Volume 66, Issue 1, 2022
Journal of Scientific Research
of
The Banaras Hindu University

Development of Anti-bacterial Nano Filling Material for Dental Caries

Hema V.R[1], Ezhilarasi P[2], and Vanavil B[*3]

[1]Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil-626126, Tamilnadu, India, hemaramer@gmail.com
[2]Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil- 626126, Tamilnadu, India, ezhilarasi@klu.ac.in
[*3]Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil, 626126, Tamilnadu, India, b.vanavil@klu.ac.in

Abstract: **Dental caries is the most common human infection and is widespread among civilized populations. Caries incidence augmented intensely due to the availability of processed sugar. Dental caries treatment involves biomaterials that could modify the mechanical, physiological, and chemical environments in the oral cavity. Clinical success in the cure of dental caries may lie in the development of biomaterials that can inhibit bacterial biofilm formation in the teeth. Clove is known for ages in the treatment of tooth decay. Nanoparticles are reported to exhibit anti-bacterial activity and are hence used in several medical applications. In this perspective, nanoparticles were biosynthesized using the clove and were characterized using FT-IR and SEM analyses. Further, dental filling material exhibiting anti-bacterial activity was fabricated using glass ionomer and synthesized nanopowder. This study reveals that developed nano-dental filling material has the potential to be used in dental applications for the prevention of caries.**

Index Terms: **Biomaterials, Dental Caries, Glass Ionomer, Nanoparticles, Nano-dental filling**

I. Introduction

Dental caries is a bacterial infection, commonly known as tooth decay or cavity. In dental caries, the hard tissues of the teeth such as dentin, enamel and cementum get demineralized and destructed (Forssten et al., 2010). *Streptococcus mutans*, *Streptococcus sobrinus*, and Lactobacilli are the group of bacteria in charge of dental caries (Loesche, 1996; Solanki, 2012). Organic acids produced by these bacteria through the anaerobic metabolism of sugars derived from the diet result in demineralization (Moynihan & Petersen, 2004). Development of cariopathogenic biofilms occurs by preliminary adhesion of these specific oral bacteria and *Streptococcus mutans* is predominantly accountable for the commencement of tooth decay and further progression of the proven lesion. A bio filling is an approach to renovate a tooth back to its normal function and shape which is dented by decay. Materials employed for dental fillings are amalgam, composite resins, cast gold, ceramics, and glass ionomer (Solanki, 2012). The adhesion and proliferation of dental pathogens can be restricted at a very early stage and secondary caries can be prevented by an effective dental filling that exhibits anti-bacterial properties. Metal nanoparticles are recognized to hold strong inhibitory and anti-bacterial effects and are used as a bactericide agent to coat hospital equipment (Subhankari & Nayak, 2013). Due to its exceptional physicochemical and optoelectronic properties, metal nanoparticles find innumerable applications in catalysis, medical diagnostic imaging, pharmaceuticals, and medicine. Silver nanoparticles exhibit both anti-bacterial and anti-inflammatory activities which can facilitate faster-wound healing. Due to this, silver nanoparticles have been incorporated into wound dressings, pharmaceutical preparations, and medical implants (Shah et al., 2015). Metal nanoparticles can be manufactured through chemical, physical, and biological routes. High energy requirements and the usage of toxic reagents for the synthesis would not recommend the physical and chemical approaches for the synthesis of nanoparticles. This issue can be overcome by employing biological mediators like bacteria, fungi, algae, actinomycetes, and plants for the production of metal nanoparticles (Padalia et al., 2015; Ahmed & Ikram, 2015).

In view of aseptic conditions and maintenance, industrial production of nanoparticles using microorganisms could not be a

* Corresponding Author

viable option. Instead, the utilization of plant extracts is quite beneficial over the microorganism due to simplicity, less biohazard and intricate method of preserving cell cultures, a rich diversity of phytochemicals, and availability in bulk quantities (Ahmed et al., 2016). Garlic, Cloves, Neem, Miswak and Tea tree oil are used to disturb the oral pathogenic biofilm (Henley-Smith, 2013). Traditionally, clove oil is employed in dental care due to its antiseptic and analgesic nature. The pain of a toothache is alleviated by chewing clove and it is being engaged to disinfect root canals in temporary fillings. The principal constituent of the clove is eugenol, followed by β-caryophyllene, α-humulene and eugenyl acetate. Eugenol is reported to inhibition of bacterial growth associated with dental plaque (Chaieb et al., 2007). The clove oil is active against oral pathogens linked with dental caries and periodontal diseases. Earlier studies have reported antifungal, anti-carcinogenic, anti-allergic, anti-mutagenic, anti-oxidant, and insecticidal properties of clove (Razafimamonjison, et al., 2014).

