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EXTRACTION AND SYNTHESIS OF CALCIUM CARBONATE NANOPARTICLES FROM ORANGE PEELS, LEMON PEELS, AND PAPAYA PEELS AND TO STUDY THEIR APPLICATIONS. *A Sustainable Nanomaterial Synthesis and Bioactivity Assessment Study*

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ABSTRACT

This study investigates the eco-friendly synthesis of calcium carbonate nanoparticles ($CaCO_3$ NPs) from waste fruit peels using drying, calcination, and controlled precipitation methods. The synthesized nanoparticles were confirmed for their chemical composition and functional groups using Fourier Transform Infrared Spectroscopy (FTIR). The biological and pharmaceutical potential of the nanoparticles were evaluated through *in vitro* assays, including antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method, anti-inflammatory activity through protein denaturation assay, and antacid activity via acid neutralization studies. This work emphasizes the valorization of fruit peel waste as a sustainable resource for the synthesis of calcium carbonate nanoparticles and explore their potential relevance in pharmaceutical and biomedical applications.

I. INTRODUCTION

The growth of the food processing and agro-industrial sectors produces large amounts of organic waste, with the fruit peels being a significant part. These waste materials are often thrown away, leading to problems like microbial spoilage, bad odors, and greenhouse emissions (*Garg et al., 2021*). However, fruit peels are rich in minerals, organic acids, polysaccharides, and phytochemicals, making them valuable for sustainable material recovery (*Guo et al., 2021*).

Among these components, calcium-based compounds stand out. Citrus peels, such as those from oranges (*Citrus sinensis*) and lemons (*Citrus limon*), contains calcium in forms like calcium citrate, calcium oxalate, and carbonate precursors. Papaya (*Carica papaya*) peels provide calcium along with phenolic compounds and organic acids that help with mineral precipitation.(*Jung et al., 2021)*. These properties make fruit peels low-cost, renewable sources for calcium carbonate ($CaCO_3$) production, which fits with green chemistry and circular economy principles.

Calcium carbonate is commonly used in the pharmaceutical, nutraceutical, biomedical, cosmetic, and food industries due to its biocompatibility, non-toxicity, chemical stability, and ability to neutralize acid(*Bae et al., 2023*). Traditional methods of producing it from limestone and chemical processes as energy-draining and harmful to the environment. This has led to a search for more sustainable options. At the nanoscale, $CaCO_3$ has a larger surface area, better dissolution, more reactivity, and improved biological interaction, making it good for drug delivery, antacids, and calcium supplements. Its three forms- calcite, aragonite, and vaterite, they vary in stability, shape, and biological activity, affecting their performance.

Green synthesis using the natural sources, especially fruit peels, presents an eco-friendly method by using natural biomolecules as agents to nucleate, precipitate, and stabilize compounds. Unlike conventional methods that depends on single-source biological materials or chemical precursors, this study focuses on comparing multiple type of fruit peels. Each fruit peels have its own unique mix of plant chemicals, and minerals which can affect the shape, surface chemistry, and health benefits of the nanoparticles. By combining the waste recovery with the nanoparticle production and thorough evaluation of their health benefits.

This research looks at a sustainable way to create $CaCO_3$ nanoparticles that have antioxidant, anti-inflammatory, and antacid properties. This approach tackles environmental issues linked to agro-industrial waste and offers an affordable, eco-friendly source of nanomaterial for pharmaceuticals and biomedical use.

II. Materials and Method

All the reagents used were of analytical grade.

