LAWSONIA ALBA MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND ITS ANTI CARIOGENIC ACTIVITY

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ABSTRACT:

Background: Silver nanoparticles comprise a very promising approach for the production of new anti microbial systems. *Lawsonia alba* has a wide range of medicinal applications due to its wound healing, anti microbial, antioxidants and anti inflammatory properties. **Aim:** The aim of this study is to evaluate the anticariogenic activity of *Lawsonia alba* mediated Ag Nps. **Materials and Methods:** In the present study, *Lawsonia alba* is used to synthesise silver nanoparticles and were characterized using UV-visible spectrophotometer. The anticariogenic activity of oral pathogens namely, *staphylococcus aureus, streptococcus mutans, Enterococcus faecalis* and *Candida albicans* were evaluated by agar well diffusion method. **Results:** In this present study, maximum zone of inhibition obtained in gram positive *S.mutans* with a zone diameter of 20mm at conc of 100 μL and minimum zone of inhibition obtained in *S.aureus* with a zone diameter of 15mm at same concentration. The maximum Zone of inhibition obtained in gram negative bacteria *E.faecalis* with a zone diameter of 15mm at concentration of 100 μL and lowest zone of inhibition obtained in *C.albicans* with a zone diameter of 11mm at concentration of 100 μL. **Conclusion:** Silver nanoparticles synthesised using *Lawsonia alba* have been shown to have excellent anticariogenic activity and can be used in a wide range of dental applications.

KEY WORDS: Silver nanoparticles; *Lawsonia alba*; oral pathogens; agar well diffusion assay; anti cariogenic activity; green synthesis; eco friendly.

INTRODUCTION:

One of the most active research fields of modern material science is nanotechnology. The development of secure confinement in the knowledge of materials and experimental protocols used for nanomaterial synthesis is one of the main facets of the nanotechnology field's concerns (Bayda *et al.*, 2019). Nanoparticles with dimensions in the range of 1-100nm have attracted a continuous trickle of interest as a result of their peculiar properties, ability to form superstructures and applications superior to their bulk counterparts (Ganta *et al.*, 2020). Nanostructured noble metals have recently gained interest in a range of scientific and medical uses, including molecular imaging, drug distribution, material production, and medical devices for diagnosis and treatment (Khan, Saeed and Khan, 2019). Metal nanoparticles have been prepared using a variety of techniques, the most common of which are chemical reduction, ultraviolet and microwave radiation, as well as photochemical and sonoelectrochemical methods (Swain, 2016). Noble metals like Au, Ag, Pd, Pt, and Cu have long been used to make stable colloids that can be used in optoelectronics, catalysis, photothermal therapy, surface enhanced Raman scattering (SERS) detection, and biological labelling (Lee and Jun, 2019). At present, silver nanoparticles have received high attention due to their extraordinary biological activities. Drug delivery, bio-labeling, sensing, food preservation, wound healing, water purification, and cosmetics are only a few of the applications (Rajakumari *et al.*, 2020). Moreover, silver nanoparticles have other interesting applications such as textiles, electronics, catalysis and paints. AgNPs are also used in the manufacture of surgical masks (Ahmed and Mustafa, 2020). According to studies, silver nanoparticles (AgNPs) can be used successfully against multidrug-resistant bacteria due to their small scale and comparatively wide surface area in contrast to volume, which allows them easy to interact with substances and enhances their antibacterial efficacy (Wang, Hu and Shao, 2017). The recent strategy for improving antibiotic efficacy is to combine them with AgNPs to control microbial infections, as indicated by AgNPs' damage to microbial deoxyribonucleic acid (Jackson *et al.*, 2020).