Glass ionomer cement (GICs) is a group of biomaterials used in modern dentistry which has been applied for the filling of cavities formed as a consequence of tooth decay. GICs are used as cavity liners, fissure sealants, and cement for prosthetic dental restoration. Biocompatibility, in-built adhesion to enamel and dentine, marginal grounding of the tooth surface before application and flexibility of shades to match with the patient's natural dentition make GIC more favorable. Commercially offered GIC consists of a powder with alumina-silica-based glass filler particles with calcium fluoride, minor salts, freeze-dried poly (vinyl) phosphonic acid, and a liquid comprising polyacrylic and tartaric acids. GICs are less susceptible to moisture (Hook et al., 2014). GIC has to be reloaded with fluoride at intervals to maintain the anti-caries activity. In this perspective, the formulation of GIC with anti-bacterial mediators will lead to improvement of the performance of GIC in battling the oral cavity (Fernandez et al., 2021). Nanoparticles find applications in dentistry such as dental implants, dental filling, whitening of teeth, anti-sensitivity agent, polishing of enamel and prevention of caries Priyadarsini et al., 2018). Silver nanoparticles are commercially used in dental adhesives and sealers (Fernandez et al., 2021). Iron oxide nanoparticles are also reported to suppress oral biofilms (Liu et al., 2021).

Taking this in view, the present study was undertaken to synthesize silver and iron nanoparticles using clove which is being used in the treatment of dental plaque traditionally. These nanoparticles are further embedded into glass ionomer cement leading to the fabrication of a novel anti-microbial dental filling material.

II. MATERIALS AND METHODS

2.1 Preparation of Clove Bud Extract

Clove was purchased from the local market, washed, cleaned with distilled water and dried. Dried cloves were minced, ground and extracted with 10ml of sterile distilled water. Crushed cloves were heated at 70-80°C for 2-3 hours and the extract was filtered using Whatman's No.1 filter paper. The filtrate was recovered and centrifuged at 5000 rpm for 10 minutes. The recovered supernatant was utilized for the production of nanoparticles (Pattanayak et al., 2013).

2.2 Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by combining precursor and reducing agents at a ratio of 9:1. Freshly prepared clove extract and 0.001M aqueous $AgNO_3$ solution were mixed at the above ratio and were incubated for 24 h to proceed with the reduction of Ag ions (Banerjee et al., 2014).

2.3 Synthesis of Iron Nanoparticles

Iron nanoparticles were synthesized by combining precursor and reducing agents at a ratio of 1:1. 50 ml of filtered aqueous clove extract was mixed with 50 ml of freshly prepared 0.001M aqueous $FeCl_3$ solution for the reduction of Fe ions to nanoparticles (Hariprasad et al., 2016).

2.4 Characterization of Biologically Synthesized Nanoparticles

The functional groups of clove extract existing on the surface of copper and iron nanoparticles that are accountable for the reduction of metal ions into nanoparticles were investigated using FT-IR spectra (Shimadzu) recorded from 4000 to 400 cm^{-1}. The morphology of nanoparticles was revealed using SEM study (Carl Zeiss, Germany).

2.5 Fabrication of Nano Dental Composite

GIC was gifted by the local dentist and the matrix was prepared by mixing powder and liquid in the ratio of 2:2. GIC is mixed with 5mg, 10mg silver nanoparticle, and 10mg iron nanoparticle powders respectively. GIC without nanoparticles is used as a control.