2.1 Sample collection and extraction

Fresh orange, green papaya, and lemon peels were air-dried, ground to obtain fine powder. Suspend each peel powder of 10 gram in 100 mL Distilled Water, heat at 80–90 °C for 20 min, cool to room temperature, and filter through Whatman paper to obtain separate orange, papaya, and lemon extracts.(Anantharaman, 2016)

2.2 Synthesis of calcium carbonate Nanoparticle (per extract)

In a conical flask, mix 10 mL of 1 M $Ca(NO_3)_2$ solution with 10 mL of the corresponding fruit-peel extract. Stir the mixture at 300–400 rpm for 30 min at ambient temperature using magnetic stirrer. Add 10 mL of 1 M Na_2CO_3 solution drop-wise, while maintaining stirring until the pH reaches ≈ 9-10 and a milky-white $CaCO_3$ precipitate forms. Filter the precipitate using Whatman paper and wash 7–8 times with Distilled Water until the wash water is neutral (pH ≈ 7). Transfer the wet cake to a petri dish and dry in a Hot-air oven at 60 °C for 6–8 hours. The dried $CaCO_3$ nanoparticles were collected in Eppendorf tubes and stored for further characterization and analysis.

2.3 Characterization

FTIR analysis of calcium carbonate nanoparticles was performed using an ATR-FTIR spectrophotometer (PerkinElmer, USA; Model RX1) to identify surface functional groups and confirm carbonate bonding.

2.4 DPPH Antioxidant assay:

The DPPH scavenging ability of $CaCO_3$ NPs was measured according to the protocol with some modification. For orange, lemon- and papaya-mediated $CaCO_3$ nanoparticles, sample solutions were prepared at concentrations of 50, 100, 200, 300, and 500 µg/mL(“Green Synthesis of Calcium Carbonate Nano Scale Particles Using Benincasa Hispida (Thunb.) Cogn. Pulp and Their Qualities,” 2024). For Each 1 mL sample was mixed with 1.4 mL of 0.1 mM DPPH solution, incubated in the dark for 30 min and the absorbance read at 490 nm; % radical scavenging activity was calculated as:

$$\%\,\boldsymbol{RSA} = (A_{control} - A_{sample}) / A_{control} \times 100$$

2.5 Anti-inflammatory assay using BSA (Bovine Serum Albumin)

The anti-inflammatory activity of calcium carbonate nanoparticles ($CaCO_3$ NPs) was evaluated using the bovine serum albumin (BSA) protein denaturation method. A 1% (w/v) BSA solution was prepared in phosphate-buffered saline (PBS, pH 7.4). $CaCO_3$ NP stock solutions (1 mg/mL) were prepared in PBS and sonicated for 10–15 min. Working concentrations of orange, lemon- and papaya-mediated $CaCO_3$ nanoparticles, were prepared at concentrations of 50, 100, 200, 300, and 500 µg/mL by serial dilution.(“Green Synthesis of Calcium Carbonate Nano Scale Particles Using Benincasa Hispida (Thunb.) Cogn. Pulp and Their Qualities,” 2024). The reaction mixture (5 mL) consisted of 2 mL of 1% BSA, 2 mL of $CaCO_3$ NP solution, and 1 mL of PBS. The control contained 2 mL of BSA and 3 mL of PBS, while PBS alone served as the blank. Samples were incubated at 70°C for 20 min and then cooled to room temperature. Absorbance was measured at 650 nm using a colorimeter. The percentage inhibition of protein denaturation was calculated using the equation:

$$\%\boldsymbol{Inhibition} = \left(\frac{A_{sample}}{A_{control}} - 1\right) \times 100$$

2.6 Antacid Capacity Assay

The antacid capacity of $CaCO_3$ nanoparticles was evaluated using an acid neutralization followed by back titration method with slight modifications to pharmacopeial procedures.(*OTC Monograph_M001-Antacid Products for OTC Human Use 10.14.2022*, n.d.) Briefly, 50 mg (0.05 g) of $CaCO_3$ nanoparticles was dispersed in 25 mL distilled water in a 100 mL conical flask and stirred to obtain a uniform suspension. To this, 25 mL of 0.1 N HCl was added, and the mixture was stirred for 15 minutes at room temperature to allow complete acid neutralization, as indicated by effervescence due to carbon dioxide release. Subsequently, 2–3 drops of phenolphthalein indicator were added, and the excess acid was titrated against 0.1 N NaOH until a persistent faint pink endpoint was observed. The volume of NaOH consumed was recorded. Antacid capacity was calculated by determining the difference between the volume of NaOH consumed by the blank and the sample; Results are reported as milliequivalents (mEq) of acid per gram of sample.

