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GREEN SYNTHESIS OF CALCIUM CARBONATE NANOPARTICLES USING CITRULLUS LANATUS EXTRACT: PREPARATION AND CHARACTERIZATION

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ABSTRACT:

Background: Calcium carbonate nanoparticles have been gathering increased attention in the field of biomedicine due to their potential in anticancer treatment and remineralisation abilities. The present study aims to synthesize calcium carbonate nanoparticles using plant extracts of
Citrullus lanatus.
Methodology: Calcium hydroxide and sodium carbonate solutions were used to produce calcium carbonate solution was reduced to calcium carbonate nanoparticles using Citrullus lanatus extract. The prepared nanoparticles were then characterized using scanning electron microscopy, energy dispersive X-ray analysis and Fourier transform Infrared Spectroscopy
Results: The prepared nanoparticles showed amorphous rod shaped morphology in the SEM analysis. FTIR analysis showed the characteristic carbonate peaks at 1480-1410 cm-1 and between 880-860 cm-1 . EDAX analysis showed the presence of calcium, carbon and oxygen content in the prepared nanoparticles.
Conclusion: Citrullus lanatus extracts can be used for the synthesis of calcium carbonate nanoparticles, which have a promising avenue in the field of biomedical research. Further studies are necessary in order to develop these nano-particles into a product for clinical use.

Keywords: Citrullus lanatus extracts, Nano particles, BioMedical Research, Nanomaterials

1. INTRODUCTION

Nanomaterials have been attracting a lot of attention over the past decade due to their numerous applications in a variety of sectors, that include electronics, textiles, different areas of food and agriculture, the environment, healthcare, etc.[1][2][3][4]([4,5] These applications require a limited number of intrinsic and distinct properties of nanomaterials, such as their high surface area to volume ratio, low cost of synthesis, ease of surface modification, and high catalytic efficiency. Nanomaterials tend to be suitable for controlled chemical reactions, targeted drug delivery, effective energy and mass transfer, and other industrial and biomedical applications because of these properties. Based on their chemical makeup, nanomaterials can be broadly divided into metallic, metal oxide, carbon-based, and semiconductor-based particles. Among these, metal oxide-based nanomaterials have evolved significantly as a result of their broad spectrum of industrial applications, including catalysis, plasmonics, and fuel production.
Nanomedicine is the carefully regulated application of nanotechnologies and nanoparticles in medical care, opening up new avenues for the detection and treatment of human diseases.Nanoparticles can be found in nature and are used in everyday life, such as in toothpaste, dietary supplements, and sprays for coating, cleaning, and impregnation. They can also be found in cosmetic products, such as sunscreens [in which titanium dioxide (TiO2) or

zinc oxide (ZnO) particles are added as ultraviolet (UV) light filters] and sun screens. Some nations have tested and granted licenses for the use of silicon dioxide (SiO2), magnesium oxide (MgO), and titanium dioxide (TiO2) as food additives. The use of nanotechnology has enormous potential to enhance daily life. The development of metal nanoparticles has sparked significant interest among scientists and nanotechnologists due to their microbicidal properties. Efforts have been made to synthesize green nanoscale objects using various metals, such as silver, copper, zinc, titanium, tin (Sn), and gold.[6] While greenly synthesized gold has numerous applications in various engineering and technological fields, silver nanoparticles (AgNPs) synthesized by green methods show potential applications in the biomedical field, particularly in developing antimicrobials (AgNPs).[7,8][9] Traditionally, a key challenge in nanoparticle synthesis is developing environmentally friendly and sustainable methods. Conventional synthesis approaches often involve hazardous chemicals and high-energy processes, adversely affecting the environment and human health. To address these issues, researchers have turned toward green synthesis, a sustainable approach utilizing natural sources, such as plant extracts, as reducing and stabilizing agents for nanoparticle synthesis.[10] Calcium carbonate (CaCO3) nanoparticles have garnered significant attention due to their excellent biocompatibility, biodegradability, ease of preparation, and pH sensitivity [11]. CaCO3 exists in several forms: an amorphous calcium carbonate (ACC) phase, two hydrated metastable phases (calcium carbonate hexahydrate and monohydrocalcite), and three anhydrous crystalline polymorphs (calcite, aragonite, vaterite)[12]. Among these, the ACC phase exhibits the highest solubility and serves as the precursor to the anhydrous crystalline polymorphs, which readily crystallize in solutions to form these polymorphs.[13]

