustainable material. Nanoparticles have significant applications in medicine, cosmetics, environmental purification, drug delivery, and industrial products, making them an important area of research in modern nanotechnology.*

Keywords: Nanoparticles, Eggshell waste, Calcium oxide (CaO), Silver nanoparticles, antimicrobial activity, Nanotechnology, Environmental applications

I. INTRODUCTION

1.1 What are Nanoparticles?

A nanoparticle is an extremely small particle with a diameter of less than 100 nm. Despite its minute size, it behaves as a single unit in terms of its properties and transport characteristics. Nanoparticles are widely used in various sectors because their small size imparts unique physical, chemical, and biological properties. These distinctive characteristics make nanoparticles suitable for applications in fields such as medicine, pharmaceuticals, electronics, agriculture, environmental science, and cosmetics. Consequently, nanotechnology and nanoparticle research have become some of the most extensively studied areas in modern science [1].

Nanoparticles are very special because it makes relation between bulk material and atoms or molecules, properties of bulk material do not depend on their size. If we reduce size of any material to nanoscale then its physical and chemical properties also change according to its size. Surface area of nanoparticle is important than area of atom, that why its physical property is different [2]. High surface area to volume ratio is very important for speeding the chemical reaction happen at rate proportional to available surface area of reactant, therefore nanoparticles are more capable of giving reactions. Nanoparticles can be of different types depending on their size, texture, and properties. Generally, they are classified into the following groups: The physical and chemical characteristics of substances at the nanoscale often diverge significantly from their macroscopic or "bulk" counterparts. several ways for variation including shifts in optical properties, thermal stability, catalytic activity, electrical transport, and magnetic behaviour. Furthermore, a material's mechanical durability and atomic arrangement may transform when reduced to a nanometre scale [3]. A parallel example can be seen in silver: while bulk silver melts at about 962°C, silver nanoparticles can start to melt at a much lower temperature, around 200–400°C it depends on their size of the material. Nanoparticles come in many different forms and are made from a huge range of materials. This study advances green nanotechnology [4].

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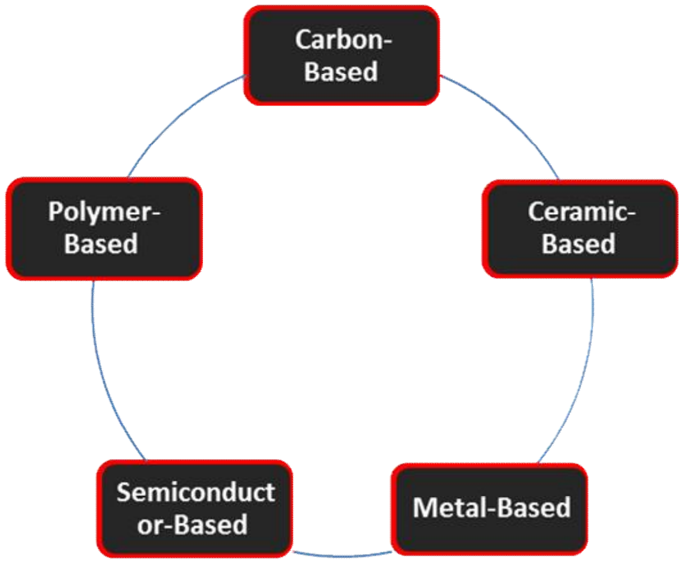

Metal nanoparticles:

Metal nanoparticles (MNPs) are microscopic pieces of pure metal or alloys, usually between 1 and 100 nanometres. Because of their tiny size and massive surface area, they behave differently than bulk metals which showing unique electrical, optical, and chemical traits that make them incredibly useful in medicine, electronics, and green energy [5].