$$\boldsymbol{Antacid\ capacity}\ (\boldsymbol{mEq/g}) = (V_{blank} - V_{sample}) \times \frac{N_{NaOH}}{\boldsymbol{Weight\ of\ sample}(\boldsymbol{g})}$$

III. RESULTS AND DISCUSSION

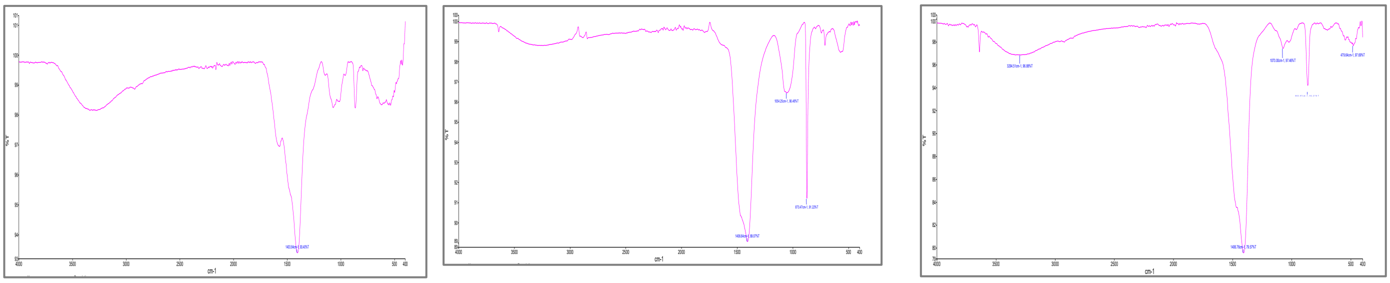
3.1 Green synthesis of calcium carbonate nanoparticles

Calcium carbonate nanoparticles ($CaCO_3$ NPs) were synthesized via a green synthesis method utilizing aqueous extracts derived from orange, lemon, and papaya peels. Phytochemicals inherent to these agro-waste materials, including organic acids, polyphenols, flavonoids, and polysaccharides, served as natural reducing, stabilizing, and capping agents. This approach facilitated nanoparticle formation while circumventing the need for hazardous chemicals. The employment of fruit peel extracts promotes sustainability and valorizes agricultural waste.

3.2 FTIR analysis

The synthesized $CaCO_3$ nanoparticles were characterized using Fourier Transform Infrared (FTIR) spectroscopy to confirm functional groups, carbonate bonding, and polymorphic structure.

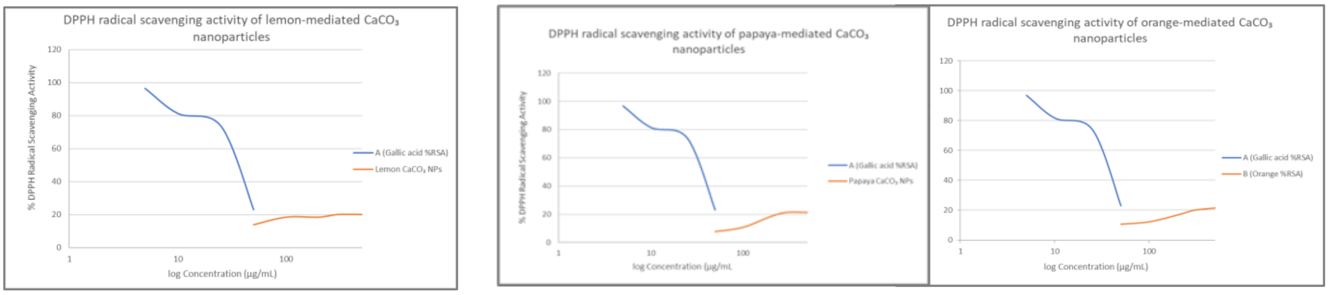
All samples exhibited a strong absorption band in the 1400–1500 cm^{-1} region, corresponding to the asymmetric stretching vibration (ν_3) of carbonate (CO_3^{2-}) ions, and a distinct band near ~870 cm^{-1}, attributed to the out-of-plane bending vibration (ν_2), are characteristic of calcium carbonate formation. Characteristic absorption peaks around ~1080 cm^{-1} and 744–748 cm^{-1} indicate the presence of the vaterite polymorph of calcium carbonate. The preferential formation of vaterite suggests that the phytochemicals in the peel extracts influenced nucleation and crystal growth during the green synthesis process. Broad absorption bands in the region of 3200–3500 cm^{-1} correspond to O–H stretching vibrations, indicating the presence of hydroxyl groups from polyphenols, alcohols, or adsorbed moisture.(Luo et al., 2020). Minor variations in peak intensity among the orange, lemon, and papaya peel–derived samples reflect differences in crystallinity and organic interactions, attributable to the distinct biochemical compositions of the extracts.