In this present study *Lawsonia Alba* plant has been used as a biological source for synthesizing silver nanoparticles. *Lawsonia alba* is a dwarf shrub that is well known for its cosmetic use due to the presence of unique active principles in the leaves. Henna or mehndi are two common names for it. It's a North African and South-West Asian native that's also grown in West Africa (Sharma, Goel and Bhatia, 2016). It is an ornamental hedge and dye plant that has been used for Centuries in many civilizations and cultures (Abd-el-Malek *et al.*, 1973). *L. alba* leaf paste is used to dye hair and palms of hands, as well as to avoid skin inflammation. As a colouring agent, it includes lawsone (Badoni Semwal *et al.*, 2014). Wounds, ulcers, asthma, bronchitis, lumbago, rheumatalgia, inflammations, diarrhoea, dysentery, leucoderma, scabies, boils, anaemia, haemorrhages, fever, hair loss, and greying hair are all treated with the leaves of this herb (M, Mungle A N. Ittadwar A M Mungle A N. Ittadwar A M and ijrbat, 2018). The secondary metabolites including saponins, triterpenoids and dioxin derivatives isolated from *Lawsonia inermis* plant had an antibacterial effect against *Pseudomonas aeruginosa (Gull et al., 2013).*

Oral health is related to physical well-being and life satisfaction. Oral infections may result from either an endogenous source that produces microorganisms that are normally present in the mouth, such as plaque-related dental caries and periodontal disease, or an exogenous source that produces microorganisms that are not normally found in the oral microflora (Ali *et al.*, 2020). The use of nano-sized antimicrobials provides the possibility to monitor the formation of these oral biofilms through the use of nanoparticles with biocidal, anti-adhesive and delivery capabilities (Barma, 2020). This is why the current work stands remarkable as it deals with green synthesis of Ag Nps using *Lawsonia alba* extract. Our team has extensive knowledge and research experience that has translated into high quality publications (Princeton, Santhakumar and Prathap, 2020),(Mathew *et al.*, 2020),(Sridharan *et al.*, 2019),(R *et al.*, 2020),(Antony *et al.*, 2021),(Sarode, Gondivkar, Sarode, *et al.*, 2021),(Hannah R *et al.*, 2021),(Chandrasekar *et al.*, 2020),(Subramanyam *et al.*, 2018),(Jeevanandan and Thomas, 2018),(Ponnulakshmi *et al.*, 2019), (Sundaram, Nandhakumar and Haseena Banu, 2019),(Alsawalha *et al.*, 2019),(Yu *et al.*, 2020),(Shree *et al.*, 2019),(Zafar *et al.*, 2020),(Karunagaran *et al.*, 2019),(Sarode, Gondivkar, Gadbail, *et al.*, 2021),(Raj Preeth *et al.*, 2021),(Prithiviraj *et al.*, 2020). (Krishnan and Lakshmi, 2013; Soh and Narayanan, 2013; Lekha *et al.*, 2014; Dhinesh *et al.*, 2016; PradeepKumar *et al.*, 2016)The aim of the present study is to determine their anti cariogenic activity against oral pathogens such as *S.mutans*, *S.aureus*, *E.faecalis* and *C.albicans* which are responsible for the cause of tooth caries and nosocomial infections.

MATERIALS AND METHODS:

Preparation of plant extract:

Leaves of *Lawsonia alba* were purchased commercially. About 1g of *Lawsonia alba* leaf powder was dissolved in distilled water and boiled for 5-10 min at 60-70^0 (Figure-1). The solutions were then filtered using whatman No 1 filter paper. The filtered extract was collected and stored in 4 ^{0} C for further use.

Synthesis of Silver Nanoparticles:

About 1 mmol of silver nitrate was prepared in 90 ml of distilled water. The plant extracts of Lawsonia alba were added with the metal solution and this solution was made into a 100 ml solution. The colour change was observed visually and photographs were taken for the record. The solution was then kept in a magnetic stirrer for synthesis of nanoparticles. The synthesis of nanoparticles is primarily characterized using UV-visible spectroscopy (Figure-2). 3 ml of the solution is taken in a cuvette and scanned in a UV-visible spectrometer under 300 nm to 700nm wavelength. The results were recorded for graphical analysis.

