Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.14.044

Formulation And Characterisation of Nanosilver-Chitosan Biocomposite

Dakshinya M[1], Sandhya Sundar[2]*, Rajesh Kumar Shanmugam[3], Ramya Ramadoss[4], Suganya Paneerselvam[5], Pratibha Ramani[6]

[1]Saveetha Dental College and Hospitals, Saveetha Institute of medical and Technical Sciences(SIMATS), Saveetha University,Chennai-600077, Tamil Nadu,India

[2]Senior lecturer, Saveetha Dental College and Hospitals, Saveetha Institute of medical and Technical Sciences (SIMATS), Saveetha University, Chennai- 600077, Tamil Nadu, India

[3]Professor, Saveetha Dental College and Hospitals, Saveetha Institute of medical and Technical Sciences (SIMATS), Saveetha , Chennai- 600077, Tamil Nadu, India, No.162, Poonamalle high road, Velappanchavadi, Chennai-600077

[4]Professor, Department of Dental Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of medical and Technical Sciences (SIMATS), Saveetha University, Chennai- 600077 No.162, Poonamalle high road, Velappanchavadi, Chennai-600077, Tamil Nadu, India

[5]Senior lecturer, Department of Dental Anatomy, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai – 600077, Tamil Nadu, India

***Corresponding author:** Sandhya Sundar, Senior lecturer, Saveetha Dental College and Hospitals, Saveetha Institute of medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India

Submitted: 17 March 2023; Accepted: 16 April 2023; Published: 08 May 2023

ABSTRACT

Background: Green synthesis of nanoparticles is gaining popularity. When this eco-synthesis of nanoparticles is mediated by commonly available natural resources like tamarind seed, it can combine with other natural products easily to give composite materials with many advantages.

Aim: To formulate, synthesize and assess the antimicrobial and anti-inflammatory property of bionanocomposite being incorporated in silver nanoparticles and chitosan.

Materials and Methods: Silver nanoparticles are prepared by adding tamarind seed and converted as Nanocomposite by homogeneously mixing with chitosan. The anti-inflammatory and antimicrobial property of the prepared bio-nanocomposite is evaluated by assessment of protein denaturation and zone of inhibition against most common oral pathogens respectively.

Results: Anti Inflammatory activity measured by Bovine Serum Assay and Egg Albumin Assay showed that the 75% of protein denaturation was inhibited by 50 microlitre of the prepared Bionanocomposite.In Anti microbial activity,The maximum zone of inhibition of common oral pathogens like S.mutans,S.aureus,E.faecalis and C.albicans is 100 microlitre of concentration from the prepared Nanocomposite.

Conclusion: The study was successful in formulating a Nanocomposite material which is highly eco friendly with less toxicity.This novel Biomaterial can be formulated as Mouthwash in future which mainly focuses on preventing tooth decay and promoting the health of gums.

Keywords: *Mouthwash, Tamarindseed, chitosan, stains, eruption, decay.*

J Popul Ther Clin Pharmacol Vol 30(14):e336–e343; 08 May 2023.

INTRODUCTION

Common preferences of mouthwash used by people to maintain oral hygiene is chlorhexidine and fluoride.But tamarind seed mediated mouthwash is still unknown and not used by more number of people.It's exposure of teeth and its effect,it's biocompatibility is yet to be discovered.Chitosan on the other view is a natural biomaterial used for antimicrobial and anti inflammatory activity[1].The aim of in vitro study is to evaluate the efficacy of the mouthwash and its effects that can be used later and to know its impact that will cause in the environment[2] .On to the spread of the infectious disease in attribute to the pathogenic bacteria and rise of antibiotic resistance there is a demand for synthesis of unique antimicrobial agents which is increased.Nanoparticles usually act as an antimicrobial agent consisting of large surface area to volume ratio which can give a better adhesion with bacterial cells.These nanoparticles produce nano composite with high antimicrobial efficacy. Bionanocomposite can be utilized in antimicrobial agents,bio nanocatalysts,photocatalytic activity and drug delivery.As a natural polymer extracted from marine environment,chitosan is an amino polysaccharide taken by deacetylation of chitin(poly-N-acetyl-d-glucosamine).It got from the name after second most abudant natural polymer after cellulose.It enables numerous amine and hydoxyl groups which creates synthesis of bio composite with various uses.

