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Green Synthesis of Strontium Oxide Nanoparticles using Citrus limon Extract: Characterization and Evaluation of its Antimicrobial Efficacy

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Abstract:
Aim: The aim of this study is to synthesize Strontium oxide nanoparticles (SrO-NPs) using Citrus limon fruit extract and evaluate their physicochemical characteristics and antimicrobial efficacy against common oral pathogens.

Materials and methods: The extract of Citrus limon was combined with a Strontium chloride solution to synthesize SrO-NPs. The NPs were characterized using Fourier transform infrared (FTIR) analysis, scanning electron microscopy (SEM), and energy-dispersive electron microscopy (EDAX). Antimicrobial activity was assessed using the agar well diffusion method against Staphylococcus aureus, Enterococcus faecalis and Streptococcus mutans.

Results: FTIR analysis confirms the presence of functional groups from lemon extract in SrO-NPs. SEM reveals well-defined nanoparticles with spherical shapes and uniform size distribution. EDAX microscopy confirms the elemental composition of SrO-NPs. SrO-NPs exhibit potent antimicrobial effects against oral pathogens at 100 µL.

Conclusion: The synthesis of SrO-NPs using Citrus limon fruit extract proves effective, with lemon extract facilitating the production of stable nanoparticles with antimicrobial properties. However, further research is needed to understand their behavior in clinical settings and integrate them into practical applications.

Keywords: Nanoparticles, green synthesis, Citrus limon, Strontium oxide, antimicrobial efficacy

Introduction:

Nanotechnology has emerged as a powerful tool in diverse medical and dental realms, encompassing applications such as dental remineralization, regeneration of soft and hard tissues, delivery systems for orthodontic drugs, addressing hypersensitivity, as well as treating periodontal disease and dental caries [1]. Nanotherapeutics present an opportunity to manage biofilms by harnessing the biocidal characteristics of nanoparticles [2]. The reduced diameter of nanoparticles enhances their mechanical attributes and augments their antimicrobial efficacy due to the increased surface-to-volume ratio. Numerous mechanisms have been suggested to elucidate the means by which nanoparticles regulate bacterial growth in the oral cavity. These include metal ion release, oxidative stress, and nonoxidative mechanisms [3–5]. However, a contact-mediated biocidal effect remains the most frequently known mechanism. Nanoparticles offer a distinct advantage over conventional antimicrobial approaches in that they are not influenced by antibiotic resistance mechanisms. This is because nanoparticles establish direct contact with the bacterial cell wall, bypassing the need for penetration [6,7].

There has been an increased focus in contemporary research and development within materials science and technology, revolving around the advent of "green synthesis" methodologies [8,9]. Essentially, these methods entail the production of materials and nanomaterials through processes that emphasize regulation, control, cleanup, and remediation. These methods generate nanoparticles (NPs) utilizing extracts derived from plants, bacteria, fungi, algae, and other organisms abundant in phytochemicals that acts as both reducing and stabilizing agents in the process [10]. Strontium, a trace metal with properties similar to calcium and magnesium, plays vital roles in the human body. Its properties make it valuable in bone and tissue engineering as well as dentistry [11,12]. Recently, there has been growing interest in formulating nanoparticles containing strontium due to their diverse applications, including drug delivery, bioimaging, cancer therapy, bone engineering, and environmental management [13–15].

Flavonoids play a major role in providing antioxidants. Citrus fruits have the most flavonoids during their development [16]. The antioxidants in citrus fruits, including flavonoids, carotenoids, terpenes, and limonoids, help reduce oxidative stress [17]. Citrus limon, commonly known as lemon, is a key crop widely utilized in fruit production. The industrial processing of lemons generates significant quantities of by-products rich in nutrients and bioactive compounds, which hold potential value for the food industry [18]. Citrus limon contains high amount of phenolic and fibrous compounds that are responsible for its antioxidant, antimicrobial and anti-inflammatory properties [19]. The phytochemicals present in the extract inhibit nanoparticle overgrowth and reduce aggregation during colloidal synthesis. Moreover, they can modulate and enhance the properties of the synthesized nanoparticles, optimizing their functionality for various applications [20,21].

The study focused on the green synthesis of Strontium oxide nanoparticles (SrO-NPs) using the aqueous extract of Citrus limon, analyze the physicochemical characteristics of the synthesized nanoparticles and evaluate its antimicrobial efficacy against common oral microbes.