Anticariogenic activity

The anticariogenic activity was determined by the well diffusion method (Figure-3). The mueller Hinton agar plates were prepared and each plate was swabbed with four different oral pathogens such as S.*mutans*, S.*aureus*, E. *faecalis* and C.*albicans*. A gel puncture was used to cut four wells on each petri plate. To the first three wells, silver nanoparticles with lawsonia alba were added in different concentrations of 25 µL, 50 µL, 100 µL. The anticariogenic activity was determined based on the measurement of the diameter of the zone of inhibition formed around the well and the mean values were calculated.

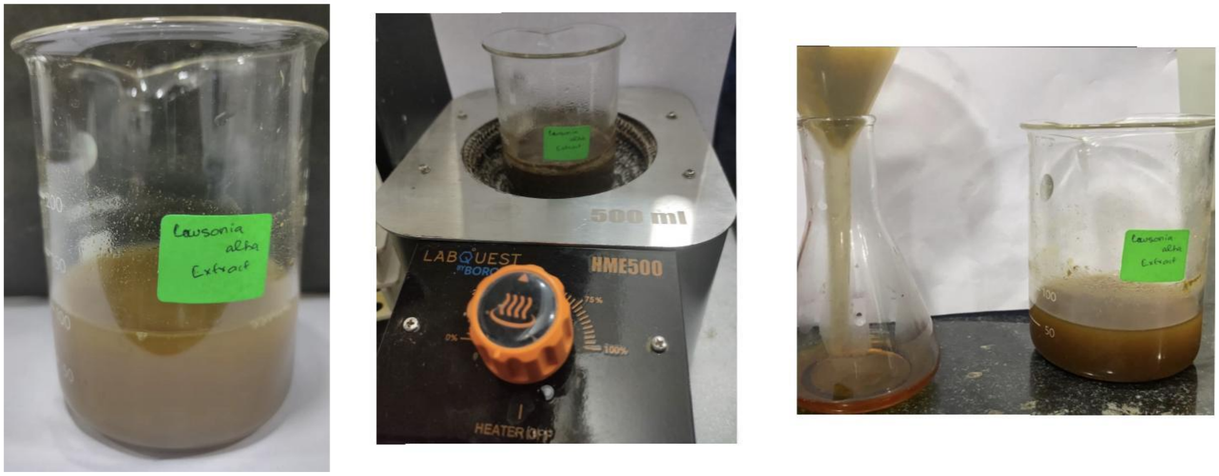

Figure-1: Synthesis of Lawsonia alba mediated Silver nanoparticles

Figure-2: Characterization of Silver nanoparticles is done by using UV visible spectroscopy. The graphical representation of the red line indicates the wavelength at which the nanoparticles are synthesized. The wavelength ranges from 300-700 nm. The synthesis of AgNps was confirmed by formation of a peak at 400 nm.

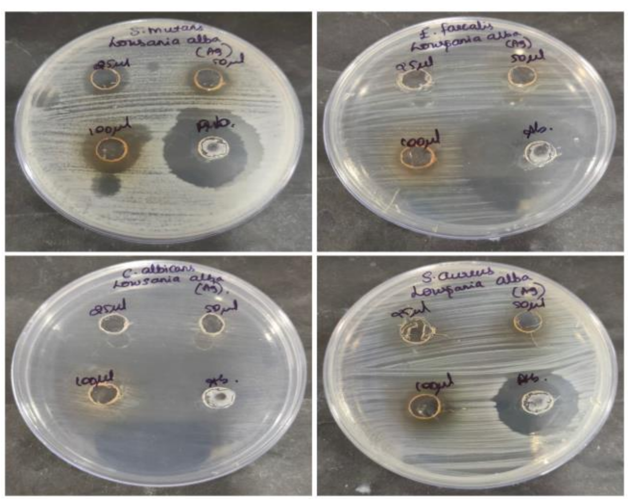

Figure-3: Anticariogenic activity of Lawsonia alba mediated silver nanoparticles against oral pathogens (*S.mutans*, *E.faecalis*, *C.albicans*, *S.aureus*)

RESULTS AND DISCUSSION:

Visual observation:

The leaf extract of *Lawsonia alba* was visually observed (Figure-2). It was gold colour first and when it was added with the metal solution, it turned into a dark brown extract which was indicated with the formation of silver nanoparticles.