(a) (b) (c)

Figure 1: FTIR analysis with a scan range of 400-4000 cm^{-1} of calcium carbonate nanoparticles synthesized using (a) lemon peel extract, and (b) papaya peel extract, (c) orange peel extract.

3.3 Assessment of Antioxidant activity: *DPPH* Assay



(a) (b) (c)

Figure 2: Antioxidant potential of $CaCO_3$NPs (a) lemon peel extract, and (b) papaya peel extract, (c) orange peel extract.

The antioxidant activity of green-synthesized calcium carbonate ($CaCO_3$) nanoparticles mediated by orange, lemon, and papaya extracts was assessed using the DPPH radical scavenging assay, with gallic acid as the reference standard.(Brand-Williams, W et al., 1995) Scavenging activity was evaluated over a $\log_{10}$ concentration range of 5–500 µg/mL to examine dose-dependent behaviour.

Orange-mediated $CaCO_3$ nanoparticles exhibited a gradual increase in radical scavenging activity, rising from approximately 10% at lower concentrations to ~21% at 500 µg/mL, indicating effective phytochemical surface functionalization. Lemon-mediated nanoparticles showed a consistent increase in scavenging activity from ~13–14% to nearly 20%, suggesting uniform incorporation of antioxidant compounds. Papaya-mediated $CaCO_3$ nanoparticles displayed the highest scavenging activity among the three samples, increasing from ~7–8% to ~21–22%, reflecting the contribution of bioactive constituents present in papaya extract. Gallic acid demonstrated a steep concentration-dependent scavenging response at lower concentrations, confirming its strong antioxidant capacity and validating the assay performance. The logarithmic concentration scale enabled clear differentiation between the scavenging profiles of the reference antioxidant and the green-synthesized nanoparticles.

The present findings confirm that green-synthesized $CaCO_3$ nanoparticles exhibit measurable and concentration-dependent antioxidant activity, primarily governed by surface-associated phytochemicals introduced during the synthesis process. Since calcium carbonate is inherently inorganic, the observed radical scavenging activity arises from bioactive compounds adsorbed or capped on the nanoparticle surface, rather than from the $CaCO_3$ matrix itself. The differences in scavenging efficiency among the samples reflect variations in the phytochemical composition of the plant extracts used, including phenolics, flavonoids, organic acids, and reducing sugars. Papaya-mediated nanoparticles demonstrated comparatively higher activity, likely due to richer phytochemical content capable of effective hydrogen or electron donation. Lemon- and orange-mediated nanoparticles showed slightly lower but consistent activity, indicating stable surface functionalization.

Gallic acid, a well-established phenolic antioxidant, exhibited rapid and efficient radical scavenging due to its multiple hydroxyl groups, serving as a reliable benchmark for comparison. The distinct response patterns observed between gallic acid and $CaCO_3$ nanoparticles emphasize the supplementary antioxidant role of green-functionalized inorganic nanoparticles, rather than direct equivalence to small-molecule antioxidants.

Importantly, the gradual and sustained scavenging response of $CaCO_3$ nanoparticles across the tested concentration range suggests their suitability for applications where controlled or moderate antioxidant activity is beneficial, such as biomedical coatings, drug delivery systems, food packaging materials, and nutraceutical formulations. Overall, the results demonstrate that fruit-mediated green synthesis is an effective strategy for imparting biologically relevant antioxidant properties to $CaCO_3$ nanoparticles, reinforcing their potential in sustainable nanomaterial development.