The mechanism of the inhibitory effect of Ag ions on microorganisms is partially known. Several studies have reported that the positive charge of Ag ions is crucial for its antibacterial activity through the electrostatic attraction between negatively charged microbial cell membranes and positively charged nanoparticles. In contrast, Sondi and Salopek-Sondi reported that the antibacterial activity of Ag nanoparticles against Gram-negative bacteria depends on the concentration of Ag nanoparticles and is closely related to the formation of "pits" in the cell wall bacterial. Silver nanoparticles that accumulate in the bacterial membrane then cause permeability leading to cell death. However, since these studies included positively charged silver ions and negatively charged silvernanoparticles, they were insufficient to explain the antibacterial mechanism of positively charged silver nanoparticles. Therefore, we expect that there is another possible mechanism. Amuro et al. showed that metal depletion can induce the formation of irregularly shaped pits in the outer membrane and impair membrane permeability, which is caused by the gradual release of lipopolysaccharide molecules and membrane proteins.[3]

Comparing to all the antimicrobial nano materials,silver nanoparticles is having the substantial antimicrobial capabilities.It is very good in anti microbial and anti inflammatory activity against a wide variety of drug resistant micro organism,generate ROS that deactivate the bacteria membrane[4].

Tamarind seed(Tamarindus Indica L.,)are the tropical fruits widely consumed everywhere and a long economic impact.Large quantities of waste products from non edible parts have anti microbial and anti inflammatory activity[5].

Tamarind seed is rich in vitamin C,tartaric ,malic and citric acids.It is flattened,glossy and orbicular to rhomboidal.It is a cheaper source of protein having favourable amino acid composition and to alleviate protein.Malnutrition and present teeth from further decay and eruption[6].Pain and fever are the most common effects connected with inflammation,through Aspirin,Indomethacin,Phenyl butazone etc,they have prevent us from swelling.Traditionally,Tamarind seed is utilized in asthma,leprosy,tuberculosis and anti oxidant activity.Also they are rich in aspirin.phenolic compounds,alkaloids and glycosides,flavonoids,tannins and saponins that are responsible for anti inflammatory and analgesic activity ([6,7].Similarly,the zone of inhibition**,** duration of inhibition**,** and cell viability were dependent on extracts obtained from tamarind seed mouthwashes**.**[8].

Our team has extensive knowledge and research experience that has translate into high quality publications(Vishnu Prasad et al. 2018; Ramesh Kumar et al. 2011; Ganapathy et al. 2022; Arumugam et al. 2021; Mohanavel et al. 2020; Muthukrishnan 2021; Chellapa et al. 2020; Markov et al. 2021; Felicita 2017; Uthrakumar et al. 2010)

Thus, the study aims to formulate and assess the antiinflammatory and antimicrobial property of a bionanocomposite of tamarind seed mediated nanosilver with chitosan.

J Popul Ther Clin Pharmacol Vol 30(14):e336–e343; 08 May 2023.

MATERIALS AND METHODS

Preparation of Bionanocomposite - tamarind seed mediated nanosilver with chitosan

4.5 g of powdered tamarind seed in a conical flask is taken and 150 ml of distilled water added to the powder of tamarind seed. Then the tamarind seed solution is boiled for 10 mins in the heating mantle and filtered using a filter cloth in order to derive the tamarind seed extract from it.Then 70ml of distilled water is taken and poured into a conical flask of 0.0168 g silver nitrate particles.Now 30 ml of tamarind seed extract is added to silver nitrate which is further converted to silver nanoparticle.Aluminium foil is covered by it.After few hours of observing ,6 centrifuge tubes with 14 ml of the tamarind seed sample is being centrifuged for 10 mins in 8000 rpm.Now the pellet is collected and being discarded as the supernatant.

FIGURE 1: Preparation of Bionanocomposite - tamarind seed mediated silver nanoparticles with chitosan.

Anti-Inflammatory Activity Of The Prepared Bio-Nanocompsite.