2.6 Anti-bacterial Activity of Nanoparticles and Fabricated Nano Dental Filling

The anti-bacterial activity of silver and iron nanoparticles synthesized with clove extract was deliberated using a disc diffusion method against *E. coli* and *Bacillus cereus* as test organisms. The anti-bacterial activity of nanocomposite was investigated by the modified disc diffusion method. Sterile LB agar plates were seeded with *E. coli* and *Bacillus cereus*. Glass ionomer cement fabricated with nanoparticles was made in the form of disc and glass ionomer cement without nanoparticles was employed as control. The petriplates were incubated at 37ºC for 24 hours and the zone of inhibition was determined.

III. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles

With the addition of aqueous extract of clove (Figure 1a) to the colorless solution of silver nitrate (Figure 1b), there was an immediate change in color to brown (Figure 1c) and after 24 h of incubation, the color turned to dark brown (Figure 1d) owing to the conversion of silver ions into silver nanoparticles (Ahmed et al., 2016).

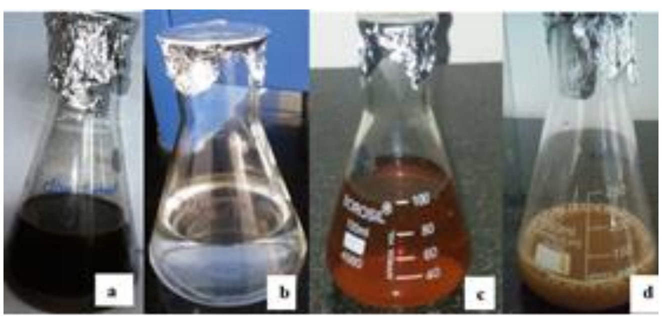
3.2 Synthesis of Iron Nanoparticles

With the addition of aqueous clove bud extract (Fig. 2a) to the golden color, ferric chloride solution (Fig. 2b) the color changed to black color immediately (Fig. 2c) owing to the conversion of iron ions into iron nanoparticles (Pattanayak et al., 2013).

3.3 Characterization of Nanoparticles using FT-IR Spectrum

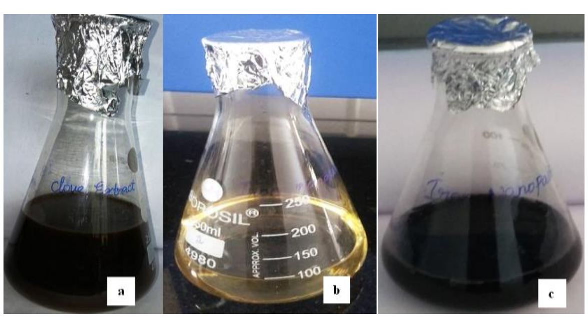
FT-IR analysis was done to ascertain the reducing biomolecules present in the clove extract for the synthesis of nanoparticles. Figure 3 depicts the FT-IR spectrum of clove extract. Table I represents the FT-IR signal assignment of clove extract.

The reduction process involves plant metabolites like proteins, sugars, organic compounds, etc. The plant extract contains abundant functional groups such as C=C (alkenyl), C=N (amide), C-H and COO- (carboxylic group), O-H (phenolic and alcohol), N-H (amine) that are responsible for nanoparticle synthesis (Hariprasad et al., 2016). Figure 4 and Figure 5 show the FT-IR spectrum of silver and iron nanoparticles produced using aqueous clove bud extract respectively. Tables II and III represent the signal assignment for the FT-IR spectrum of silver and iron nanoparticles respectively.



(a) Aqueous clove extract (b) Silver nitrate solution (c) Immediate change of color to brown (d) Silver nanoparticle synthesis after 24 h