Materials and methods:

Preparation of extract

The lemons were carefully selected and sourced from a fruit vendor and prepared for extraction. Each lemon was meticulously cleaned and cut into small fresh pieces, followed by crushing them to release the pulp. The extracted pulp was filtered using a Whatman number 42 filter, in order to remove any solid particles or residues. Additionally, the filtered juice was centrifuged for an hour to separate any remaining particulate matter or sediment from the extract, enhancing its purity and transparency. Finally, the clarified lemon extract was carefully transferred to a clean container and stored in the refrigerator.

Preparation of SrO solution

Strontium oxide nanoparticles were synthesized using a green synthesis approach mediated by the lemon extract. 20mmol of strontium chloride was weighed and added to 90mL of distilled water in a beaker. The resultant solution was agitated continuously with temperature maintained at 50-60°C.

Addition of lemon extract to SrO Solution

10mL of the lemon extract was added to the diluted SrO solution. The solution mixture was placed on a magnetic stirrer and stirred continuously at 600 rpm speed for 48 hours. Then, centrifugation was performed at 8000 rpm for 10 minutes. The resultant pellet was collected, and the supernatant was discarded. The pellet obtained was dried in a hot air oven, after which it was scraped to obtain powdered Strontium oxide nanoparticles (SrO-NPs). 100mg of this nanoparticle powder is added to 10mL of distilled water for further analysis.

Characterization ofSrO-NPs

The synthesized nanoparticles were analyzed using the following characterization techniques:

Fourier Transform Infrared (FTIR) Spectroscopy: The synthesized SrO-NPs were mixed with Potassium bromide and compressed to form a pellet. This resultant naanoparticle pellet was subjected to FTIR (Thermo Nicolet, Avatar 330, Waltham, MA, USA) analysis in order to identify the functional groups in the synthesized SrO-NPs.

Scanning Electron Microscopy (SEM): The synthesized SrO-NPs were applied onto a sample holder,coated with a thin layer of conductive material. The morphology of the NPs was then evaluated using high-resolution SEM (JEOL USA Inc., Peabody, MA, USA).

Energy-Dispersive Electron (EDAX) microscopy: The SrO-NPs were subjected to EDAX analysis to identify their elemental characteristics. The NPs were deposited onto a suitable substrate and a scanning electron microscope equipped with an EDX detector (Bruker Germany, D8 Advance Diffractometer, Leipzig, Germany) was used for analysis.

Antimicrobial evaluation against oral pathogens:

The methodology was according to *Ramesh et al.[22]*. The antimicrobial efficacy of the synthesized SrO-NPs was assessed against Staphylococcus aureus, Enterococcus faecalis and Streptococcus mutans, employing agar well diffusion method. The Mueller-Hinton agar (MHA) was prepared with double distilled water at pH of 7, and autoclaved for 15 minutes at 121°C. The sterilized medium was poured into three petri dishes and was allowed to solidify at room temperature. After the medium is hardened, a sterile cotton swab was used to transfer an inoculum of freshly prepared microbial culture (about 106cfu/ml) onto the three MHA plates, corresponding to the designated test bacteria, respectively.

Four wells with a diameter of 9mm were drilled into the MHA medium using a micropipette. Three wells were filled with SrO-NPs solution with 25 µL, 50 µL, and 100 µL volumes and the fourth well was filled with 0.2% Chlorhexidine (CHX) solution as a control. The solutions were allowed to diffuse at room temperature for 4 hours, followed by incubation of the culture plates for 24 hours at 37°C. Following the incubation period, the diameter of the zone of inhibition was measured was each tested medium.

Results:

Fourier Transform Infrared (FTIR) Spectroscopy:

The FTIR spectrum exhibited distinct peaks indicative of various functional groups (Figure 1). The presence of these peaks at specific wavenumbers confirmed the successful synthesis of the nanoparticles and the contribution of functional groups derived from the lemon extract in their formation. The infrared spectrum exhibit the absorption peaks ranging from 850 to 2800 cm^{-1}. The absorption peak at 2872.69 cm^{-1} revealed carbon residues, while the peaks at 2359.73 cm^{-1} and 2335.95 cm^{-1} show oxygen-carbon elongation. The absorption range at 1164.56 cm^{-1} and 1063.29 cm^{-1} indicate stretching mode of hydroxyl groups. The peak at 878.80 cm^{-1} indicate hydrogen-carbon bending.