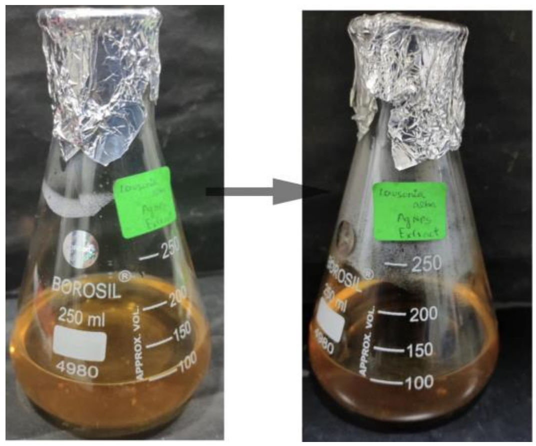

Figure-4: *Lawsonia alba* mediated Ag Nps synthesis and colour transition from golden yellow colour to canary yellow.

UV-vis spectroscopy:

The bio-reduction of pure $AgNo_3$ to Ag Nps was characterised by using UV-vis-spectroscopy. The presence of nanoparticles was confirmed by obtaining a spectrum in the visible range of 300-700nm. The absorption spectrum of the incubated solution, which showed a peak at 400nm confirmed the formation of Ag Nps as shown in Figure-2.

The anticariogenic activity of *Lawsonia alba* mediated silver nanoparticles was investigated against oral pathogens namely, *S.mutans, E.faecalis, C.albicans and S.aureus* recovered from dental caries and nosocomial infected patients. The results obtained are recorded and inhibitory activity of *Lawsonia alba* mediated Ag Nps is calculated for anticariogenic activity and shown in table 1. It was found that *Lawsonia alba* mediated Ag Nps caused inhibition of oral pathogens in a dose dependent manner. The graph seen in Figure-4 depicts maximum zone of inhibition obtained in gram positive bacteria *S.mutans* with a Zone diameter of 20mm at concentration of 100 µL and minimum zone of inhibition obtained in *S.aureus* with a zone diameter of 15mm at same concentration. The maximum Zone of inhibition obtained in gram negative bacteria *E.faecalis* with a zone diameter of 15mm at concentration of 100 µL and lowest zone of inhibition obtained in *C.albicans* with a zone diameter of 11mm at concentration of 100 µL. The results revealed that *Lawsonia alba* mediated Ag Nps show effective anti cariogenic activity.

Table-1 depicts the anticariogenic activity of Silver nanoparticles with *Lawsonia alba* at various concentrations compared with the antibiotic (amoxicillin).

Concentration	25 µL	50 µL	100 µL	Antibiotic
E.faecalis	9	12	15	25
S.mutans	15	16	20	27
C.albicans	9	11	11	12
S.aureus	12	13	15	25