Citrullus lanatus, commonly known as watermelon, is widely consumed for its refreshing taste and nutritional benefits. It is rich in vitamins, minerals, antioxidants, and bioactive compounds with potential biological properties. Compounds such as polyphenols, flavonoids, and terpenoids in watermelon have demonstrated antibacterial, antifungal, and antioxidant activities. Consequently, watermelon extract has garnered interest as a natural source for environmentally friendly nanoparticle manufacturing [14].

Using watermelon extract in nanoparticle synthesis offers several advantages. Firstly, it serves as an eco-friendly and cost-effective alternative to conventional reducing and stabilizing agents. The bioactive compounds in the extract can act as reducing agents, facilitating the conversion of metal ions into nanoparticles, and as stabilizing agents, preventing agglomeration and loss of nanoparticle activity. Moreover, watermelon extract's biological properties contribute to the potential antimicrobial efficacy of the synthesized nanoparticles. Watermelon extract has shown antibacterial activity against various pathogenic bacteria, including Escherichia coli, certain Streptococcus strains, and Pseudomonas aeruginosa. These antimicrobial properties are attributed to bioactive compounds that disrupt bacterial cell membranes, inhibit enzyme activity, and interfere with bacterial adhesion and biofilm formation. Incorporating watermelon extract in nanoparticle synthesis may enhance the antimicrobial effectiveness of the resulting nanoparticles against target pathogens such as Streptococcus mutans [15].

Traditional methods of nanoparticle synthesis have the additional drawback of using toxic precursor chemicals, such as sodium borohydride, potassium bitartrate, methoxypolyethylene glycol, and hydrazine; toxic solvents, such as sodium dodecyl benzyl sulfate; and toxic by-products. As science advances, more environmentally benign methods for making metal nanoparticles are discovered, using biologically derived agents (derivatives of bacterial and fungal substances) to transform metal salts into nanoparticles. These substances act as in vitro

capping and reducing agents. Utilizing natural substances, such as phytochemicals, has been suggested as a potential cancer management technique, as they exhibit a wide range of biological activities, are inexpensive, and have few undesirable side effects.
The present study aimed to synthesize and characterize calcium carbonate nano-particles using Citrullus lanatus extract.

2. MATERIALS AND METHODS

Watermelon seeds and red flesh were obtained from the native Indian variety of watermelon, known for its high phenolic content. After thoroughly washing with distilled water, the fruit was cut into smaller pieces and exposed to sunlight for seven days to achieve complete drying.Using a clean mortar and pestle, the dried watermelon pieces were ground into a fine paste, which was then collected in a clean beaker. This paste was diluted with 100 mL of distilled water and stirred for 15 minutes to ensure the proper extraction of bioactive compounds from the watermelon to prepare its aqueous extract. This was then used for nanoparticle synthesis.

Calcium carbonate nanoparticles were synthesized by mixing 100 mL of 1.47 mol/L aqueous calcium chloride and 100mL of 1.06 mol/L sodium carbonate solution. This resulted in formation of calcium carbonate solution.