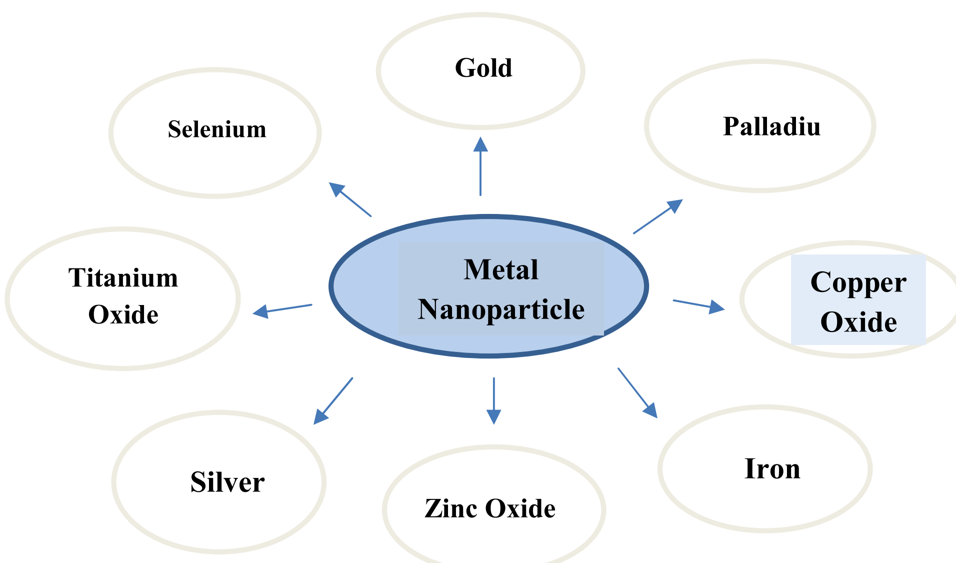

Fig 1.1 Types of Metal Nanoparticles

1.2 Why Silver Nanoparticles?

Among the various types of metal nanoparticles silver nanoparticles (AgNPs) are study because occupy a unique position due to their antimicrobial properties. Silver has been recognized for thousands of years for its ability to inhibit the growth of microorganisms and bacteria. In ancient times, silver vessels were commonly used to store water and keep it fresh for extended periods. At the nanoscale, the antimicrobial effectiveness of silver is significantly enhanced because of its increased surface area and greater interaction with microbial cells. As a result, silver nanoparticles exhibit strong antibacterial, antifungal, and antiviral activities, making them valuable in biomedical, pharmaceutical, and cosmetic applications. [6].

Silver nanoparticles release silver ion into surrounding environment as these ions are very toxic that kill bacteria, fungi and viruses. They damage the bacterial cell wall, interfere with cells energy production and disrupt its DNA to make it impossible for bacteria to develop resistance as they do with antibiotics. This is very big advantage at a time when antibiotics resistance becomes one of the biggest health threats in the world [7]. Outside their antimicrobial power, AgNps are also very good electrical conductor, having very unique optical properties, and are being studied in cancer therapy, wound dressing, water purification, food packaging and also textile coating's ability to use silver at such small concentration and yet get huge effect makes AgNps one of the most commercially very important nanoparticles in the world today [8].

1.3 Why Use Eggshell Waste?

Universal, the egg represents a prime source of protein. Higher egg production will generate great quantities of eggshell waste that will end up in the environment. Eggshell is a solid waste produced by households and industries. Mostly traditional methods for making silver nanoparticles involves unsafe reducing agents like sodium borohydride, which is very dangerous to handle and also very harmful for environment. The synthesis process needs high temperature, good equipment's and they generate harmful chemical wastes that needs proper disposal, Hen Eggshell are composed of 94-97 % calcium carbonate with small amount of magnesium carbonate, calcium phosphate and protein. When the egg shells treated at high temperature around 800-900°C, the calcium carbonate decomposes into calcium oxide, which is also knows as quicklime [9].

Calcium oxide is very strong alkaline compound with powerful and strong reducing and stabilizing properties. When calcium oxide mixed with silver nitrate solution, it gives alkaline environment that help to converts silver ion into silver atoms, which later combine together to form a group of nanoparticles. The protein and organic compounds present in eggshell membrane helps to stabilize these nanoparticles and prevent them from clumping [10]. The most existing this here is that the eggshells are most useless and waste product. Globally million tons of eggshells are discarded every year from home, bakeries, restaurants and food industries. Most of the waste are dumped where it decomposes and can generate ammonia and hydrogen sulphide gases, resulting into air and soil pollution. By finding valuable use of eggshell waste, this project not only produce useful nanoparticles, but it also helps to solve actual environmental problems [11].

1.4 How Waste Material Can Reduce Pollution

The most important idea behind this project is the principle of green chemistry are follows that is to use naturally available, low-toxicity, and preferably waste materials to carry out chemical processes that would otherwise require harmful reagents. This is especially very important in nanotechnology [12]. The eggshells that would have ended up in a waste now serve a useful purpose, reducing solid waste. Secondly eggshell-derived CaO acts as a natural reducing and stabilizing agent, there is less need for synthetic toxic chemicals, reducing chemical waste [13]. Third, the synthesis process is relatively simple and can be carried out at mild conditions, meaning lower energy consumption compared to many industrial nanoparticle production methods [14]. On large scale, the silver nanoparticles produced through this method can be used for environmental cleanup. AgNPs are being studied for water purification [15]. They are also used in textile coatings to prevent the growth of odour-causing bacteria, reducing the need for washing which in turn saves

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water. those nanomaterials can then be applied to reduce pollution and improve public health. It is a small-scale demonstration of how sustainable thinking can change the way we approach material science [16].