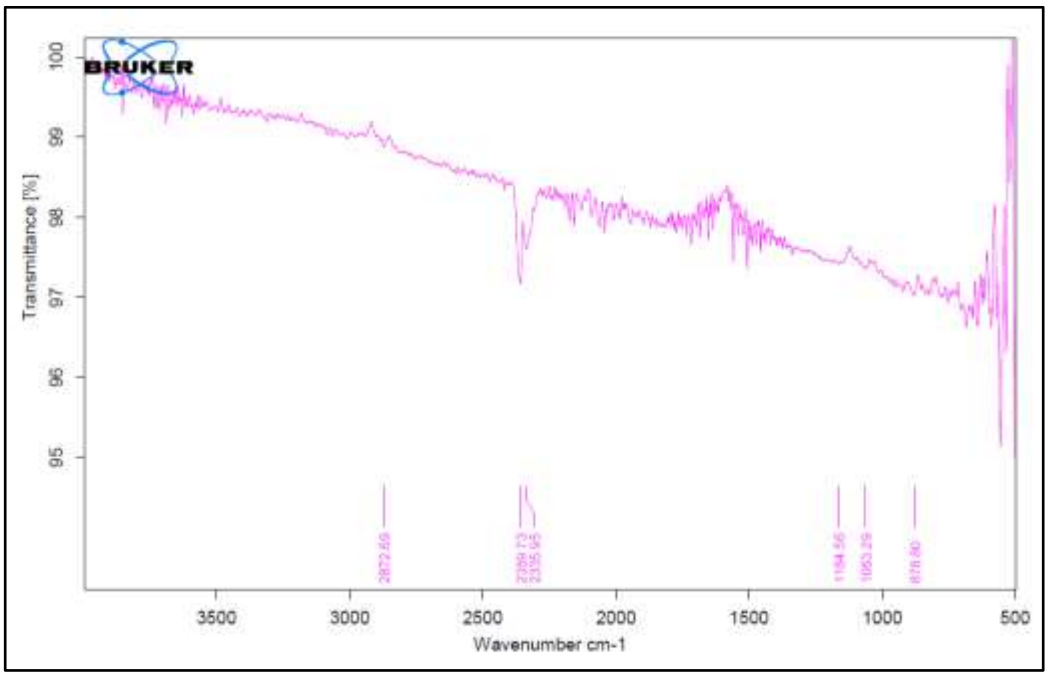

Figure 1: FTIR spectroscopy analysis of SrO-NPs

Scanning Electron Microscopy (SEM):

SEM analysis was conducted to investigate the morphology of the synthesized nanoparticles. The SEM images show nanoparticles with spherical shapes with a mean particle size of 55nm (Figure 2 and 3). The SEM examination visually confirmed the structure and surface properties of the synthesized nanoparticles.

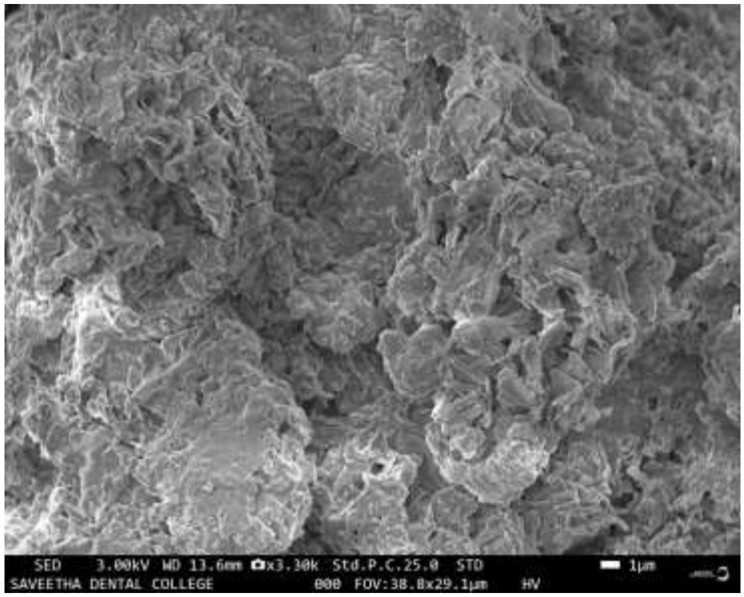

Figure 2: SEM image of SrO-NPs (at 3,300x magnification)