3.4 Anti-inflammatory assay using BSA Assay

Protein denaturation can be considered to be one of the causes of inflammation. As a result, agents or chemicals that significantly reduce protein denaturation may have potential anti-inflammatory effects. BSA was taken to check whether the test samples prevent its denaturation. Evaluation of anti-inflammatory activity was done at different concentrations of CaCO3 NPs i.e., 50, 100, 250 and 500 µg/mL.("Green Synthesis of Calcium Carbonate Nano Scale Particles Using Benincasa Hispida (Thunb.) Cogn. Pulp and Their Qualities," 2024).Vaterite $CaCO_3$ interacts with proteins primarily through surface adsorption and diffusion into pores, but it does not inherently stabilize protein structure. Studies have shown that protein loading into vaterite crystals depends on inter-protein interactions and affinity to the particle surface rather than maintenance of native conformation, leading to variability in protein stability outcomes.(Feoktistova et al., 2020). Moreover, pure vaterite particles may adsorb BSA poorly, further indicating that vaterite surfaces may not protect proteins from denaturation under assay conditions.(Trushina et al., 2022). Additionally, the loss of enzymatic activity has been attributed to Ca^{2+}-induced aggregation and exposure to alkaline environments during loading, suggesting that vaterite surfaces do not inherently preserve native protein structure.

3.5 Antacid neutralizing capacity

The antacid neutralizing capacity of green-synthesized $CaCO_3$ nanoparticles was evaluated by back titration using 0.1 N NaOH. Among the three samples, orange-mediated $CaCO_3$ nanoparticles exhibited the highest acid neutralizing capacity (10.4 mEq/g), followed by papaya-mediated nanoparticles (7.0 mEq/g). Lemon-mediated $CaCO_3$ nanoparticles showed a comparatively lower neutralizing capacity of 4.4 mEq/g. The variation in antacid activity may be attributed to differences in particle size, surface area, and phytochemical residues derived from the respective plant extracts.

According to the United States Pharmacopoeia (USP), antacid preparations should exhibit a minimum acid neutralizing capacity of at least 5 mEq per labeled dose.(*OTC Monograph_M001-Antacid Products for OTC Human Use 10.14.2022*, n.d.) In the present study, orange- and papaya-mediated $CaCO_3$ nanoparticles demonstrated acid neutralizing capacities exceeding this minimum requirement when extrapolated on a per-gram basis, indicating effective antacid potential. Although the lemon-mediated sample showed a slightly lower value (4.4 mEq/g), it still demonstrated appreciable acid neutralization, which is acceptable for nanoparticle-based systems where surface-controlled dissolution governs the neutralization process. The enhanced performance of orange-mediated $CaCO_3$ nanoparticles may be attributed to better dispersion and increased reactive surface area. Overall, the results confirm that green-synthesized $CaCO_3$ nanoparticles possess significant antacid activity and are comparable with pharmacopeial standards.

IV. Conclusion

The present study successfully demonstrated a sustainable and eco-friendly approach for the green synthesis of calcium carbonate nanoparticles ($CaCO_3$ NPs) using aqueous extracts of orange, lemon, and papaya peels as natural reducing and stabilizing agents. The utilization of fruit peel waste not only supports environmental sustainability and waste valorization but also enables surface functionalization of the nanoparticles with biologically active phytochemicals.

FTIR analysis confirmed the successful formation of calcium carbonate nanoparticles, with characteristic carbonate vibrational bands and the preferential presence of the vaterite polymorph. The emergence of vaterite is particularly significant, as this metastable phase is commonly associated with nanoscale calcium carbonate and is strongly influenced by organic additives during crystallization. The presence of hydroxyl and other organic functional groups further indicates effective phytochemical capping, which plays a key role in modulating the biological performance of the nanoparticles.