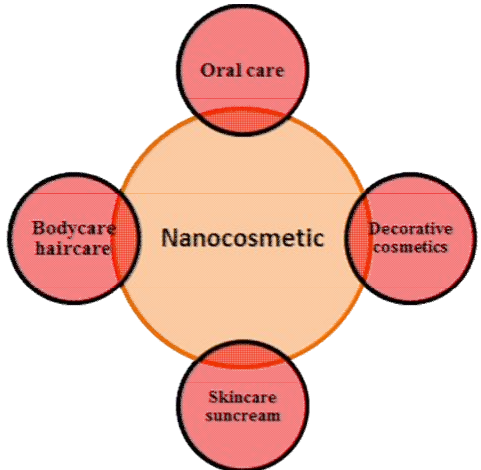

Fig 1.2 Nanomaterials in various Cosmetic Applications.

The synthesis of NPs from biodegradable wastes is to reduce and reuse the waste that pollutes our ecosystem and product with good value that could be used in various applications [17]. The present study contributes to an eco-friendly, simple, and sustainable Sustainability method of NPs synthesis that could help in reducing environmental pollution [18]

II. MATERIALS AND METHODS

2.1. Chemicals: Hydrochloric acid (HCl) used for the antimicrobial analysis with different range of dilution prepared, Muller–Hinton agar powder and Agar-agar powder, Cocamidopropyl betain used as surfactant for preparation of face wash

2.2. Ecofriendly materials: Beetroot, Aloe vera gel, Neem, Glycerine, rose water, hibiscus flower extract, Rice water was added in facewash. They make natural product and safe for all types of skins.

2.3. Equipment: The technical execution of this process relies on standard laboratory hardware, conical flasks, and measuring cylinders for precise mixing. A magnetic stirrer or heating plate is used to maintain consistent reaction conditions, while a centrifuge is essential for purifying the nanoparticles.

2.4. Methodology

Method 1: Direct Process

2.4.1. Preparing the Eggshell Extract

- **Initial Cleaning:** Thoroughly wash the eggshells with deionized water to remove any dirt or organic residue.
- **Shell Processing:** Once dry, you can crush the entire shell which converts into a fine powder.
- **Deep Purification:** To ensure the highest quality which is use an ultrasonic bath to shake off any microscopic impurities.

2.4.2. Calcination process

- **The Furnace Step:** Place the powder in a muffle furnace at 800–900°C for 2 to 4 hours.

$$CaCO_3 \rightarrow CaO + CO_2 \uparrow$$

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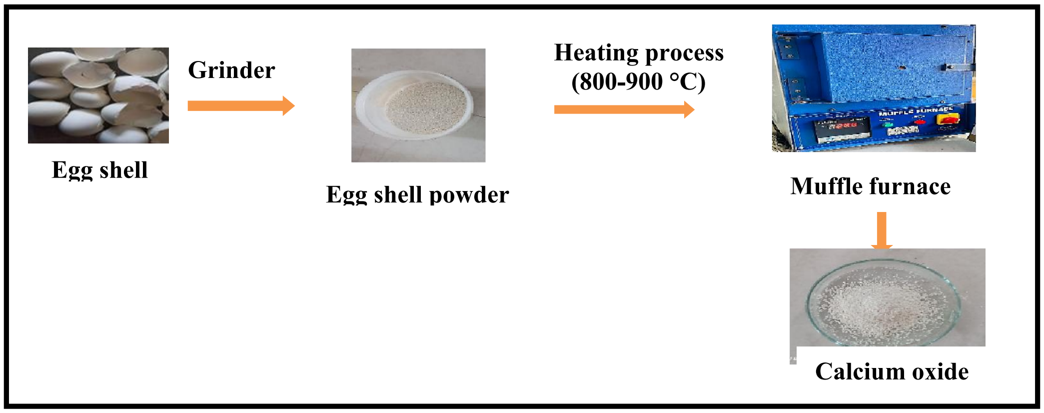