Albumin Denaturation Assay

The anti-inflammatory activity for prepared extract of bio-nanocomposite was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (Pratik Das et al.,2019). 0.05 mL of Tamarind seed of various fixation (10µL,20µL,30µL,40µL,50µL)was added to 0.45 mL bovine serum albumin(1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control. Percentage of protein denaturation was determined utilizing following equation,

% inhibition= Absorbance of control-Absorbance of sample/
Absorbance of control *100

Egg Albumin Denaturation Assay

A 5ml solution was made which was comprised of 2.8ml of freshly prepared phosphate buffered saline of pH - 6.3, 0.2 ml of egg albumin extracted from hens egg. Specific concentrations were prepared separately for tamarind seed-chitosan as (10µL,20µL,30µL,40µL,50µL). Diclofenac sodium was used as the positive control. Then the mixtures were heated in water bath at 37ºC for 15 minutes. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm.

Anti microbial activity of the prepared Bio-nanocompsite

Anti microbial activity of respective nanoparticles against the strain S.mutants,S.aureus,E.faecalis. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller hinton agar were prepared and sterilize for 15 minutes at 121oC. Media poured into the sterilized plates and let stable for solidification. The wells were cut using 9mm sterile polystyrene tip and the test organisms were swabbed. The nanoparticles with different concentration (25µL, 50 µL ,100 µL)were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hours at 37 °C. After the incubation time the zone of inhibition were measured. For Candida albicans is used as test

pathogen by agar well diffusion assay. Rose Bengal Agar is used to prepare the fungal medium. The prepared and sterilized medium was swabbed with test organism and nanoparticles with different concentration (25µL, 50 µL ,100 µL)were added to the wells and in the fourth well standard antibiotic flucanazole was loaded. The plates were incubated at 37°C for 48-72hours. After the incubation time the zone of inhibition were measured.

RESULTS

The anti inflammatory activity of the mouthwash obtained is checked using the BSA (Bovine Serum Albumin)assay and EA(Egg albumin) assay which increases with increase in concentration of the substrate and attained till 75% of inhibition to 50 micro litre of concentration. The anti microbial activity is checked by the different zone of inhibition occupied mostly by C.Albicans attaining till 12 mm of zone of inhibition followed by S.Aureus microbes occupying 9mm of inhibition.

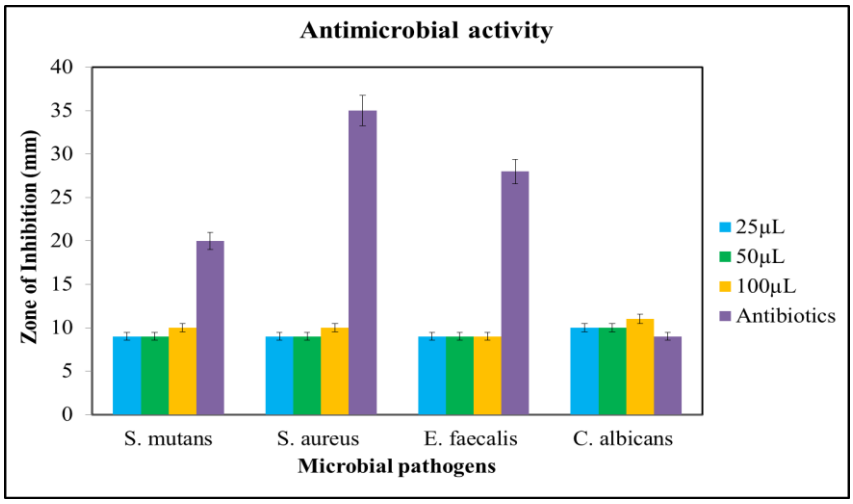

FIGURE 2: It indicates the antimicrobial activity of four microbes in 25 micro litres,50 micro litres,100 micro litres and antibiotics respectively.Here on increasing the concentration of microbial pathogens,the percentage of zone of inhibition increases or decrease and sometimes remains same.It shows in this graph that S.aureus have high zone of inhibition in antibiotics.C.albicans have higher concentration in zone of inhibition.We can state that the sample we prepared have the effect I.e C.Albicans is mostly inhibited from the sample.