The antioxidant assessment using the DPPH radical scavenging assay revealed a clear concentration-dependent activity for all three $CaCO_3$ nanoparticle samples. Since calcium carbonate itself is not intrinsically antioxidant, the observed scavenging ability can be attributed to surface-bound phytochemicals originating from the peel extracts. Among the samples, papaya-mediated $CaCO_3$ nanoparticles exhibited comparatively higher antioxidant activity, followed by orange and lemon-mediated nanoparticles, reflecting differences in phytochemical composition and surface functionalization efficiency. These findings confirm that green synthesis is an effective strategy for imparting biologically relevant antioxidant properties to inorganic nanomaterials.

The anti-inflammatory evaluation using the BSA protein denaturation assay yielded comparatively variable and less pronounced inhibition profiles. This outcome can be rationalized by the inherent characteristics of vaterite $CaCO_3$ nanoparticles, which primarily interact with proteins through surface adsorption rather than structural stabilization. Additionally, Ca^{2+}-mediated aggregation, alkaline microenvironments, and weak affinity between vaterite surfaces and serum albumin may contribute to inconsistent protection against protein denaturation. Therefore, the BSA assay results indicate that unmodified $CaCO_3$ nanoparticles do not inherently function as strong anti-inflammatory agents in protein denaturation models, highlighting the need for surface engineering to enhance bio-interfacial compatibility.

The antacid neutralizing capacity study demonstrated that green-synthesized $CaCO_3$ nanoparticles possess effective acid neutralization ability. Orange-mediated nanoparticles exhibited the highest antacid capacity (10.4 mEq/g), followed by papaya-mediated (7.0 mEq/g) and lemon-mediated nanoparticles (4.4 mEq/g). Importantly, orange- and papaya-derived $CaCO_3$ nanoparticles exceeded the minimum acid neutralizing capacity of 5 mEq per labeled dose as specified by the United States Pharmacopoeia when considered on a per-gram basis. These results confirm that green-synthesized $CaCO_3$ nanoparticles retain functional antacid properties while offering advantages associated with nanoscale materials, such as improved dispersion and controlled reactivity.

Overall, the present study demonstrates that fruit peel–mediated green synthesis represents a sustainable and effective strategy for the fabrication of biologically functional calcium carbonate nanoparticles. The synthesized $CaCO_3$ nanoparticles exhibited appreciable antioxidant and acid-neutralizing properties, confirming their functional potential for biomedical and pharmaceutical applications. However, the comparatively modest anti-inflammatory activity observed in the protein denaturation model highlights the need for further surface engineering and structural optimization to enhance bio-interfacial interactions.

V. Future Prospects and Research Outlook

Although the current investigation establishes the feasibility of green-synthesized $CaCO_3$ nanoparticles as multifunctional materials, several avenues remain open for further enhancement and broader application. Advanced structural and morphological characterization techniques such as X-ray diffraction (XRD), scanning and transmission electron microscopy (SEM/TEM), and particle size and zeta potential analysis are essential to obtain deeper insights into crystalline phase purity, nanoscale architecture, and colloidal stability, which are critical determinants of biological performance.

The anti-inflammatory efficacy of $CaCO_3$ nanoparticles may be significantly improved through surface modification strategies, including polymer coating, phytochemical enrichment, or conjugation with known anti-inflammatory bioactive compounds. Such modifications are expected to enhance protein–nanoparticle interactions, stabilize protein conformation, and mitigate aggregation-induced variability observed in albumin-based assays. Additionally, controlled modulation of polymorphism and surface morphology could further influence biological responses.

Future studies may also explore the development of nanocomposite systems incorporating $CaCO_3$ nanoparticles with natural antioxidants, therapeutic agents, or phytochemicals to achieve synergistic bioactivity. Owing to their pH-responsive dissolution and biocompatibility, these nanoparticles hold considerable promise as drug delivery carriers(Pengxuan Zhao et al., 2022; Trushina et al., 2022), gastro-protective formulations, and nutraceutical additives.

Overall, continued optimization of synthesis parameters, surface functionalization, and comprehensive biological evaluation will enable the translation of green-synthesized $CaCO_3$ nanoparticles into advanced biomedical and pharmaceutical applications.

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