Fig 2.1 Preparation of the Eggshell Extract

2.4.3. Silver Nanoparticle Synthesis

Solution: Dissolve silver nitrate ($AgNO_3$) in distilled water. This creates the silver ion base needed for the reaction.
Blending the Extract: Slowly pour your eggshell or collagen extract into the silver solution. Place the mixture on a heating plate at 70°C–80°C for 30 to 60 minutes, keeping it under constant motion with a magnetic stirrer.
Formation Nanoparticles: After mixing the $AgNO_3$ and the calcium oxide filtrate to finalize the nanoparticle formation.
Final Collection: Use high-quality Whatman filter paper to capture the finished nanoparticles from the liquid. Let them air-dry at room temperature to obtain the final powdered product.

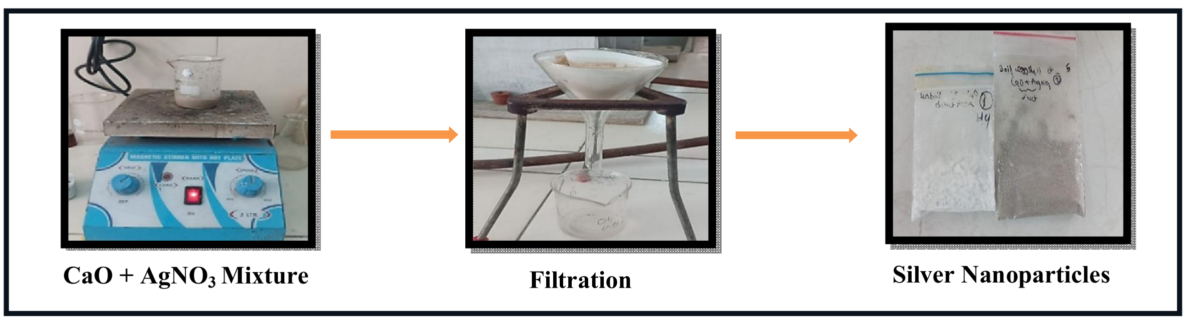

Fig 2.2 Silver Nanoparticle Synthesis

Method 2 : Digestion Process

2.4.4. Preparing the Eggshell Powder

In this method also same process to preparing the eggshell powder such as Cleaning, Dry the eggshell and Grinding to convert fine powder.

2.4.5. Calcination process

- The Furnace Step: Place the powder in a muffle furnace at 800–900°C for 2 to 4 hours.
- The Reaction: This high heat triggers a chemical change where the calcium carbonate ($CaCO_3$) releases carbon dioxide (CO_2), leaving behind pure Calcium Oxide (CaO).

$$\mathbf{CaCO_3 \rightarrow CaO + CO_2 \uparrow}$$

2.4.6. Silver Nanoparticle Integration

- Silver Solution: Mix silver nitrate ($AgNO_3$) into distilled water to create a liquid silver base.

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- The Blend: Slowly stir your eggshell extract into the silver solution. Heat this on a hot plate at 70–80°C for about an hour while keeping it under constant motion with a magnetic stirrer.

2.4.7. Final Refinement
- Digestion: Let the mixture sit (or apply gentle heat) to allow the particles to settle and grow to the correct size. This step helps improve the purity of your final product.
- Filtering: Use high-grade filter paper to separate the solid nanoparticles from the remaining liquid.
- Washing: Rinse the captured solids to remove any leftover chemical traces, then dry the powder completely.