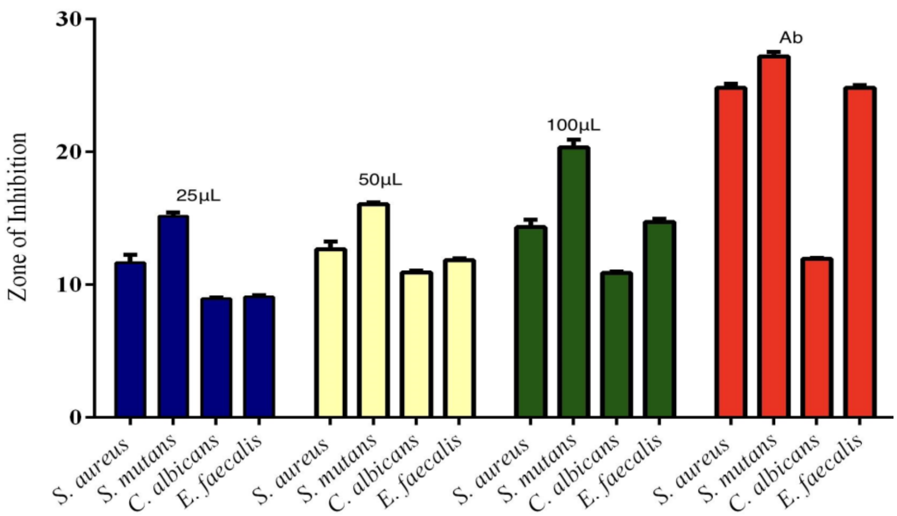

Figure-5: The bar graph represents the comparison between the *Lawsonia alba* (Ag Nps) and standard antibiotic (amoxicillin). The X- axis represents the concentration of the *Lawsonia alba* (Ag Nps) and the standard amoxicillin and the Y-axis represents the zone of inhibition against the oral pathogens. The anticariogenic activity of AgNps which showed that *S. mutans* was the most sensitive bacteria followed by *E. faecalis, S.aureus and C.albicans.*

Since oral bacteria have highly complex adherence mechanisms, they colonise and cause disease primarily in the oral cavity (Nasim, Kamath and Rajeshkumar, 2020). Commercially available antibacterial agents such as chlorhexidine, fluorides, and various antibiotics that can be used to avoid dental caries. However, they have a variety of side effects that include nausea, vomiting, and diarrhoea and that an attempt has been made to find medicines made from naturally occurring substances with the minimum side effects (Aronson, 2009). Synthetic chemicals may be replaced with natural ingredients derived from plants used in herbal medicine. About 80% of people in developing countries use traditional medicine which includes substances obtained from medicinal plants (Veeresham, 2012). Herbal medicines are usually thought to be harmless. However, in order to prevent fatal effects, it is appropriate to assess their biological safety before use. There is no doubt about Lawsonia alba's pharmacological properties, but toxicological testing is still needed. Lawsonia alba extracts were tested in mice in vivo for acute toxicity, and no mortality was observed (Chowdhury, Kubra and Ahmed, 2014).

In previous literature, *omani henna* extract was evaluated against *S. aureus, E. coli, P. aeruginosa, Shigella sonnei* and *C.albicans*. It was found to be the best anti microbial activity against *Shigella sonnei* but it failed to show antifungal activity against *C. albicans* (Habbal *et al.*, 2005). But in our study, *Lawsonia alba* mediated Ag Nps have a good antimicrobial activity against *C.albicans* at Conc of 100 µL. Another study reported, aqueous extracts show less antimicrobial activity against *Escherichia coli ATCC 25922*. However, *Staphylococcus aureus ATCC 25923* and *Candida albicans ATCC 10231* were sensitive toward leaf extract of *Lawsonia inermis* (Hadef and Boufeldja, 2019). In our study, a combination of silver nanoparticles with *Lawsonia alba* showed better anticariogenic activity against *E.faecalis, S. aureus and C. albicans*. *L.inermis* exhibited antimicrobial activity against Gram-positive bacteria while was ineffective for Gram- negative bacteria (Papageorgiou *et al.*, 1999). While in our study, *Lowsonia alba* had antimicrobial activity against Gram positive bacteria as well as Gram negative bacteria *(S.mutans, E.faecalis, C.albicans, S.aureus)*. Another study reported that *Phyllanthus emblica* exhibited strong antibacterial activity against a wide range of bacteria such as *B.cereus, S.aureus, P. aeruginosa* and *E.coli* compared to *Lawsonia alba* (Khan *et al.*, 2013). Using Ag Nps alone against *S.mutans* was found to be significant bactericidal and bacteriostatic activity and these effects were similar to commercially available chlorhexidine (Santos *et al.*, 2014). Similar study, performed by silver nanoparticles with *Phyllanthus emblica* and *Cinnamomum verum* was found to be good antibacterial activity against *S. aureus* followed by *Lactobacillus, E. faecalis* and *S.mutans (S et al., 2020)*. While in our study, silver nanoparticles formulation with *Lawsonia alba* produces significant anti cariogenic activity against dental pathogens.

