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# Development And Characterisation of Morinda Citrafolia Coupled Hydroxyapatite Nano Particle as an Anticariogenic and Remineralising Agent

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## ABSTRACT

**Aim**
This study looked at how morinda citrifolia coupled hydroxyapatite nanoparticle was developed. It also checked its features as an agent to prevent tooth decay.
**Materials and Methods**
Five grams of M.citrifolia powder was made by grinding the noni fruit and drying it. Five grams of HAP (hydroxyapatite) were prepared using the wet chemical condensation method. This HAP was then finely ground. It was sent for SEM (scanning electron microscopy) and EDS (energy-dispersive X-ray spectroscopy) to detect HAP nanoparticles and check the elements. After HAP was ready, it was combined with Morinda citrifolia and sent for FTIR (Fourier-transform infrared spectroscopy) to find the functional groups. This helped determine the substances present. Finally, S.mutans and LB broth were prepared. They were then sent for PCR analysis and compared with a control group and the HAP+Morinda sample.
**Results**
FTIR results showed a strong bond between Morinda citrifolia and hydroxyapatite nanoparticles. EDX results provided their chemical makeup. SEM results showed their clear physical features. Real-time PCR analysis showed a decrease in the nucleic acid expression of Lactobacillus and S.mutans samples after treatment with the Morinda citrifolia-coupled hydroxyapatite nanoparticles.
**Conclusion**
This study shows a good bond between Morinda citrifolia and hydroxyapatite nanoparticles, seen with FTIR and EDX analyses. SEM results show their clear shape and size. Real-time PCR analysis shows a clear drop in the nucleic acid expression of Lactobacillus and S. mutans after treatment. This suggests they can kill germs. The Morinda citrifolia-coupled hydroxyapatite nanoparticles look promising for use in dentistry and medicine. More study is needed to understand how they work and if they can be used in clinics.

**Keywords:** Nanobiotechnology, Nanoparticles, Morinda citrifolia, Dental caries, Hydroxyapatite (HA), Antimicrobial properties, Biocompatibility, Caries prevention, Bioactive compounds, Nanoparticle synthesis, Plant-based synthesis, Tooth remineralization, Anticancer effects In vitro studies, Sustainable materials

## Introduction

Nanobiotechnology uses living materials to make very tiny particles. These particles have special features [1]. These tiny particles are smaller than 100 nanometers. They are used in many different areas. Compared to older ways of making nanoparticles, like chemical and physical methods, nanobiotechnology is better for the environment. It also costs less [2]. Dental caries, also known as tooth decay, is a common problem with oral health. It happens when the outer layer of the tooth, called enamel, loses its minerals. This remains a big health concern worldwide [3]. Even with new methods in dental care, finding good and lasting ways to prevent tooth decay is still important [4]. Natural compounds and very small materials (nanomaterials) are now seen as hopeful options for this [5].

Plants offer an easier and better way to make nanoparticles compared to using tiny living cells (microbial cells) [6]. Unlike methods that use microbes, making nanoparticles from plants does not involve hard steps. These hard steps include making nanoparticles inside cells and many cleaning steps [7].
Morinda citrifolia, often called Indian mulberry, is a plant from warm regions. It has a long history of being used in traditional medicine. It contains many active substances. These include anthraquinones, iridoids, and terpenoids. These substances have been reported to fight bacteria, reduce swelling, and protect against cell damage [8]. These qualities suggest that Morinda citrifolia could be a part of dental products to stop tooth decay.
M. citrifolia has shown many effects against cancer in different cancer models [9]. It works in many ways. These include stopping cancer growth, reducing how fast cells multiply, causing cancer cells to die (apoptosis), stopping new blood vessel growth, stopping cells from moving, and changing swelling and body defense responses. This suggests M. citrifolia might be a useful medicinal plant for cancer treatment. It affects many body processes. More careful studies on how well it works and if it is safe are needed. This would help move it towards future studies in humans. Such studies could further prove the use of M. citrifolia in cancer therapy [9].
Hydroxyapatite (HA) is a natural mineral that is safe for the body. It is very similar to the part of human tooth enamel that is not alive. HA nanoparticles have been studied a lot for their possible uses in dentistry. These include helping teeth get back their minerals (tooth remineralization) and making new dental materials. The very small size of HA particles makes them more active in the body. It also allows them to enter the mouth better [10].
By combining the active properties of Morinda citrifolia with the ability of HA nanoparticles to help teeth remineralize, a theory was formed. It was thought that Morinda citrifolia-coupled HA nanoparticles could be a new and strong agent to prevent tooth decay. This study aimed to find out how to make these nanoparticles. It also aimed to check their features and how well they prevent tooth decay in lab tests (in vitro).