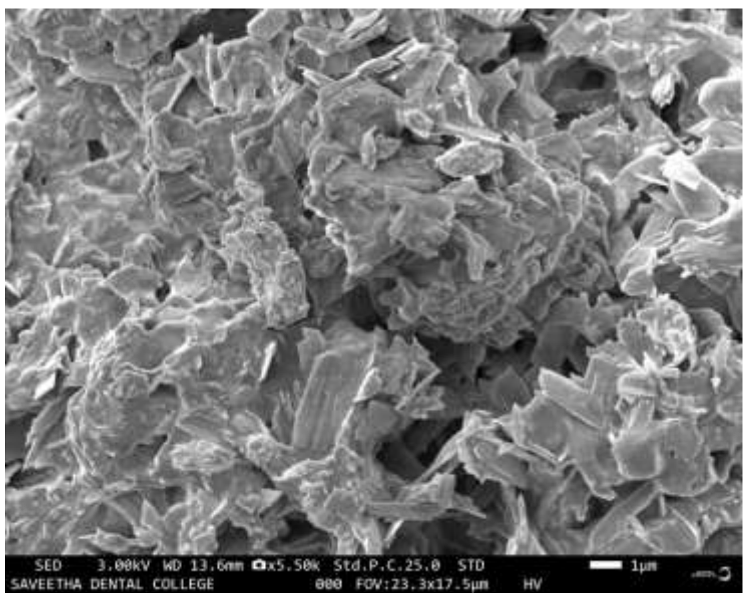

Figure 3: SEM image of SrO-NPs (at 5,500x magnification)

Energy-Dispersive Electron (EDAX) microscopy:

The EDAX analysis consistently displayed peaks, within the SrO crystallite spectrum, confirming the presence of strontium and oxygen and validating the successful production of SrO-NPs. Due to the inert and pure nature of the material throughout the synthesis process, no supplementary peaks were observed in the analysis. The EDAX results show presence of 52.9

wt% of carbon, 21.4 wt% of oxygen, 15.3 wt% of strontium, 9.5 wt% of chloride and 0.9 wt% of potassium (Figure 4).

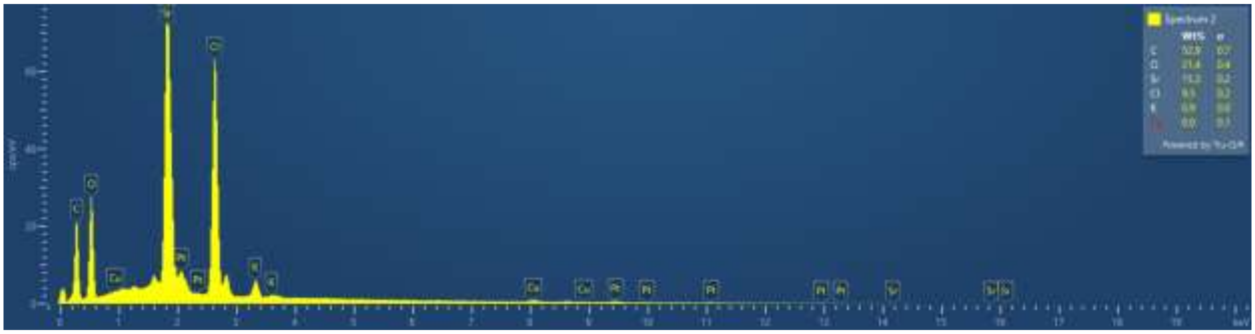

Figure 4: EDAX results showing elemental composition of SrO-NPs

Antimicrobial evaluation against oral pathogens:

The results revealed potent antimicrobial effects of SrO nanoparticles against S. aureus across different concentrations, that is 25 µL, 50 µL, and 100 µL (Table 1), yielding zone of inhibition diameters of 11 mm, 13 mm, and 16 mm, respectively. Additionally, when tested at higher concentrations, the synthesized nanoparticles exhibited significant antibacterial activity against E. faecalis and S. mutans.