The present study was done to evaluate the anticariogenic activity of *Lawsonia alba (Ag Nps)* extract against limited microbes. Further, it shows moderate anticariogenic activity compared to amoxicillin. However, it is environmentally friendly, quick, and simple to synthesise, as well as effective against bacteria with multidrug resistance. Thus, Ag Nps may potentially be used as a non-toxic alternative to antibiotics.

CONCLUSION:

In our present study, *lawsonia alba* mediated silver nanoparticles showed excellent anticariogenic activity and these green synthesis of silver nanoparticles was of low cost and convenient to carry out. Thus, it is a promising new approach incorporated as an oral health care product for the prevention of oral pathogens in the near future.

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REFERENCE:

1. Abd-el-Malek, Y. A. *et al.* (1973) 'Antimicrobial principles in leaves of Lawsonia inermis L', *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Zweite naturwissenschaftliche Abt.: Allgemeine, landwirtschaftliche und technische Mikrobiologie*, 128(1), pp. 61–67.
2. Ahmed, R. H. and Mustafa, D. E. (2020) 'Green synthesis of silver nanoparticles mediated by traditionally used medicinal plants in Sudan', *International Nano Letters*, pp. 1–14. doi: 10.1007/s40089-019-00291-9.
3. Ali, S. J. *et al.* (2020) 'Antifungal Activity of Selenium Nanoparticles Extracted from Capparis decidua Fruit against Candida albicans', *Journal of Evolution of Medical and Dental Sciences*, pp. 2452–2455. doi: 10.14260/jemds/2020/533.
4. Alsawalha, M. *et al.* (2019) 'Novel mathematical modelling of Saudi Arabian natural diatomite clay', *Materials Research Express*, 6(10), p. 105531.
5. Antony, J. V. M. *et al.* (2021) 'Particle size penetration rate and effects of smoke and smokeless tobacco products - An invitro analysis', *Heliyon*, 7(3), p. e06455.
6. Aronson, J. K. (2009) *Meyler's Side Effects of Antimicrobial Drugs*. Elsevier.
7. Badoni Semwal, R. *et al.* (2014) 'Lawsonia inermis L. (henna): ethnobotanical, phytochemical and pharmacological aspects', *Journal of ethnopharmacology*, 155(1), pp. 80–103.
8. Barma, M. D. (2020) 'Synthesis of Triphala Incorporated Zinc Oxide Nanoparticles and Assessment of its Antimicrobial Activity Against Oral Pathogens : An In-Vitro Study', *Bioscience Biotechnology Research Communications*, pp. 74–78. doi: 10.21786/bbrc/13.7/14.
9. Bayda, S. *et al.* (2019) 'The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine', *Molecules*, p. 112. doi: 10.3390/molecules25010112.
10. Chandrasekar, R. *et al.* (2020) 'Development and validation of a formula for objective assessment of cervical vertebral bone age', *Progress in orthodontics*, 21(1), p. 38.
11. Chowdhury, M. M. H., Kubra, K. and Ahmed, S. R. (2014) 'Antimicrobial, Phytochemical and Toxicological Evaluation of Lawsonia inermis Extracts against Clinical Isolates of Pathogenic Bacteria', *Research Journal of Medicinal Plant*, pp. 187–195. doi: 10.3923/rjmp.2014.187.195.
12. Dhinesh, B. *et al.* (2016) 'An assessment on performance, emission and combustion characteristics of single cylinder diesel engine powered by Cymbopogon flexuosus