**Materials and Methods**

Five grams of M.citrifolia powder was prepared by grinding the noni fruit and drying it. This made it into a powder. Five grams of HAP (hydroxyapatite) were prepared using the wet chemical condensation method. The HAP was then finely ground using a mortar and pestle. It was sent for SEM (scanning electron microscopy) and EDS (energy-dispersive X-ray spectroscopy). This was done to detect HAP nanoparticles and for elemental analysis of HAP. After the HAP was ready, it was combined with Morinda citrifolia. This combination was sent for FTIR (Fourier-transform infrared spectroscopy). This was to detect the functional groups and determine the substances present in the extract. Finally, S.mutans and LB broth were prepared. They were then sent for PCR analysis. The results were compared with a control group and the HAP+Morinda sample.

**Results**

**FTIR**
FTIR analysis of the Morinda citrifolia-coupled hydroxyapatite nanoparticles showed specific peaks. These peaks were linked to O-H, C-H, and C=O stretching. This confirmed that Morinda citrifolia components were present. The spectrum also showed peaks that indicated hydroxyapatite. This suggested that the two materials successfully combined. No new, clear peaks appeared in the fingerprint region. This suggests that the interaction between Morinda citrifolia and hydroxyapatite was mainly physical, not chemical. From FTIR results, a strong bond was seen between Morinda citrifolia and hydroxyapatite nanoparticles.

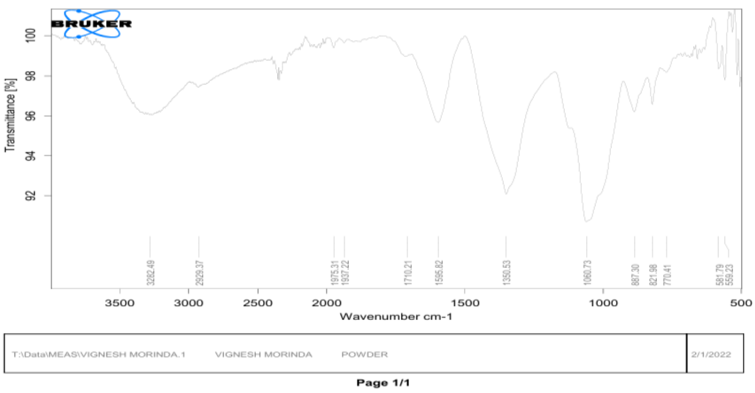


**FIGURE 1:** Figure showing the FTIR analysis for Morinda citrifolia-coupled hydroxyapatite nanoparticles

**EDX**

EDX analysis of the Morinda citrifolia-coupled hydroxyapatite nanoparticles confirmed the presence of expected elements. These included calcium, oxygen, carbon, nitrogen, and magnesium. The calcium peak showed high intensity. This meant hydroxyapatite was the main part in the composite. Finding organic elements suggested that Morinda citrifolia components successfully combined. No major impurities or unwanted substances were found. From these EDX results, the chemical makeup of the Morinda citrifolia-coupled hydroxyapatite nanoparticles was provided