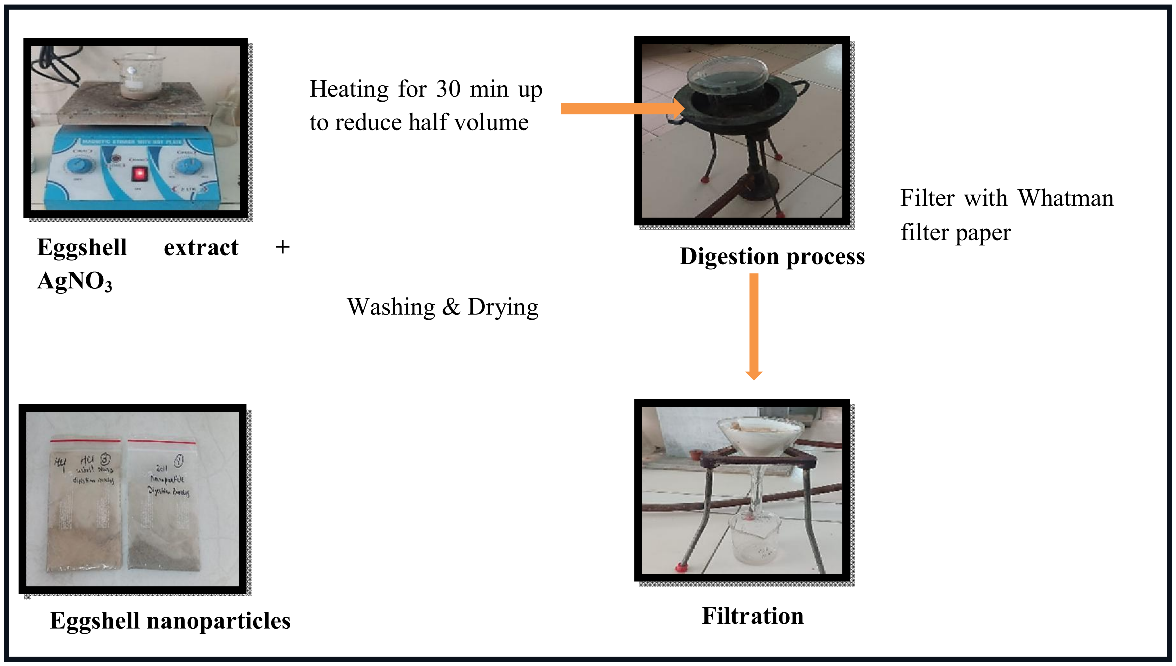

Fig 2.3 Preparation of silver nanoparticles from boiled and unboiled eggshell

2.5. Antimicrobial testing of eggshell nanoparticles by Muller-Hinton agar
2.5.1. Preparation of Muller-Hindon Agar Medium
To prepare the solid culture medium, 7.6 g of Muller–Hinton agar powder and 4 g of agar–agar powder was precisely weighed and mixed in 50ml distilled water with constant stirring. To ensure complete dissolution of all components, the mixture was heated gently until it formed a perfectly clear solution. The formulated medium was subsequently sterilized in an autoclave at 121°C for two hours. Finally, the sterilized agar was poured into sterile Petri dishes and left to solidify at room temperature.

2.5.2. Preparation of Bacterial Culture
To inoculate the media, fresh bacterial cultures of Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa were first prepared. Sterile cotton swabs were then immersed into each respective bacterial suspension. Finally, the swabs were used to spread the cultures evenly across the entire surface of the prepared Muller–Hinton agar plates to ensure uniform microbial growth.

2.5.3. Formation of Wells

Using a sterile pipette tips wells neatly punched into the solidified agar plates. Each well was then carefully labelled to clearly differentiate between the various samples and experimental controls.

2.5.4. Addition of diluted nanoparticles

Using a micropipette, the prepared eggshell nanoparticle sample was carefully introduced into the designated wells, with the flexibility to inoculate varying concentrations if required by the experimental design. For comparative analysis, a standard antibiotic solution was introduced into specific wells to serve as the positive control, while sterile distilled water was utilized as the negative control.

2.5.5. Incubation

The inoculated plates were then placed in an incubator and maintained at 37°C for a 24-hour period to allow for bacterial growth and the development of inhibition zones.

2.5.6. Observation of Zone of Inhibition

Following the incubation period, the plates were visually examined for the presence of clear zones of clearing around the wells, which indicate bacterial growth inhibition. The overall antimicrobial efficacy of the eggshell nanoparticles was then evaluated and determined based on the respective sizes of these measured zones.