Fig. 1. Silver Nanoparticles Synthesis using Aqueous Clove extract



(a) Aqueous clove extract (b) Ferric chloride solution (c) Iron nanoparticles

Fig. 2. Iron Nanoparticles Synthesis using Aqueous Clove Extract

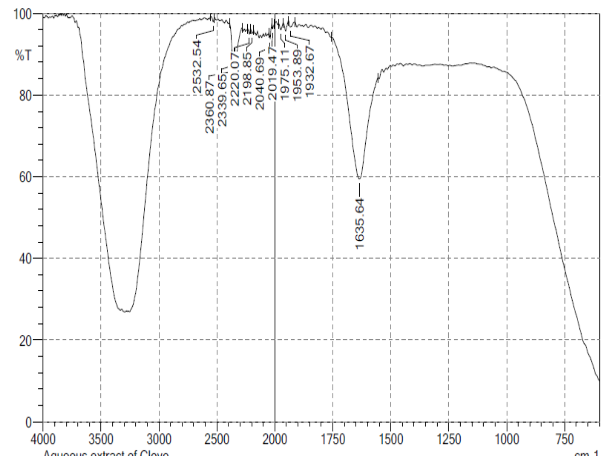


Fig. 3. FT-IR Spectrum of Clove Extract

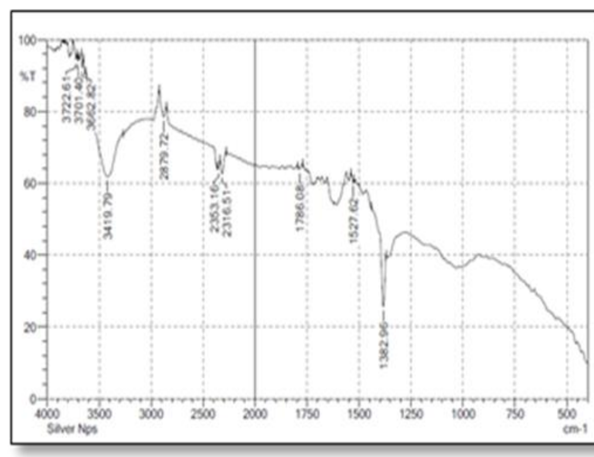


Fig. 4. FT-IR Spectrum of Silver Nanoparticles Produced using Clove Extract

3.4 SEM Analysis

The SEM images of silver and iron nanoparticles synthesized using clove extract are shown in Figures 6 and 7 respectively. As evident from the figures, the silver nanoparticles are spherical with sizes ranging between 44.28 nm to 56.09 nm. Iron nanoparticles are also spherical with sizes ranging between 25.14 nm and 28.11nm.

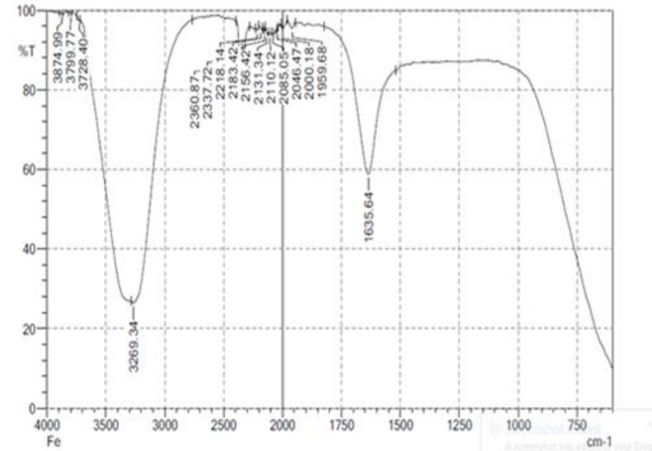


Fig. 5. FT-IR Spectrum of Iron Nanoparticles Produced using Clove Extract

Table I. Signal Assignment for FT-IR Spectrum of Clove Extract

Wavenumber	Functional group
1635.64 cm^{-1}	Bending vibration of C=O groups of beta diketones
2360.87cm^{-1}, 2082 cm^{-1} 2335.80 cm^{-1}	CH stretching modes of alkanes
2198.85 cm^{-1}	N-H stretching of tertiary amine

Table II. Signal Assignment for FT-IR Spectrum of Silver Nanoparticles

Wavenumber	Functional group
1381.03 cm^{-1}, 1361.74 cm^{-1}	Bending vibration of nitro groups of N-O
2879.72 cm^{-1}	CH stretching modes of alkanes
3589.53 cm^{-1}, 3564.45 cm^{-1}	O-H stretching vibration of phenol/carboxylic group

Table III. Signal Assignment for FT-IR Spectrum of Iron Nanoparticles

Wavenumber	Functional group
3269.34 cm^{-1}	C-C stretching vibration of the alkenyl group
2360.87 cm^{-1}, 2337.72 cm^{-1}, 2082 cm^{-1}	CH stretching modes of alkanes.
1635.64 cm^{-1}	C-O stretching vibration due to ketones, aldehyde, carboxylic acid