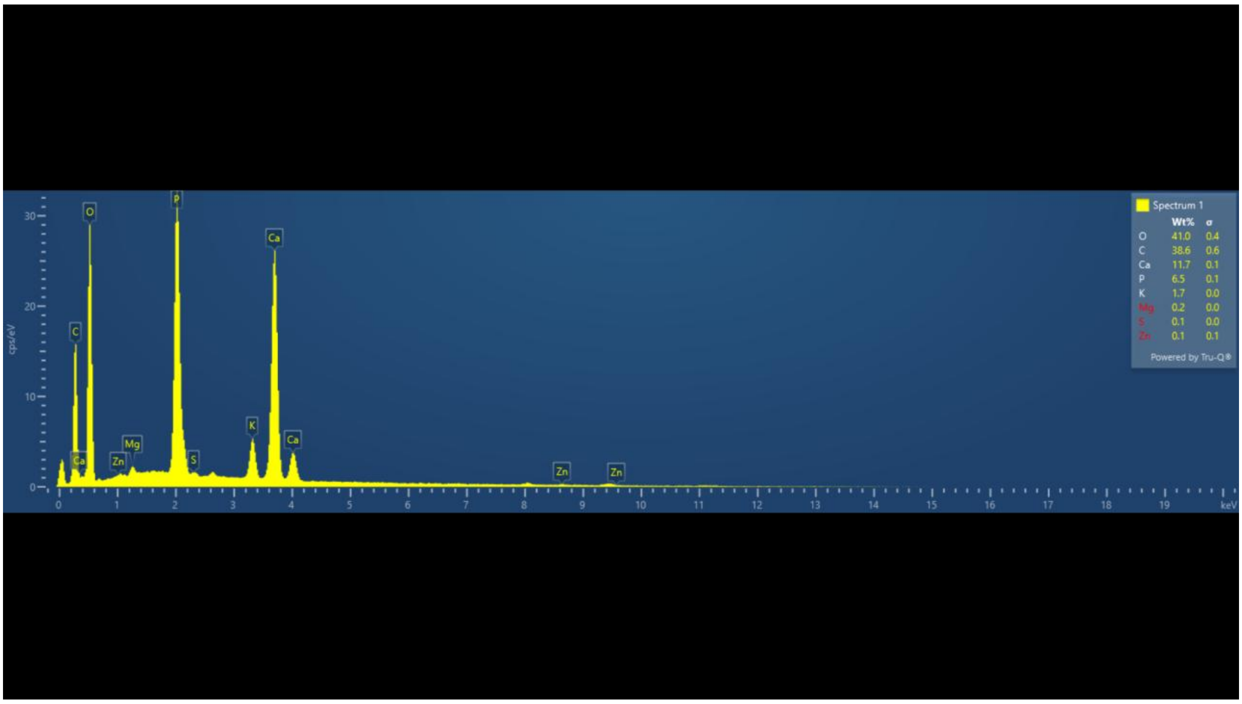


**FIGURE 2:** Figure 2 showing EDX analysis of the Morinda citrifolia-coupled hydroxyapatite nanoparticle

**SEM**

SEM analysis showed that Morinda citrifolia-coupled hydroxyapatite nanoparticles were not spread evenly. They had different sizes and irregular shapes. The particles showed a rough surface and tended to clump together (agglomeration). Both crystalline and amorphous features were present. This suggested a complex interaction between the organic and inorganic parts. From the SEM results, the clear physical characteristics of the Morinda citrifolia-coupled hydroxyapatite nanoparticles could be seen. Also, the crystallization of this compound was confirmed from the analysis. It also showed the ability to help teeth regain minerals (remineralising capability).

**PCR Analysis**

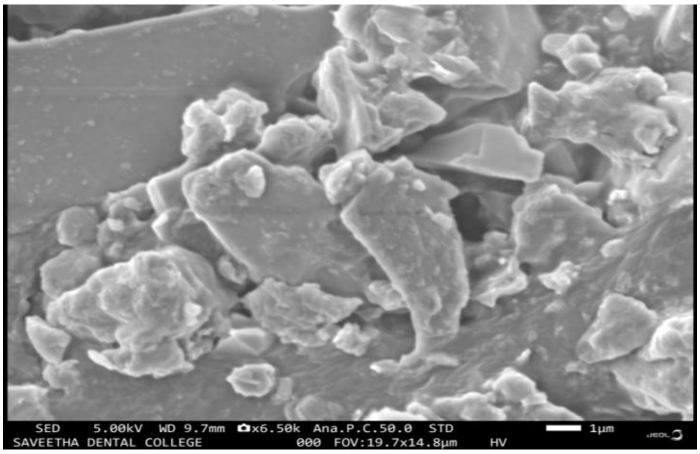


**FIGURE 3:** Figure 3 Showing Morinda citrifolia-coupled hydroxyapatite nanoparticles with varying sizes and irregular shapes

**PCR Analysis**

Fresh LB broth was used for growing more bacteria. Bacteria grown overnight at 37°C were used to isolate total RNA. A total RNA isolation reagent (TRIR) from Ab gene house, United Kingdom, was used for this. Briefly, 2µg of RNA was copied using the reverse transcriptase RT kit from Eurogentec, Seraing, Belgium. The sequence of the primers used in this study is given in Table 2. 16s r-RNA served as a reference gene (housekeeping gene). Genes were increased in number using SYBR green mastermix from Takara, Japan, in a qRT-PCR system (CFX96 Touch Real-Time PCR Detection System) from Bio-Rad, USA. The PCR protocol was as follows: starting heat treatment at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, 59-60°C for 30 seconds, and 72°C for 30 seconds.