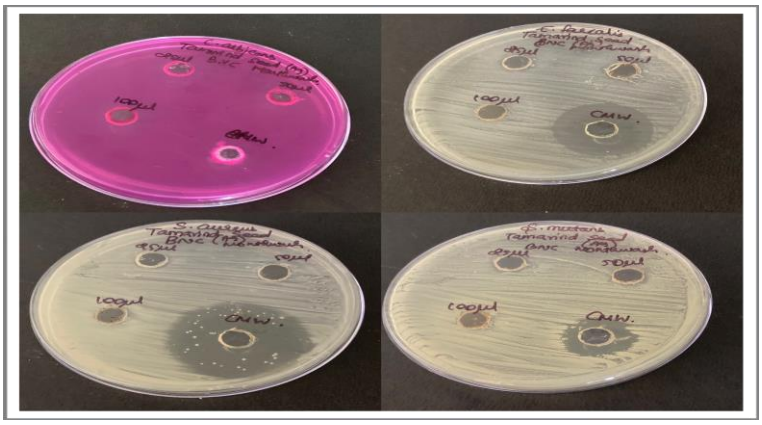

FIGURE 3: Antimicrobial assay shows the zone of inhibition in four different microbes.First microbe taken is C.Albicans with concentration of 25 micro litre,50 micro litre,100 micro litre and CMW where much inhibition is not seen.Second microbe taken is E.Faecalis show much inhibition in CMW than 25,50,100 micro litre.Third microbe and fourth microbe is S.Aureus,S.mutans also showing much inhibition in CMW.

J Popul Ther Clin Pharmacol Vol 30(14):e336–e343; 08 May 2023.

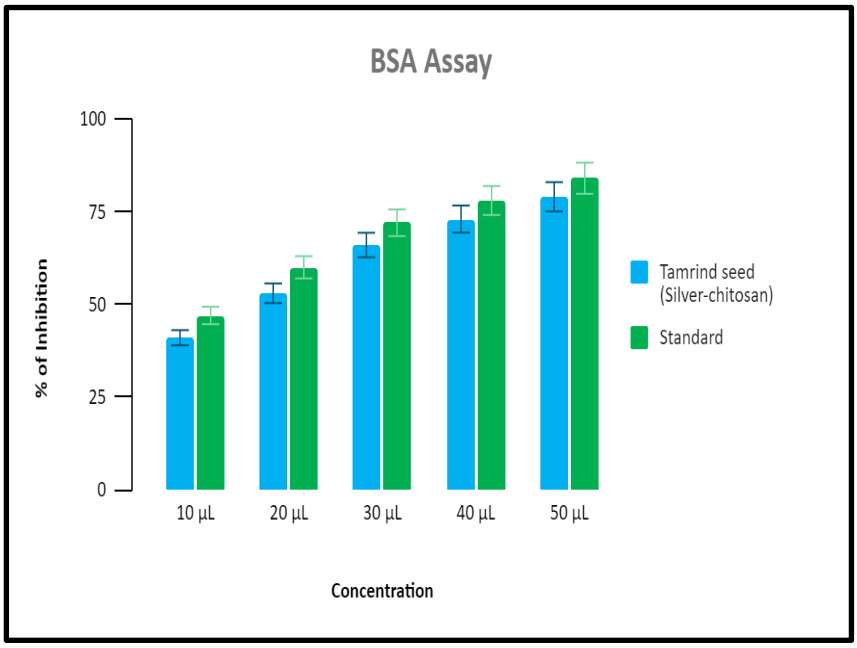

FIGURE 4: Anti inflammatory of Bovine Serum Albumin Assay is showing increase in concentration for increase in percentage of inhibition finally reaching 75% of inhibition for 50 micro litre concentration.