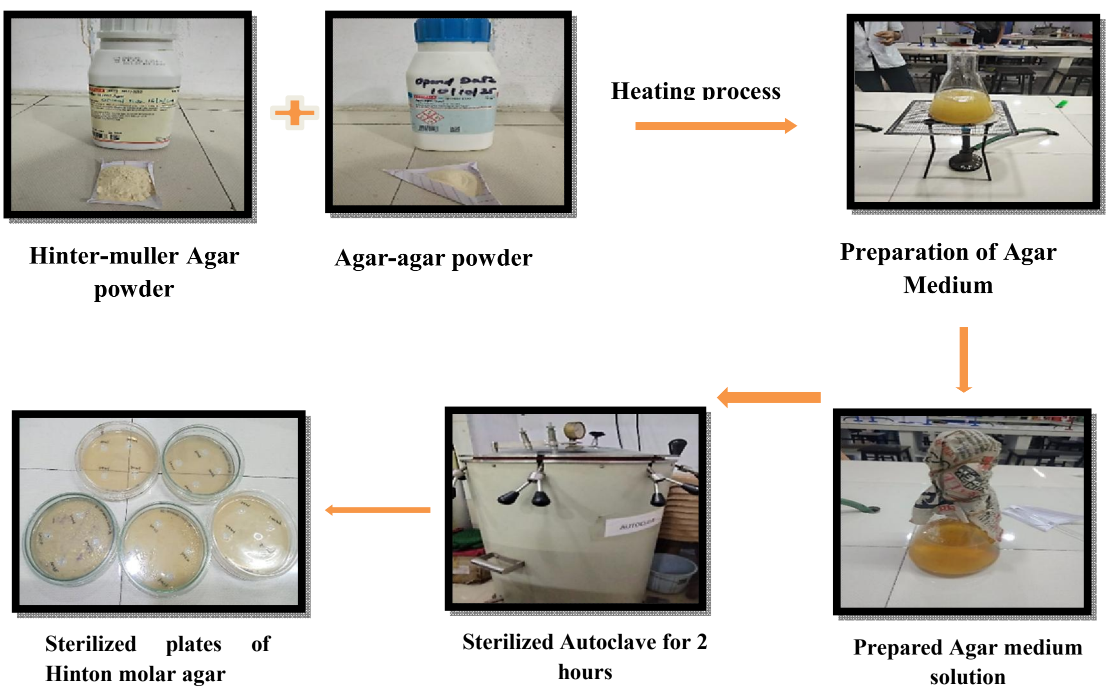

Fig 2.4 Preparation and Sterilization of Mueller–Hinton Agar Medium

Cocamidopropyl betain

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2.6. Preparation of Face Wash Using Silver Nanoparticles

Procedure:

Step 1: Preparation of Base Solution

1. Take 50 ml distilled water in a clean beaker.
2. Add 20 ml added surfactant slowly (Cocamidopropyl betain)
3. Stir gently using a magnetic stirrer or glass rod to avoid foam formation.

Step 2: Addition of Moisturizing Agents

1. Addition of glycerine to the solution. Then added 20 ml aloe vera gel.
2. Beetroot is added to the above mixture. Beetroot is a dark pinkish-red vegetable rich in vitamin C and iron. It also contains antioxidant properties that help impart a natural rosy tint, reduce the appearance of dark spots, and promote healthy, glowing skin.
3. Stir the mixture continuously until a uniform and homogeneous solution is obtained.


Aloe vera gel


Glycerin


Beet


Neem


Mixing process

Step 3: Addition of Silver Nanoparticles

1. Take 0.1–0.5 g of synthesized silver nanoparticles.
2. Disperse the nanoparticles in 10 ml distilled water to form a suspension.
3. Add this suspension slowly to the face wash mixture while stirring continuously.
4. Maintain the concentration of AgNPs at a safe level throughout the formulation process.

Step 4: pH Adjustment

1. Measure the pH of the solution using pH paper.
2. when the solution is basic medium then a small amount of citric acid solution dropwise was added. Adjust the pH to 5.5 – 6.5, which is suitable for skin.

Step 5: Addition of Fragrance (Optional)

To enhance the formulation, two to three drops of lavender oil were added with rose water. The mix properly to complete uniform solution.

Step 6: Final Mixing and Storage

Mixture was stirred continuously for 10 to 15 minutes. Once fully blended, the prepared face wash was transferred into a clean, airtight bottle for containment. Finally, the product was stored at room temperature to maintain its stability.

2.7. Preservative:

Preservatives are added to cosmetic formulations to prevent the growth of bacteria, fungi, and other microorganisms. They help to maintain its safety, quality, and effectiveness during storage and use. Neem extract was used as a preservative that Mix the ingredients thoroughly for 10–15 minutes. Maintain the pH which is suitable for skin and supports product stability.

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IV. RESULTS

4.1. FTIR Analysis:

The FTIR spectrum was used to identify the functional groups present in the synthesized eggshell-derived nanoparticles. Different absorption peaks observed in the FTIR spectrum indicate the presence of metal oxide bonds, carbonate ions, hydroxyl groups, and other functional groups responsible for nanoparticle formation and stabilization.