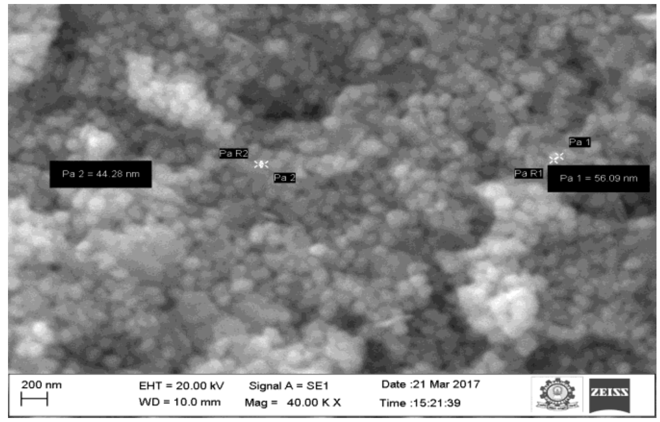


Fig. 6. SEM Image of Silver Nanoparticles

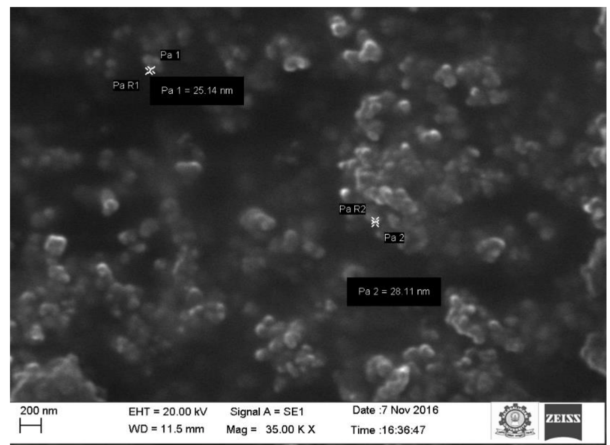


Fig. 7. SEM Image of Iron Nanoparticles

3.5 Anti-bacterial Activity of Silver and Iron Nanoparticles

The anti-bacterial activities of silver and iron nanoparticles synthesized using clove extract were evaluated using disc-diffusion assay. The silver nanoparticles exhibited anti-bacterial activity against *E. coli* and *Bacillus cereus* as observed from Figures 8 and 9. The iron nanoparticles also exhibited anti-bacterial activity against *E. coli* and *Bacillus cereus* as observed from Figures 10 and 11 respectively.

Fig. 8. Anti-bacterial Activity of Silver Nanoparticles (10mg/100µl) against *E. coli*

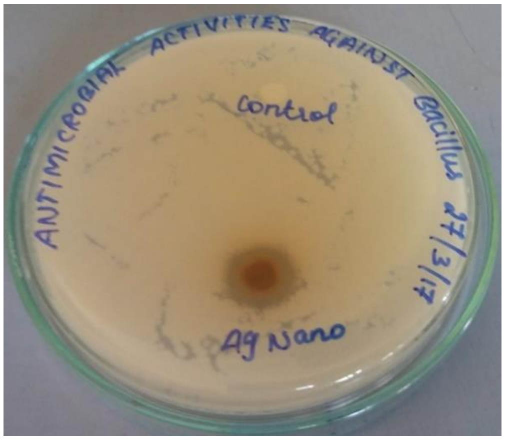


Fig. 9. Anti-bacterial Activity of Silver Nanoparticles (10mg /100µl) against *Bacillus cereus*

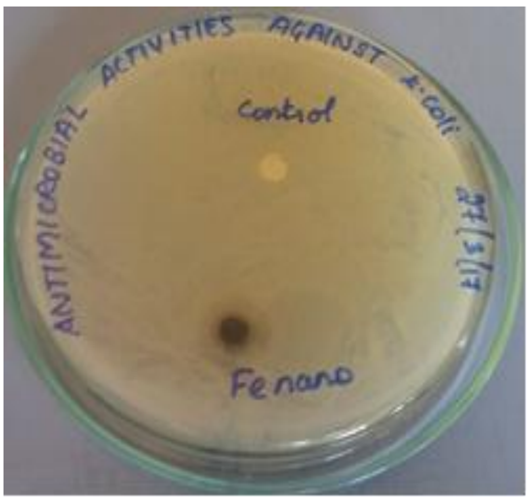


Fig. 10. Anti-bacterial Activity of Iron Nanoparticles (10mg /100µl) against *E. coli*