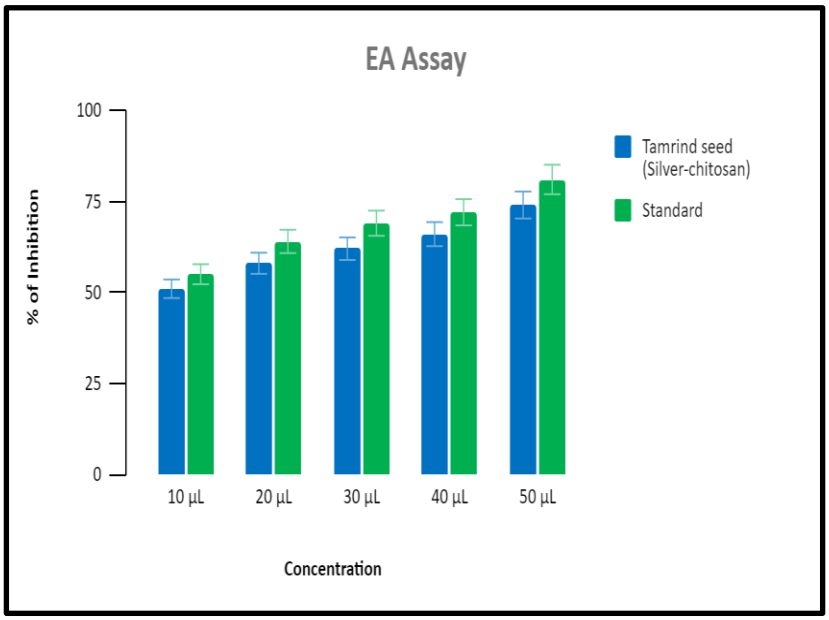

FIGURE 5: Egg albumin Assay showing increase in the zone of inhibition against common oral pathogens with increased concentration of Tamarind seed mediated silver -chitosan nanocomposite.It reaches 75% of inhibition for 50 microliter of concentration.

DISCUSSION

Inflammatory activity is a complicated process created by several indicators from bacterial infection and chemical injury to environmental pollution that result in cell injury or death.Non steroidal anti inflammatory drugs(NSAIDs) are frequently used day to day.They do not cure the conditions but remove the underlying cause of disease and only modify the inflammatory response to the disease like periodontal disease.Thus tamarind seed is analgesic due to inhibition of release of endogenous inflammatory

J Popul Ther Clin Pharmacol Vol 30(14):e336–e343; 08 May 2023.

mediators like histamine,serotonin,prostaglandin and inhibition of nociceptive transmission.It exhibit anti inflammatory process([9].Silver nanoparticles are between 1nm and 100 nm in size.While frequently described as being large percentage of silver oxide due to their large surface area to bulk silver atoms.They are antibacterial and antifungal activities for industrial purpose in water treatment and consumer production like clothing,cosmetics,food containers and many more [10].

Chitosan is a natural polysaccharide derived from chitin, mainly from the shells of crustaceans such as shrimp. It is a positively charged biopolymer which, with its amino groups, binds strongly to surfaces with negative zeta potential, such as hard dental tissues. The active chitosan molecule reacts with the enamel surface to form at least one thin, pervasive layer of chitosan. This chitosan-based layer has a thickness of a few nanometers and is therefore invisible under a scanning electron microscope.

There are two phases in nanocomposite I.e nanometer scale and polymers of biomolecules.They are used in electronic sensor and electricity generation and biomedical application and insulation[11].

Tamarind is highly valued worldwide for its nutritional value and high value for health promotion. Recently, natural sources of antioxidants and their role in the prevention and treatment of various diseases have been widely studied. The polyphenolic and flavonoid compounds prevalent in tamarind are believed to be responsible for its strong antioxidant activity. The phenolic compounds found in tamarind are beneficial for cardiovascular health and immune health, and play specific roles in antibacterial and anti-cancer activities. Flavonoids present in different parts of tamarind are known to have defense mechanisms and can be used as anti-inflammatory, anti-diabetic and anti-hyperlipidemic agents in the treatment of various human health risks. Although a wealth of data is available in the literature on the antioxidant properties of tamarind, this chapter attempts to bring all of this information together into one platform to aid in the future direction of this area of research(Barsha Devi et al,2020).

For antimicrobial activity,the pathogens that was used had high disease causing or pathogenic effect as: Candida Albicans-Present in saliva,dental plaque and proximal to cervical caries.Enterococcus Faecalis-Opportunistic pathogen causing marginal periodontitis,root canal infections,absences.Streptococcus Aureus-Important pathogen of dental caries and play a frequent role in isolating oral cavity and peri oral region.

Streptococcus mutans-Participate in bacterial adhesions to both surfaces via interaction with salivary pellicle.These pathogen load will be reduced in the mouth wash as shown by a previous study[12] also.