The relative amount was calculated from the melt and amplification curves analysis. The relative amount of each gene was found using the 2-ΔΔCT method. The expression level of each gene was calculated using the fold change. This was compared to the untreated group's normal activity level.

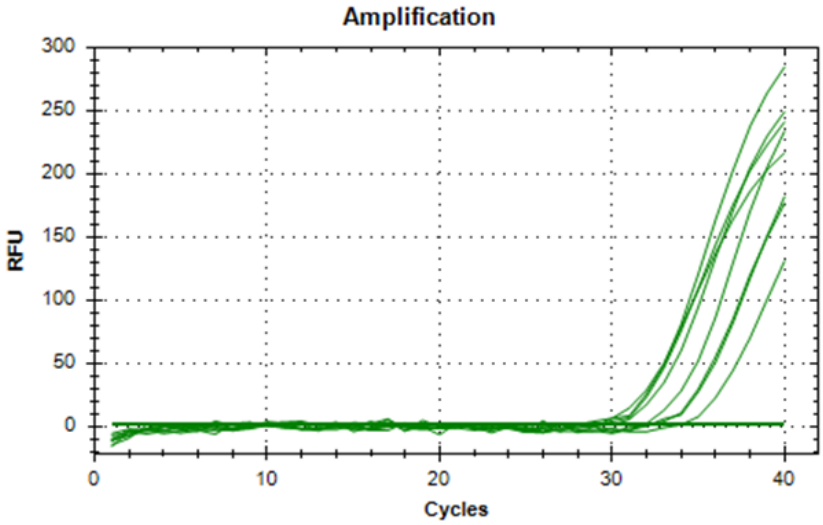


**Figure 4: Figure 4 Showing the Real time PCR cycle for the Lactobacillus untreated with nanoparticle**

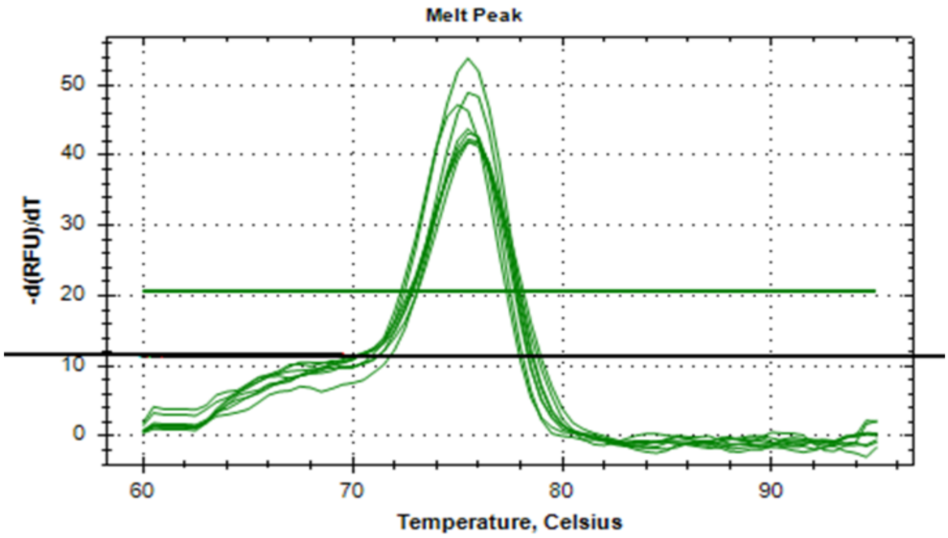


**Fig 5: Figure 5 Showing the real time PCR amplification cycle for the Lactobacillus treated with nanoparticle**

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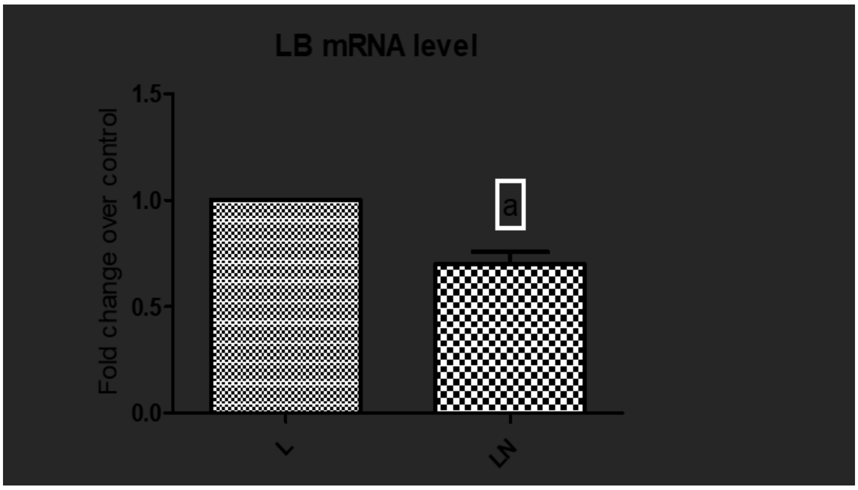


**Fig 6: Figure 6 showing control Group and experimental group of Lactobacillus untreated and treated with nanoparticle**