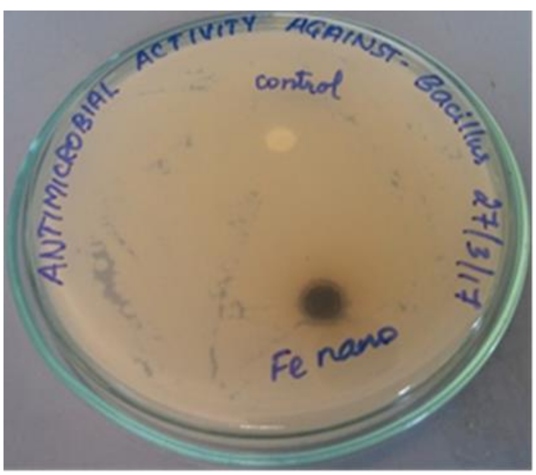


Fig. 11. Anti-bacterial Activity of Iron Nanoparticles (10mg /100µl) against *E. coli*

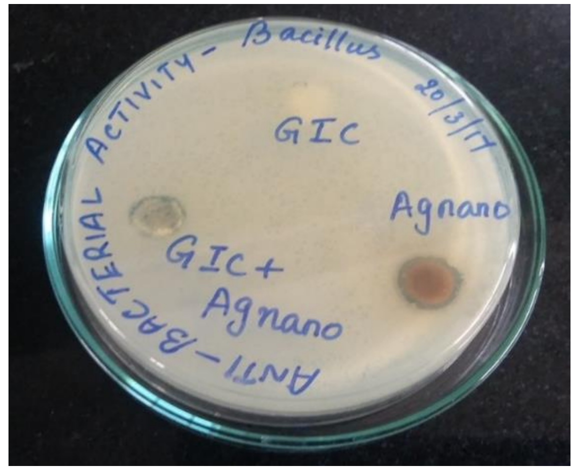


Fig. 12. Anti-bacterial Activity of Silver Nano Dental Composite I (5mg of AgNP with 2:2 ratio of powder and liquid) against *Bacillus cereus*

Table IV depicts the zone of inhibition diameter of the nanoparticles against tested bacteria.

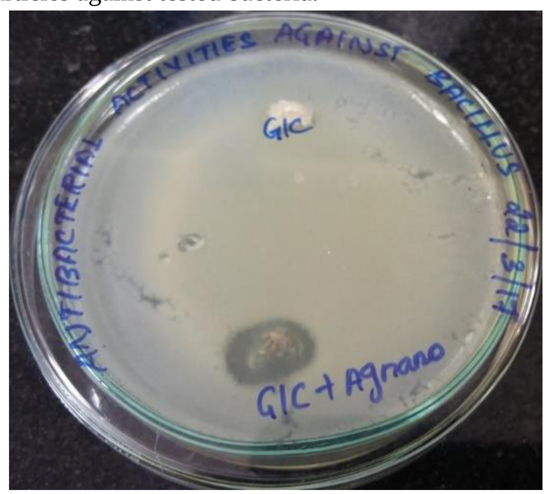


Fig. 13. Anti-bacterial Activity of Silver Nano Dental Composite II (10mg of AgNP with 2:2 ratio of powder and liquid) against *Bacillus cereus.*

Fig 14. Anti-bacterial Activity of Iron Nano Dental Composite (5mg of FeNP with 2:2 ratio of powder: liquid) against *Bacillus cereus*

Table IV. Anti-bacterial Activity of Nanoparticles against Tested Bacteria

S. No	Nanoparticle	Tested bacteria	Zone of inhibition diameter (cm)
1	Silver	*Bacillus cereus*	1
		E. coli	0.8
2	Iron	*Bacillus cereus*	0.5
		E. coli	0.2