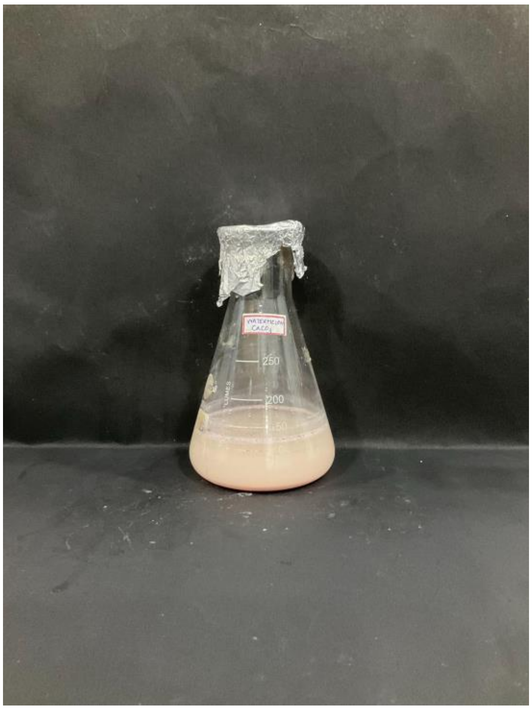

Figure 1 - Watermelon extract added to Calcium carbonate solution

2mL of the Citrullus lanatus extract was then added to this prepared solution and stirred gently to allow for uniform mixing. This resulted in generation of calcium carbonate nanoparticles which are suspended within the solution. This solution was then subjected to orbital shaking using laboratory orbital shaker for 2-3 hrs and later centrifuged at 10000 rpm for 10 mins to separate the nanoparticles from the remaining solution. (Figure 1)

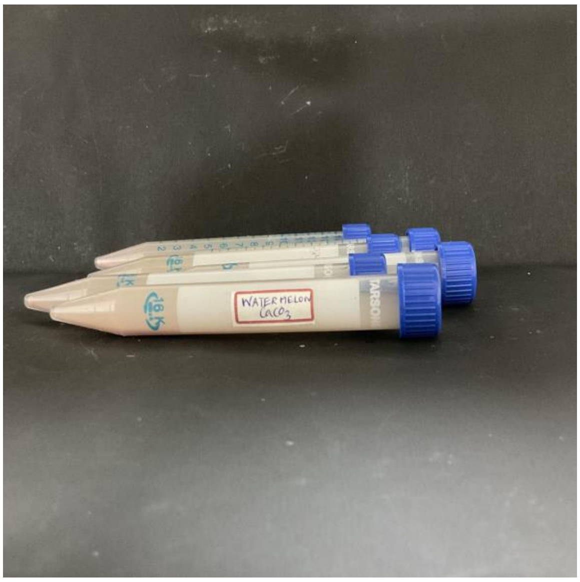

Figure 2 - Centrifuged tubes containing Calcium carbonate nanoparticles.

Early signs of reduction of metal salts into nanoparticles is by colour change, which is the end point indicator of nanoparticle synthesis. The synthesized nanoparticle was then characterized using scanning electron microscopy(SEM), Fourier transform infrared spectroscopy(FT-IR), and Energy dispersive X-Ray analysis (EDAX) (Figure 2)

3. RESULTS

Scanning Electron Microscopy

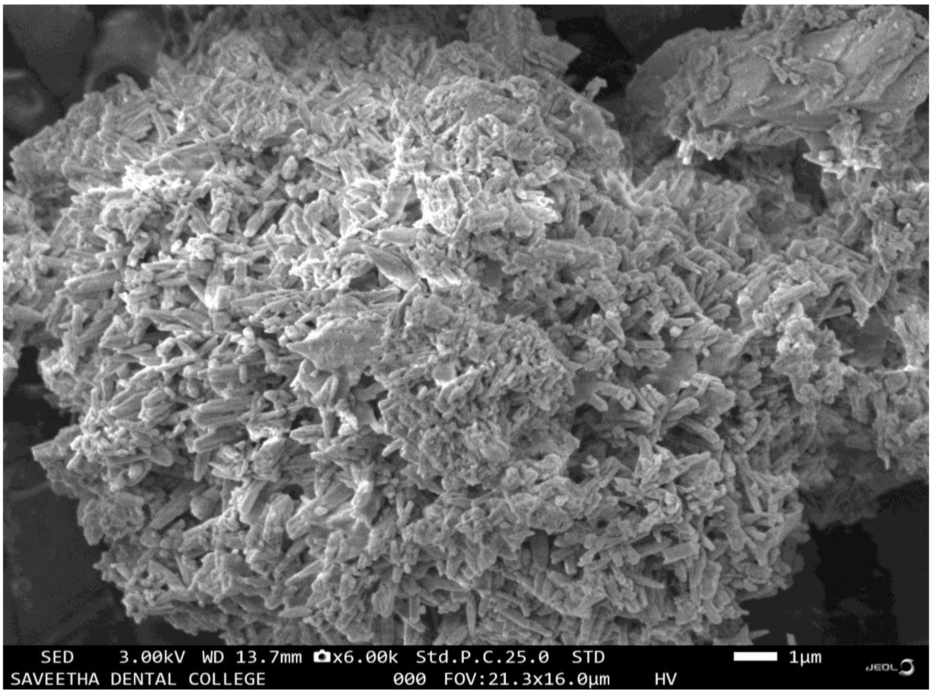

Figure 3 - SEM image of the prepared nanoparticles at 6000x magnification.