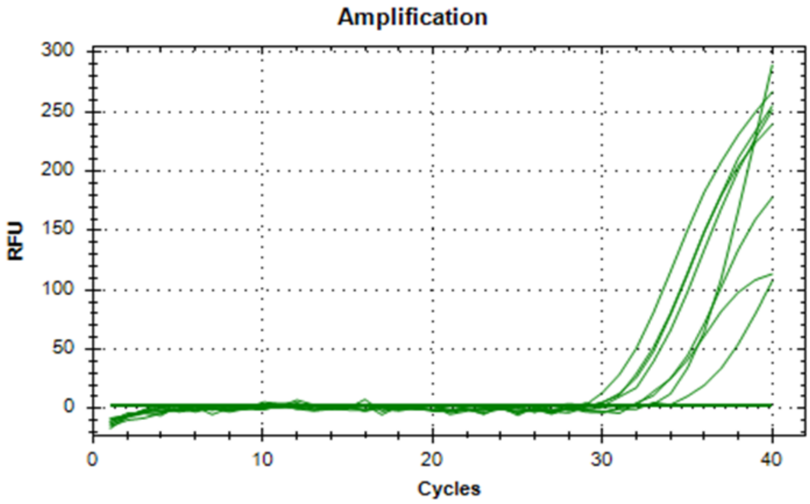


**Fig 7: Figure 7 showing the real time PCR amplification cycle of S.mutans untreated with nanoparticle**

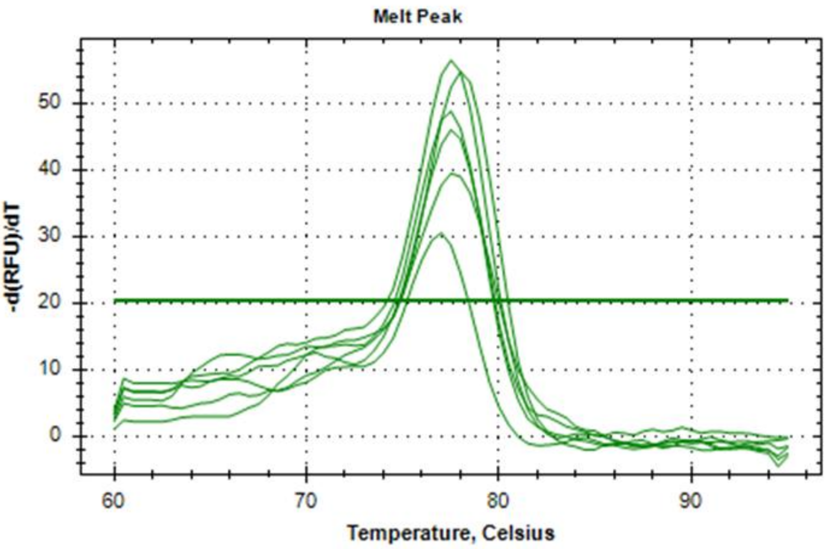


**Fig 8: Figure 8 showing the real time PCR amplification cycle of S.mutans treated with nanoparticle**

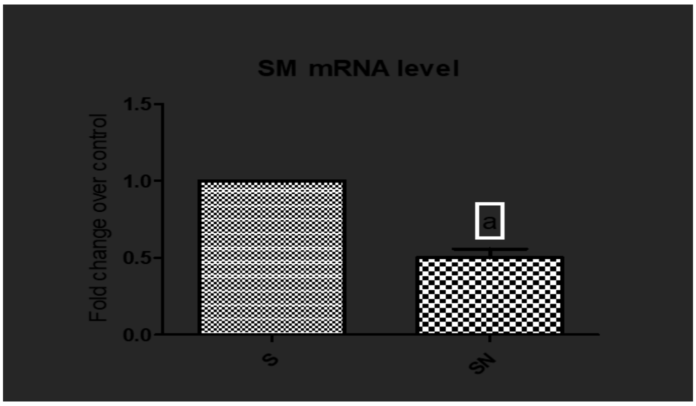


**Fig 9: Figure 9 showing the control Group and experimental group of Lactobacillus untreated and treated with nanoparticle**

**Discussion**

Developing new and effective agents to fight tooth decay has been a very important area of study in dental research. This particular study looks at the possible use of Morinda citrifolia-coupled hydroxyapatite nanoparticles. These nanoparticles appear to be a promising new option for preventing tooth decay [11, 12]. By carefully comparing the results found in this research with information from other existing studies, a clearer understanding can be gained. This comparison helps in seeing the possible good points and any challenges connected with using these special nanoparticles in dentistry. Such an understanding is crucial for moving forward with new dental treatments.

Characterization and Properties

The way the Morinda citrifolia-coupled hydroxyapatite nanoparticles were described and identified in this study matches well with what earlier research has found. Both FTIR (Fourier-transform infrared spectroscopy) and EDX analysis (energy-dispersive X-ray spectroscopy) were used. These analyses confirmed that the organic parts from Morinda citrifolia and the inorganic mineral parts, hydroxyapatite, successfully combined together. This means the components joined as intended. Also, SEM analysis (scanning electron microscopy) showed how the nanoparticles were spread out. They were not evenly spaced and had different sizes, which is called a heterogeneous distribution of nanoparticles. These observations are in line with other studies. These earlier studies have explored how hydroxyapatite-based nanoparticles are made and described for different uses in dentistry [13]. The consistency of these findings adds strength to the understanding of these new materials.

Anticariogenic Potential