Table V. Anti-bacterial Activity of Nano Dental Composite

S. No	Nanocomposite	Tested bacteria	Zone of inhibition diameter (cm)
1	Silver nanocomposite I (5mg)	*Bacillus cereus*	0.8
	Silver nanocomposite II (10mg)		1.5
2	Iron nanocomposite (5 mg)	*Bacillus cereus*	0.6

3.6 Anti-bacterial Activity of Nano Dental Composite

The fabricated dental nanocomposite was evaluated for the anti-bacterial activity to be employed as a novel dental filling material. As observed from Figures 12 and 13, GIC fabricated with silver nanoparticles showed marked anti-bacterial activity against *Bacillus cereus*. As observed from Figure 14, GIC fabricated with iron nanoparticles showed marked Anti-bacterial activity against *Bacillus cereus*. The fabricated dental nanocomposite did not show significant anti-bacterial activity against *E. coli.* Table V shows the zone of inhibition diameter of the developed nano dental composite against *Bacillus cereus*.

The physical properties of GIC were improved by the incorporation of 10% of aluminum oxide, zirconium oxide, and titanium dioxide nanoparticles (Gjorgievska et al., 2015). Anti-bacterial properties and compressive strength of silver nanoparticles in glass ionomer cement were evaluated by Paiva et al., (2018). Jowkar et al., (2019) concluded that GIC enriched with nanosilver as reassuring material for restoration due to its improved mechanical and bond strength properties. GIC modified by incorporating 2.5% MgO nanoparticles was reported as a promising material for clinical dental applications (Noori and Kareem, 2019). The addition of silver and titanium dioxide nanoparticles to conventional GIC enhanced its physicomechanical properties (Assery et al., 2020). Previous studies reported the mechanical and anti-bacterial properties of GIC formulated with nanoparticles such as silver, MgO and titanium dioxide which are synthesized using chemical methods. However, the present study investigated the formulation of GIC with silver and iron particles biosynthesized using clove extract and its antibacterial applications.

CONCLUSION

Biological production of silver and iron nanoparticles was carried out using clove extract and the synthesized nanoparticles were characterized using FT-IR spectral and SEM analysis. The anti-bacterial activity of nanoparticles synthesized using clove extract was checked against *E. coli* and *Bacillus cereus*. The synthesized nanoparticle was fabricated as a dental nanocomposite using glass ionomer cement and its anti-bacterial activity was evaluated using the disc diffusion method. Hence this study will be helpful in the development of anti-microbial dental filling material. The anti-bacterial action is due to the release of silver ions leading to oxidative dissolution in the GIC, thereby preventing the development of the oral biofilm (Fernandez et al., 2021). Further studies on the toxicity of this nanocomposite have to be evaluated for clinical use in dentistry.

ACKNOWLEDGMENT

The authors would like to acknowledge the support for analytical facilities such as FT-IR and SEM delivered by the International Research Centre, Kalasalingam Academy of Research and Education, Krishnankoil.

REFERENCES

Forssten, S. D., Björklund, M., & Ouwehand, A. C. (2010). *Streptococcus mutans*, caries and simulation models. *Nutrients*, 2(3), 290-298.

Loesche, W. J. (1996). Microbiology of dental decay and periodontal disease. Medical Microbiology, Galveston (TX), University of Texas Medical Branch at Galveston.

Moynihan, P., & Petersen, P. E. (2004). Diet, nutrition and the prevention of dental diseases. *Public Health Nutrition*, 7(1a), 201-226.

Solanki, G. (2012). Dental caries-a widely growing disease of teeth. *International Journal of Biomedical and Advance Research*, 3(2), 106-10.

Subhankari, I., & Nayak, P. L. (2013). Synthesis of copper nanoparticles using *Syzygium aromaticum* (Cloves) aqueous extract by using green chemistry. *World Journal of Nanoscience and Nanotechnology*, 2(1), 14-17.

Shah, M., Fawcett, D., Sharma, S., Tripathy, S. K., & Poinern, G. E. J. (2015). Green synthesis of metallic nanoparticles via biological entities. *Materials*, 8(11), 7278-7308.

Padalia, H., Moteriya, P., & Chanda, S. (2015). Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. *Arabian Journal of Chemistry*, 8(5), 732-741.