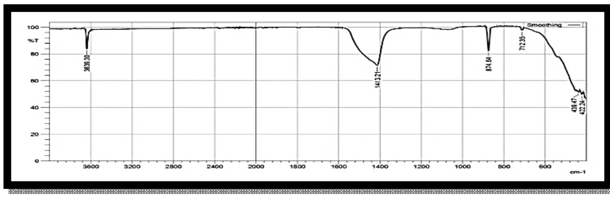

Fig 4.1 FTIR spectroscopy of unboiled

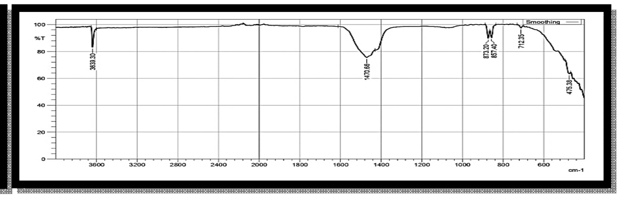

Fig 4.2 FTIR Analysis of Boiled Eggshell (CaO) with

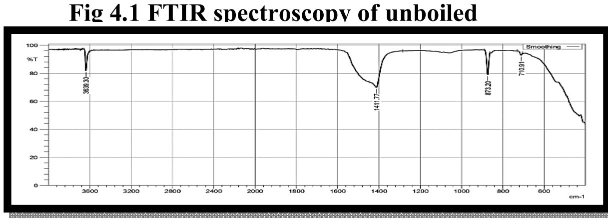

Fig 4.3 FTIR Analysis of Unboiled Eggshell (CaO)

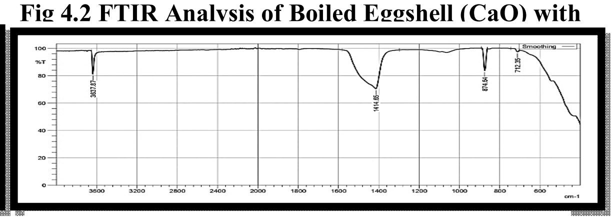

Fig 4.4 FTIR Spectrum of Boiled Eggshell

Sr. No.	Fig. No.	Functional group/Bonding	Literature Value(cm^{-1})	Observed Value(s)(cm^{-1})	Interpretation
1	Fig.4.1, Fig.4.2, Fig.4.3, Fig.4.4	O-H stretching	3600–3700	3639.30, 3639.30, 3639.30, 3627.62	Indicates hydroxyl (-OH) groups or adsorbed moisture on the eggshell surface.
2	Fig.4.1, Fig.4.3, Fig.4.4	CO_3^{2-}asymmetric stretching (ν_3)	1400–1470	1413.21, 1411.77, 1414.08	Characteristic absorption of carbonate ions confirming calcium carbonate ($CaCO_3$).
3	Fig. 4.2	CO_3^{2-} asymmetric stretching (ν_3)	1470–1490	1470.96	Indicates carbonate species present after CaO-$AgNO_3$ treatment.
4	Fig.4.1, Fig.4.2, Fig.4.3, Fig.4.4	CO_3^{2-} out-of-plane bending (ν_2)	870–875	874.64, 872.70, 872.29, 874.84	Confirms the presence of carbonate groups associated with calcite.
5	Fig.4.1, Fig4.2, Fig.4.3, Fig.4.4	CO_3^{2-} in-plane bending (ν_4)	710–715	712.35, 712.35, 710.91, 712.35	Indicates the crystalline calcite phase of calcium carbonate
6	Fig.4.1	Ca-O / Metal-O stretching	400–450	429.47, 402.42	Represents calcium-oxygen lattice vibrations of inorganic mineral phases.
7	Fig.4.2	Ca-O stretching	470–480	473.92	Suggests the formation of CaO after thermal treatment.
8	Fig.4.2	Ag-O / Metal-O vibration	430–450	439.25	Indicates interaction between silver species and CaO in the CaO-$AgNO_3$

					sample.

Table1: Comparative Study of FTIR Absorption Bands in Samples Derived from Eggshells

The above table show the FTIR Absorption Bands/functional groups in Eggshell-Derived Sample and few same functional groups are present in all figures and some are absent.

4.2. Antimicrobial Activity:

Antibacterial resistance is becoming a major global concern, highlighting the urgent need to identify new and effective antimicrobial agents from various sources. In this study, different samples were examined to determine their ability to inhibit the growth of selected pathogenic bacteria.