The PCR analysis (polymerase chain reaction analysis) performed in this study gives important information. It suggests that the nanoparticles might affect the activity of genes inside bacteria. These genes are linked to causing tooth decay, specifically in Lactobacillus and Streptococcus mutans bacteria. This observation fits well with what previous research has shown. Earlier studies have demonstrated that hydroxyapatite nanoparticles can potentially stop the growth and reduce the activity of bacteria that cause cavities [14]. This indicates a hopeful path for preventing tooth decay. However, it is important to note that more studies are still needed. These future studies would help to make a very clear connection. They would establish a definite link between the changes seen in gene activity and a proven reduction in the ability of these bacteria to cause tooth decay. Such steps are essential for making strong scientific claims.

Comparison with Other Studies

Several research studies have already looked into using hydroxyapatite nanoparticles for stopping tooth decay. For example, one notable study clearly showed how hydroxyapatite nanoparticles had the ability to lessen the formation of biofilm. Biofilm is a sticky layer of bacteria that forms on teeth. This study also showed that these nanoparticles could reduce the amount of acid produced by the bacteria that cause cavities [10]. Reducing acid production is very important because acid directly attacks tooth enamel. In a similar way, another study reported a significant finding. It stated that hydroxyapatite nanoparticles have the power to make tooth enamel stronger again. They do this by helping the teeth to regain lost minerals, a process called remineralization. This action directly helps in stopping tooth decay from getting worse and progressing further [11]. These comparisons show that the findings of the present study add to the growing knowledge about the potential benefits of using hydroxyapatite nanoparticles in dental care.

**CONCLUSION:**

This study found that Morinda citrifolia and hydroxyapatite nanoparticles bond well together, as shown by FTIR and EDX analyses. SEM showed their physical shape. PCR analysis revealed these nanoparticles strongly reduced Lactobacillus and S. mutans, suggesting they can fight germs. Overall, these nanoparticles look promising for dental and medical use. More study is needed to understand how they work and their use in clinics.

**FUTURE SCOPE:**

Further research on this study endeavors to morinda citrifolia-coupled hydroxyapatite nanoparticles from the lab to clinical practice, enhancing their utility in healthcare settings.

**CONFLICTS OF INTEREST:**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**ACKNOWLEDGMENT**

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