Ahmed, S., & Ikram, S. (2015). Silver nanoparticles: one pot green synthesis using *Terminalia arjuna* extract for biological application. *Journal of Nanomedicine & Nanotechnology*, 6(4), 1-6.

Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extracts mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of Advanced Research*, 7(1), 17-28.

Henley-Smith, C. J., Botha, F. S., & Lall, N. (2013). The use of plants against oral pathogens. *Formatex*, 30, 1375-1384.

Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A. B., Rouabhia, M., Mahdouani, K., & Bakhrouf, A. (2007). The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. *Phytotherapy Research*, 21(6), 501-506.

Razafimamonjison, G., Jahiel, M., Duclos, T., Ramanoelina, P., Fawbush, F., & Danthu, P. (2014). Bud, leaf and stem essential oil composition of *Syzygium aromaticum* from Madagascar, Indonesia and Zanzibar. *International Journal of Basic and Applied Sciences*, 3(3), 224-233.

Hook, E. R., Owen, O. J., Bellis, C. A., Holder, J. A., O'Sullivan, D. J., & Barbour, M. E. (2014). Development of a novel antimicrobial-releasing glass ionomer cement functionalized with chlorhexidine hexametaphosphate nanoparticles. *Journal of Nanobiotechnology*, 12(1), 1-9.

Fernandez, C. C., Sokolonski, A. R., Fonseca, M. S., Stanisic, D., Araújo, D. B., Azevedo, V., Portela, R. D., & Tasic, L. (2021). Applications of Silver Nanoparticles in Dentistry: Advances and Technological Innovation. *International Journal of Molecular Sciences*, 22(5), 1-21.

Priyadarsini, S., Mukherjee, S., & Mishra, M. (2018). Nanoparticles used in dentistry: A review. *Journal of Oral biology and Craniofacial Research*, 8(1), 58-67.

Liu, Y., Huang, Y., Kim, D., Ren, Z., Oh, M. J., Cormode, D. P., Hara, A. T., Zero, D. T., & Koo, H. (2021). Ferumoxytol Nanoparticles Target Biofilms Causing Tooth Decay in the Human Mouth. *Nano Letters*, 21(22), 9442-9449.

Pattanayak, M., Mohapatra, D., & Nayak, P. L. (2013). Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of *Syzygium aromaticum* (clove). *Middle-East Journal of Scientific Research*, 18(5), 623-626.

Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, 1(1), 1-10.

Hariprasad, S., Bhai, G. S., Babu, B. K., Madhu, C., Kumar, G. R., Hymavathi, A., Santhosh kumar J., & Rao, S. N. (2016). Green synthesis of copper nano particles from medicinal plants. *Indo American Journal of Pharmaceutical Sciences*, 3(8), 831-835.

Gjorgievska, E., Van Tendeloo, G., Nicholson, J. W., Coleman, N. J., Slipper, I. J., & Booth, S. (2015). The incorporation of nanoparticles into conventional glass-ionomer dental restorative cements. Microscopy and microanalysis : the official journal of Microscopy Society of America, Microbeam Analysis Society, *Microscopical Society of Canada*, 21(2), 392–406.

Paiva, L., Fidalgo, T., da Costa, L. P., Maia, L. C., Balan, L., Anselme, K., Ploux, L., & Thiré, R. (2018). Antibacterial properties and compressive strength of new one-step preparation silver nanoparticles in glass ionomer cements (NanoAg-GIC). *Journal of Dentistry*, 69, 102–109.

Jowkar, Z., Jowkar, M., & Shafiei, F. (2019). Mechanical and dentin bond strength properties of the nanosilver enriched glass ionomer cement. *Journal of Clinical and Experimental Dentistry*, 11(3), e275–e281.

Noori, A. J., & Kareem, F. A. (2019). The effect of magnesium oxide nanoparticles on the antibacterial and antibiofilm properties of glass-ionomer cement. *Heliyon*, 5(10), e02568.

Assery, M. K. A., Alshubat, A., Abushanan, A., Labban, N., Hashem, M. (2020). Nanoparticles as void fillers in glass ionomer cement for enhanced physicomechanical properties. *Materials Express*, 10(11), 1960-1964.

***