The antibacterial activity of these samples was tested using the agar well diffusion method against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Among the tested samples, Sample 2 and Sample 4 showed strong antibacterial effects against E. coli, as indicated by the formation of clear and well-defined zones of inhibition. Sample 6 and sample 3 exhibited moderate antibacterial activity against P. aeruginosa, which was observed as a less distinct inhibition zone. None of the samples were effective growth against S. aureus, as no inhibition zones were observed. the results suggest that the tested samples have selective antibacterial activity, being most effective against E. coli, moderately effective against P. aeruginosa, and ineffective against S. aureus.

P-aeruginosa:

Fig 4.5 Antibacterial activity of eggshell-derived CaO against *Pseudomonas aeruginosa* at different concentrations (25 mL, 50 mL, 75 mL and 100 mL) using agar well diffusion method.

E. coli:

Fig 4.6 Antibacterial activity of eggshell-derived CaO against *Escherichia coli* at different concentrations (25 mL, 50 mL, 75 mL and 100 mL) using agar well diffusion method.

Sample	E. coli	P. aeruginosa
Sample 2	Strong inhibition (clear zone)	No inhibition
Sample 3	No inhibition	Inhibition
Sample 4	Strong inhibition (clear zone)	No inhibition
Sample 6	No inhibition	Moderate inhibition

Table 2: Zones of inhibition observed for each sample against three bacterial strains

Samples 2 and 4 showed strong antibacterial efficacy against Escherichia coli (E. coli), as evidenced by the development of prominent and different zones of inhibition surrounding the wells. While *E. coli* is classified as a Gram-negative bacterium, it typically exhibits a higher degree of susceptibility to antimicrobial agents compared to the highly resilient Pseudomonas aeruginosa. The clear zones were observed in Sample 2 and 4. The enhanced

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performance of these specific samples can be attributed to several key factors. These samples were potentially more stable and well-dispersed, preventing aggregation and ensuring maximum surface area contact to effectively inhibit bacterial growth. P. aeruginosa is a highly resistant bacterium due to Thick outer membrane (low permeability). Only Sample 6 showing moderate inhibition suggests that slightly better interaction or higher bioavailability of active compounds in that sample.

4.3. XRD Analysis:

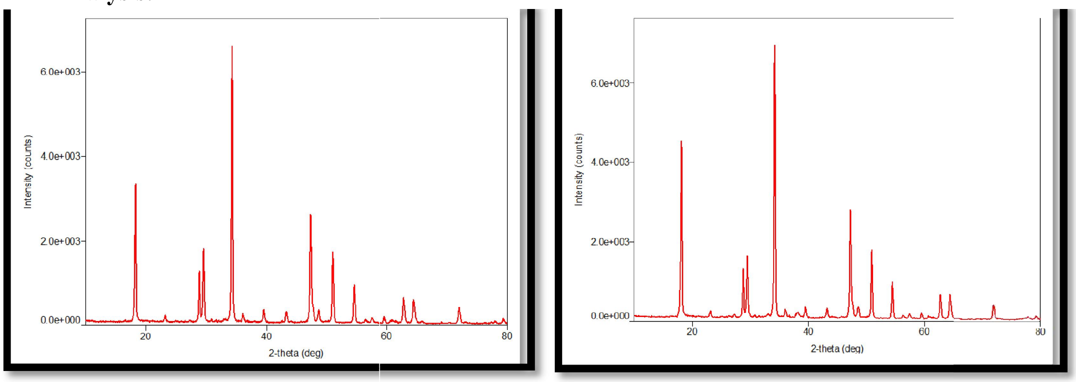

Fig. 4.7: Comparative XRD patterns showing identical crystalline phases and structural consistency of the synthesized eggshell-derived material.

The XRD results confirm that the synthesized eggshell-derived material possesses a well-crystalline structure with high phase purity. The presence of sharp diffraction peaks demonstrates successful formation of the composite and indicates good crystallographic stability. XRD patterns show nearly identical diffraction peaks, confirming that the synthesized eggshell-derived material has a consistent crystalline structure. The similarity of the peaks indicates good phase purity, structural stability, and reproducibility of the synthesis process.

4.4. After preparing the Face Wash result are shown below:

Before Applying Facewash, the skin appears slightly dull and exposed to impurities.

Presence of surface oil, dust, or environmental residues can be noticed. Skin texture looks comparatively less clean and less refreshed. This stage represents normal exposure of skin to daily environmental pollutants. After Applying Facewash, the skin looks cleaner, fresher, and more radiant. Visible reduction of dirt, oil, and surface impurities. Skin tone appears more even and refreshed that shows improved hygiene and skin clarity.

Before applying facewash

After applying Facewash

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