Organism	**Zone of inhibition (in mm)**			
	SrO-NPs			**Control (0.2% CHX)**
	25 µL	**50 µL**	**100 µL**	

S.aureus	11	13	16	42
E.faecalis	9	10	12	40
S.mutans	9	9	11	35

Table 1: Diameter of zone of inhibition indicating the antimicrobial efficacy of SrO-NPs against oral pathogens

Discussion:

Although nanoparticles show promise for their antibacterial properties, many synthesis methods are expensive and can pose risks to the environment, biological systems, and human health due to the use of toxic substances. Consequently, researchers have developed "green" nanoparticle synthesis technologies [23]. These alternatives rely on biological systems such as yeast, fungi, bacteria, and plant extracts, avoiding the use of hazardous compounds and offering a safer and more environmentally friendly approach compared to chemical methods. Plant extracts are extensively utilized for various purposes due to several factors, including their abundant and readily available sources, widespread distribution across the globe, safe handling procedures, availability of diverse metabolites with potent reducing properties, and their ability to minimize waste generation and energy consumption [24,25]. The present study aimed to synthesize strontium oxide nanoparticles (SrO-NPs) using a green synthesis approach mediated by the aqueous extract of Citrus limon fruit and evaluate their physicochemical characteristics, as well as assess their antimicrobial efficacy against common oral pathogens.

The FTIR analysis confirms the attachment of functional groups from the bioactive compounds present in the lemon extract. These functional groups played a vital role in reduction process of Strontium chloride to Strontium oxide, and further and stabilizing the synthesized SrO nanoparticles. Size and shape represent two fundamental criteria addressed during the characterization of nanoparticles. Scanning electron microscopy (SEM) was performed to determine the size, shape, distribution and surface characteristics of the synthesized SrO-NPs. The SEM images demonstrate the presence of well-defined nanoparticles with a spherical shape and uniform size distribution, suggesting successful synthesis of SrO-NPs. This outcome may assist in future development and utilization of these nanoparticles. The EDAX analysis confirms the formation of SrO-NPs, giving the elemental composition of the synthesized NPs.

Citrus limon contains alkaloids, which are known to possess antibacterial properties. Previous studies have reported the antibacterial efficacy of raw extracts derived from different parts of the lemon plant, including leaves, stems, roots, seeds, and flowers, against clinically significant bacterial strains [26]. The flavonoids found in Citrus limon exhibit a wide array of biological activities, including antibacterial, antifungal, antidiabetic, anticancer, and antiviral properties. Additionally, these flavonoids serve a defensive function against various pathogens, such as bacteria, fungi, and viruses [27]. This study investigated the antimicrobial efficacy of SrO-NPs at concentrations of 25 µL, 50 µL, and 100 µL. The most substantial antibacterial impact was observed at 100 µL concentration against S. aureus (16 mm), followed by E. faecalis (12 mm), with the least effect observed against S.mutans (11 mm). The antimicrobial efficacy of lemon extract is primarily attributed to the presence of bioactive substances such as flavonoids, terpenes, and coumarins that have strong inhibitory activity against the microorganisms [28].

The approach to drug delivery in dental treatment has shifted towards localized delivery of antibiotics, mainly due to the drawbacks associated with systemic drug administration [29]. In healthcare, nanoparticles are being used in drug delivery systems to improve the efficacy and safety of medications. By encapsulating drugs within nanoparticles, controlled release and targeted delivery to specific cells or tissues can be achieved, minimizing side effects and maximizing therapeutic benefits. This localized drug delivery system typically involves a biodegradable scaffold, allowing for the gradual release of the drug from a bioadhesive entity. Current research efforts are concentrated on developing nanodrug delivery systems for enhanced efficacy and precision in dental treatments [30]. Nanoparticles derived from phytochemicals have gained significant interest for their potential in localized drug delivery to target disease sites while minimizing toxicity and side effects [31]. These nanoparticles offer numerous advantages, including improved stability, enhanced solubility, the ability to accommodate both hydrophilic and hydrophobic substances, increased bioavailability, enhanced efficacy, dosage reduction, and improved absorption [32]. The sustained release mechanism effectively reduces microbial counts in the oral cavity, contributing to enhanced immune function and accelerated healing processes.

The current study lay emphasis the antimicrobial efficacy of lemon extract mediated SrO-NPs. However, this study was conducted in-vitro, and the findings might not entirely represent the intricate interactions within a living organism and may not precisely forecast the behavior of the synthesized nanoparticles in a clinical setting. Also, more research is needed evaluating the impact these NPs on other oral pathogens that includes bacteria, spirochetes, viruses and fungi. Further cytotoxicity analysis and laboratory studies may help development of novel products in the future.

Conclusion:

The process of producing SrO nanoparticles using Citrus limon fruit extract was seen to be effective. The fruit extract facilitates the production of stable NPs due to its dual role of reducing and stabilizing the synthesized nanoparticles. These SrO-NPs exhibit potential antibacterial properties against oral pathogens, suggesting their potential application in formulation of novel antimicrobial products. However, further research is necessary to integrate SrO-NPs into oral health products.

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