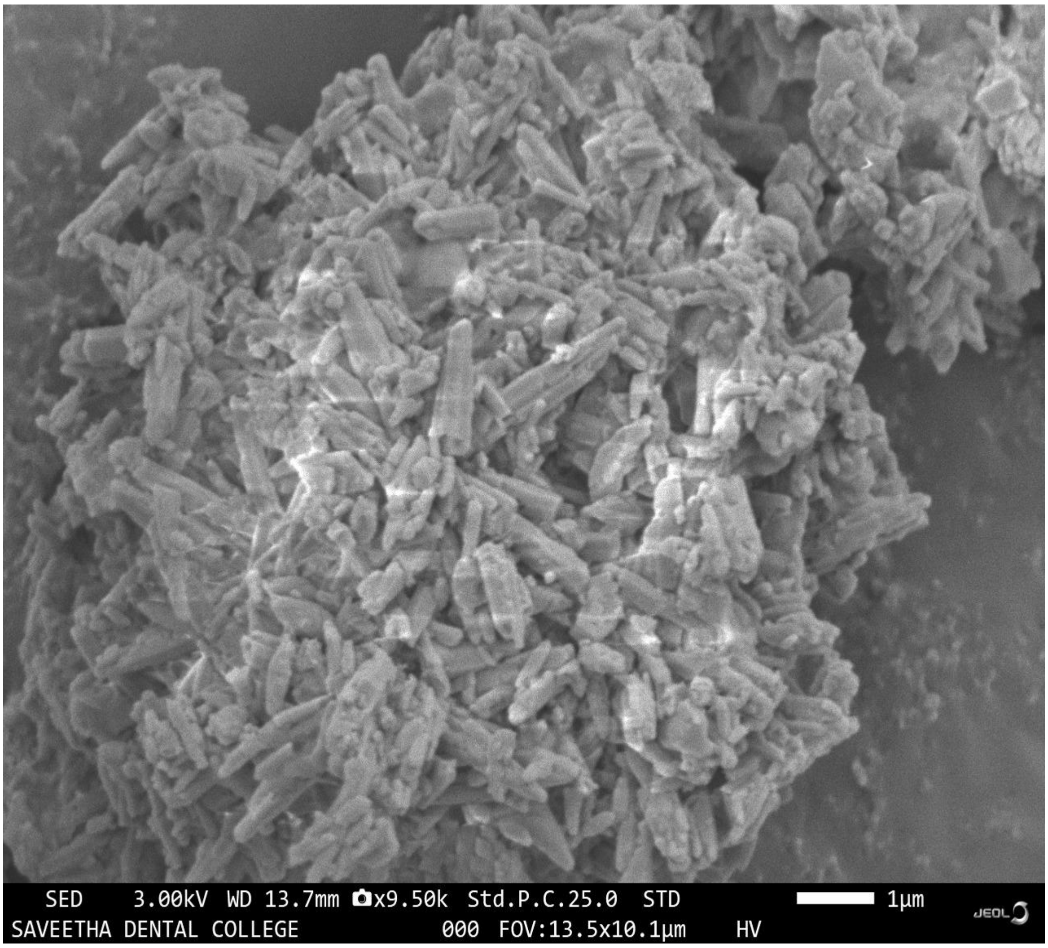

Figure 4 - SEM image of the prepared nanoparticles at 9500x magnification.

SEM analysis revealed rod shaped nanoparticles with uniform size distribution. At 6000x magnification they showed an average particle size of 70 nm. This was taken by measuring multiple nanoparticles and averaging them. (Figure 3 and 4)

Fourier Transform Infrared Spectroscopy

The function of FTIR analysis is to identify the functional groups present in the synthesized calcium carbonate nanoparticles mediated by Citrullus lanatus extract. The FTIR spectrum displayed characteristic peaks corresponding to various functional groups. Peaks at specific wavenumbers confirmed the successful synthesis of the nanoparticles and indicated the involvement of functional groups from the watermelon extract in their formation.