CONCLUSION

The prepared bio-nanocomposite is an echo friendly and safe formulation with no adverse effect on the environment. The potent antimicrobial and anti-inflammatory of the material qualifies its value in prevention of dental caries and periodontal inflammatory conditions of oral cavity.It will preserve our oral health care in daily life.This novel Biomaterial can be formulated as a Mouthwash for preventing further decay of tooth.

CONFLICT OF INTEREST

There is no conflict of interest.

Funding
Self-funded

Author Contribution

Ms.M.DAKSHINYA:Literature search,data collection,manuscript writing.

Dr.SANDHYA.S:Study design,data verification,manuscript correction.

ACKNOWLEDGEMENT

We extend our sincere gratitude to the Saveetha Dental College and Hospitals for their constant support and successful completion of this work.

REFERENCES

1. Barnett, Michael L. "The rationale for the daily use of an antimicrobial mouthrinse." The Journal of the American Dental Association 137 (2006): S16-S21.doi.10.14219/jada.archive.2006.0408

2. Nardi, Gianna Maria, Sara Fais, Cinzia Casu, Marta Mazur, Roberto Di Giorgio, Roberta Grassi, Felice Roberto Grassi, and Germano Orrù. "Mouthwash Based on Ozonated Olive Oil in Caries Prevention: A Preliminary In-Vitro Study." International journal of environmental research and public health 17, no. 23 (2020): 9106.doi.org/10.3390/ijerph17239106.
3. Ahghari, Mohammad Ali, Mohammad Reza Ahghari, Maryam Kamalzare, and Ali Maleki. "Design, synthesis, and characterization of novel eco-friendly chitosan-AgIO3 bionanocomposite and study its antibacterial activity." Scientific Reports 12, no. 1 (2022): 10491.doi.org/10.1038/s41598-022-14501-6
4. Orgil, Ola, Elinor Schwartz, Lior Baruch, Ifat Matityahu, Jamal Mahajna, and Rachel Amir. "The antioxidative and anti-proliferative potential of non-edible organs of the pomegranate fruit and tree." LWT-Food Science and Technology 58, no. 2 (2014): 571-577..doi.org/10.1016/j.lwt.2014.03.030
5. Rai, Mahendra, Alka Yadav, and Aniket Gade. "Silver nanoparticles as a new generation of antimicrobials." Biotechnology.https://doi.org/10.1016/j.biotechadv.2008.09.002
6. Othman, Siti Hajar. "Bio-nanocomposite materials for food packaging applications: types of biopolymer and nano-sized filler." Agriculture and Agricultural Science Procedia 2 (2014): 296-303.doi.org/10.1016/j.aaspro.2014.11.042
7. Joshipura, Kaumudi J., Francisco J. Muñoz-Torres, Evangelia Morou-Bermudez, and Rakesh P. Patel. "Over-the-counter mouthwash use and risk of pre-diabetes/diabetes." Nitric Oxide 71 (2017): 14-20.doi.org/10.1016/j.niox.2017.09.004
8. Devi, Barsha, and Tridip Boruah. "Tamarind (Tamarindus indica)." Antioxidants in Fruits: Properties and Health Benefits (2020): 317-332.DOI: 10.1007/978-981-15-7285-2_16.
9. Mishra K, Verma SK, Ratre P, Banjare L, Jain A, Thareja S, et al. In Silico Molecular Interaction Studies of Chitosan Polymer with Aromatase Inhibitor: Leads to Letrozole Nanoparticles for the Treatment of Breast Cancer. Anticancer Agents Med Chem. 2021;21: 1191–1199.
10. Nardi GM, Fais S, Casu C, Mazur M, Di Giorgio R, Grassi R, et al. Mouthwash Based on Ozonated Olive Oil in Caries Prevention: A Preliminary In-Vitro Study. Int J Environ Res Public Health. 2020;17. doi:10.3390/ijerph17239106
11. Ahmed F, Prashanth ST, Sindhu K, Nayak A, Chaturvedi S. Antimicrobial efficacy of nanosilver and chitosan against , as an ingredient of toothpaste formulation: An study. J Indian Soc Pedod Prev Dent. 2019;37: 46–54.
12. Potnis NS, Ali A, Pal S. Recent Developments in ROS-Based Nanotherapeutic Modalities in Preclinical Cancer Treatment. Handbook of Oxidative Stress in Cancer: Therapeutic Aspects. 2022. pp. 1–18. doi:10.1007/978-981-16-1247-3_261-1
13. Scully C. Immunity, inflammatory disorders, immunosuppressive and anti-inflammatory agents. Scully's Medical Problems in Dentistry. 2014. pp. 481–497. doi:10.1016/b978-0-7020-5401-3.00019-9
14. Ganjre AP, Bagul N, Sarode G. Merkel cell carcinoma – Beast with two Backs. Oral Oncology. 2015. pp. 880–881. doi:10.1016/j.oraloncology.2015.05.011
15. Anupama GR, Pradeepa R, Felix VP. A comparative study on quiescent beam pattern design techniques. 2012 Annual IEEE India Conference (INDICON). 2012. doi:10.1109/indcon.2012.6420709
16. Halat DH, Sarkis DK, Moubareck CA. Carbapenem-Resistant, Gram-Negative Bacilli. Antibiotic Resistance. 2016. pp. 93–119. doi:10.1016/b978-0-12-803642-6.00005-8
17. Bagul MB, Sonawane SK, Arya SS. Bioactive characteristics and optimization of tamarind seed protein hydrolysate for antioxidant-rich food formulations. 3 Biotech. 2018. doi:10.1007/s13205-018-1240-0
18. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances. 2009. pp. 76–83. doi:10.1016/j.biotechadv.2008.09.002
19. Othman SB, Ben Othman S, Trad A, Youssef H. Security architecture for at-home medical care using Wireless Sensor Network. 2014 International Wireless Communications and Mobile Computing Conference (IWCMC). 2014. doi:10.1109/iwcmc.2014.6906374
20. Rodge S. Antioxidant Activity Of Some Medicinal Plants Of Family Cucurbitaceae. World Journal of Pharmaceutical Research. 2017. pp. 946–951. doi:10.20959/wjpr20174-8097
21. Vishnu Prasad S, Kumar M, Ramakrishnan M, Ravikumar D. Report on oral health status and treatment needs of 5-15 years old children with sensory deficits in Chennai, India. Spec Care Dentist. 2018;38: 58–59.
22. Ramesh Kumar KR, Shanta Sundari KK, Venkatesan A, Chandrasekar S. Depth of resin penetration into enamel with 3 types of enamel conditioning methods: a confocal microscopic study. Am J Orthod Dentofacial Orthop. 2011;140: 479–485.
23. Ganapathy D, Ramadoss R, Yuwanati M, Karthikeyan M. Rarity of mucormycosis in oral squamous cell carcinoma: A clinical paradox? Oral Oncol. 2022;125: 105725.
24. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and