The infrared spectra of the prepared nanoparticle shows a series of absorption peaks ranging from 600 - 3000 cm^{-1} . It shows the characteristic carbonate peaks at 1480-1410 cm^{-1} and between 880-860 cm^{-1} . (Figure 5)

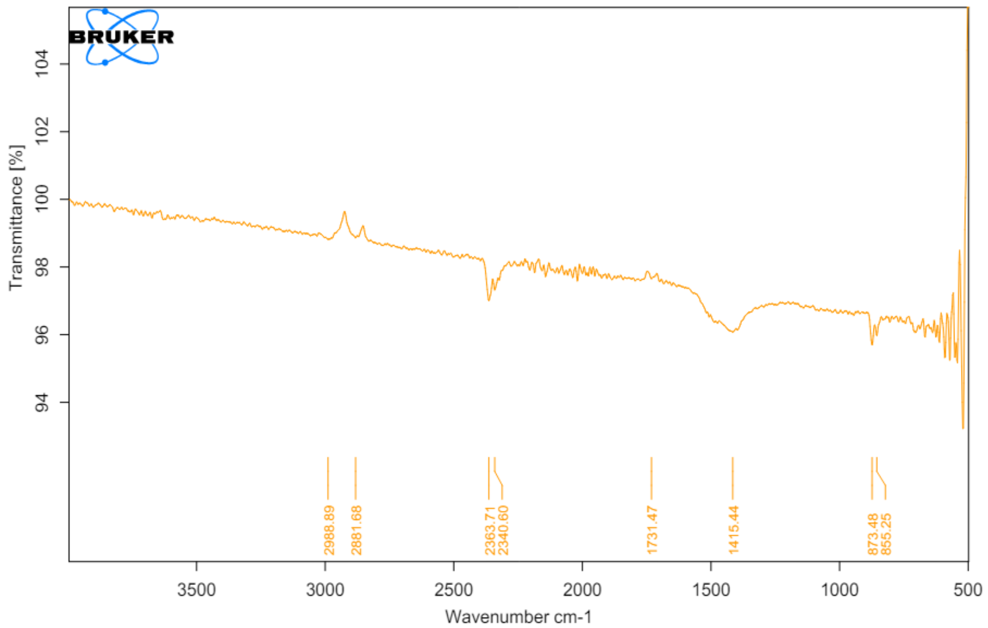

Figure 5 - FTIR analysis of the prepared calcium carbonate nanoparticles

Energy Dispersive X-Ray analysis

The JEOL JSM 7600F (JEOL USA, Inc., Peabody, MA) was used to conduct the EDAX analysis. The energy-dispersive X-ray (EDAX) analysis spectrum of the nanoparticles is shown along with the elements of oxygen and carbon. It is used to identify chemical compounds that are responsible for the production and stability of nanoparticles, as well as chemical groups that are present in the nanoparticle powder. It clearly depicts the presence of calcium, carbon and oxygen.

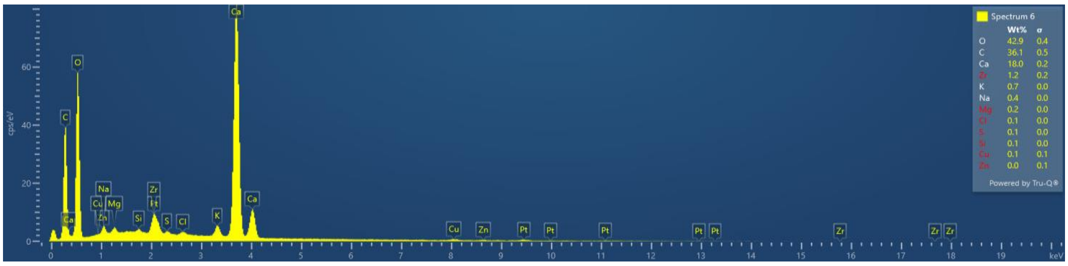

Figure 6 - EDAX analysis of the prepared nanoparticles

4. DISCUSSION

Green nanotechnology utilizes medicinal plants for the synthesis of metal and other nanomaterials with potential applications in the diagnosis and treatment of various diseases and disorders.[16] Metal nanoparticles synthesized from medicinal plants, microbes, and other food sources are recognized for their safety and cost-effectiveness.[17] However, concerns about environmental sustainability arise due to the impact on global food security and the scarcity of natural resources.[18] To address these concerns, researchers have begun using biowaste materials from various plant and fruit sources to synthesize metal-based and metallic oxide nanoparticles. (Figure 6)