neck squamous cell carcinoma. Arch Oral Biol. 2021;122: 105030.
25. Mohanavel V, Ashraff Ali KS, Prasath S, Sathish T, Ravichandran M. Microstructural and tribological characteristics of AA6351/Si3N4 composites manufactured by stir casting. Journal of Materials Research and Technology. 2020;9: 14662–14672.
26. Muthukrishnan L. Multidrug resistant tuberculosis - Diagnostic challenges and its conquering by nanotechnology approach - An overview. Chem Biol Interact. 2021;337: 109397.
27. Chellapa LR, Rajeshkumar S, Arumugham MI, Samuel SR. Biogenic Nanoselenium Synthesis and Evaluation of its antimicrobial, Antioxidant Activity and Toxicity. Bioinspired Biomim Nanobiomaterials. 2020; 1–6.
28. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther. 2021;12: 192.
29. Felicita AS. Orthodontic management of a dilacerated central incisor and partially impacted canine with unilateral extraction - A case report. Saudi Dent J. 2017;29: 185–193.
30. Uthrakumar R, Vesta C, Raj CJ, Krishnan S, Das SJ. Bulk crystal growth and characterization of non-linear optical bisthiourea zinc chloride single crystal by unidirectional growth method. Curr Appl Phys. 2010;10: 548–552.