Citrullus lanatus, commonly known as watermelon, is a widely distributed fruit rich in bioactive compounds such as lycopene, citrulline, flavonoids, and phenolic compounds. These bioactive compounds contribute to the antibacterial properties and facilitate the synthesis of nanoparticles in various ways.[19]Watermelon contains antioxidants and anti-inflammatory agents, such as lycopene, vitamin C, and flavonoids. These compounds help combat oxidative stress and inflammation, often associated with bacterial infections. By reducing oxidative stress and inflammation, these bioactive compounds support the body's immune response and potentially inhibit bacterial growth and survival.In addition to their indirect effects, these compounds can directly disrupt bacterial membranes. They can destabilize the lipid bilayer of bacterial cell membranes, leading to cell lysis and bacterial death.[20] This mechanism is particularly effective against gram-positive bacteria such as Streptococcus mutans. Moreover, the bioactive compounds interfere with bacterial adhesion and biofilm formation, making bacteria more susceptible to antibacterial treatments. Thus in addition to their ability to reduce metal oxide, they also have anti-microbial effects that make them suitable materials for aiding in nanoparticle synthesis. In the green synthesis of nanoparticles, the bioactive compounds in watermelon serve as reducing and stabilizing agents. For instance, the phenolic compounds and citrulline in watermelon extract can act as reducing agents, facilitating the conversion of metal ions into nanoparticles.

In biological systems, calcium carbonate and calcium phosphate are essential components of bones, shells, and teeth.[21] Due to their chemical similarity to these tissues, CaCO3-based drug delivery systems are believed to exhibit excellent biocompatibility. On the other hand, nanoparticles such as Au, Ag, Se, Cr, TiO2, and ZnO have been shown to increase mutation frequency and reactive oxygen species production, leading to cell apoptosis.[22] In contrast, CaCO3 nanoparticles are among the safest biomaterials, as their by-products (Ca2+ and CO32−) are already present in the bloodstream.Additionally, CaCO3 nanoparticles are stable at the normal blood pH of 7.4 but decompose rapidly in the acidic environment of tumors, thereby facilitating targeted delivery to tumor sites.[23]The preparation of CaCO3 nanoparticles typically requires only common salts and no organic solvents, making the process cost-effective.[24] Additionally, the surface of CaCO3 nanoparticles can be modified with targeting moieties, enhancing their ability to reach specific sites.

The solution precipitation method is the most established technique for preparing CaCO3 nanoparticles. This method involves the reaction between Ca2+ and CO32− in an aqueous solution, allowing for the production of large quantities of CaCO3 nanoparticles without the need for surfactants, thereby reducing production costs. Due to the mild preparation conditions, various bioactive species, including small molecule drugs, genes, and proteins, can be loaded into CaCO3 nanoparticles during the precipitation process. The present study therefore uses the solution precipitation method for synthesis of calcium carbonate nano-particles. The synthesis parameters such as pH, temperature, ion concentration, stirring speed, solvent species and additives can be used to control the size, shape and phase of calcium carbonate.

The present study is an in-vitro pilot attempt in order to check if Citrullus lanatus extract mediate calcium carbonate nanoparticle synthesis. Limitations of this study involve its in-vitro nature due to which it cannot capture the true essence of the complexities that might arise when these nanoparticles are used in a day-to-day clinical scenario. Further studies are required in order to establish them as potential drug delivery systems.

5. CONCLUSION

Within the limitations of the present study we can conclude that Citrullus lanatus extracts can be used for the synthesis of calcium carbonate nanoparticles, which have a promising avenue in the field of biomedical research. Further studies are necessary in order to develop these nano-particles into a product for clinical use.

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