biofuel', *Energy Conversion & Management*, 117, pp. 466–474.
13. Ganta, S. S. L. *et al.* (2020) 'Anti-Inflammatory Activity of Dried Ginger Mediated Iron Nanoparticles', *Journal of Pharmaceutical Research International*, pp. 14–19. doi: 10.9734/jpri/2020/v32i2830866.
14. Gull, I. *et al.* (2013) 'Phytochemical, toxicological and antimicrobial evaluation of Lawsonia inermis extracts against clinical isolates of pathogenic bacteria', *Annals of clinical microbiology and antimicrobials*, 12, p. 36.
15. Habbal, O. A. *et al.* (2005) 'In-vitro antimicrobial activity of Lawsonia inermis Linn (henna). A pilot study on the Omani henna', *Saudi medical journal*, 26(1), pp. 69–72.
16. Hadef, K. Z. and Boufeldja, W. (2019) 'Antimicrobial Activity of Lawsonia inermis Leaf Extract Collected from South of Algeria Touat (Adrar) and Tidikelt (In Salah)', *Journal of Plant Sciences*, pp. 9–16. doi: 10.3923/jps.2020.9.16.
17. Hannah R *et al.* (2021) 'Author response for "Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris—A review"'. Wiley. doi: 10.1111/odi.13937/v2/response1.
18. Jackson, K. *et al.* (2020) 'Cytotoxic potentials of silibinin assisted silver nanoparticles on human colorectal HT-29 cancer cells', *Bioinformation*, pp. 817–827. doi: 10.6026/97320630016817.
19. Jeevanandan, G. and Thomas, E. (2018) 'Volumetric analysis of hand, reciprocating and rotary instrumentation techniques in primary molars using spiral computed tomography: An in vitro comparative study', *European journal of dentistry*, 12(1), pp. 21–26.
20. Kamala, K., Kumar, G.J., Ganapathy, D., Sundramoorthy, A.K. and Sivaperumal, P., 2023. Marine Actinobacterial Streptomyces sp., mediated synthesis of silver nanoparticles: characterization and different Biological properties under In-vitro Condition. Current Analytical Chemistry, 19(7), pp.550-560.
21. Karunagaran, M. *et al.* (2019) 'Expression and distribution pattern of podoplanin in oral submucous fibrosis with varying degrees of dysplasia – an immunohistochemical study', *Journal of Histotechnology*, pp. 80–86. doi: 10.1080/01478885.2019.1594543.
22. Khan, D. A. *et al.* (2013) 'Antibacterial activity of Phyllantus emblica, Coriandrum sativum, Culinaris medic, Lawsonia alba and Cucumis sativus', *Acta poloniae pharmaceutica*, 70(5), pp. 855–859.
23. Khan, I., Saeed, K. and Khan, I. (2019) 'Nanoparticles: Properties, applications and toxicities', *Arabian Journal of Chemistry*, pp. 908–931. doi: 10.1016/j.arabjc.2017.05.011.
24. Krishnan, V. and Lakshmi, T. (2013) 'Bioglass: A novel biocompatible innovation', *Journal of advanced pharmaceutical technology & research*, 4(2), pp. 78–83.
25. Lee, S. and Jun, B.-H. (2019) 'Silver Nanoparticles: Synthesis and Application for Nanomedicine', *International Journal of Molecular Sciences*, p. 865. doi: 10.3390/ijms20040865.
26. Lekha, L. *et al.* (2014) 'Schiff base complexes of rare earth metal ions: Synthesis, characterization and catalytic activity for the oxidation of aniline and substituted anilines',

Journal of organometallic chemistry, 753, pp. 72–80.
27. Mathew, M. G. *et al.* (2020) 'Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial', *Clinical oral investigations*, 24(9), pp. 3275–3280.
28. M, M. A. N. I. A. M. A. N. I. A., Mungle A N. Ittadwar A M Mungle A N. Ittadwar A M and ijrbat (2018) 'LAWSONIA INERMIS L.: PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES. A REVIEW', *International Journal of Researches in Biosciences and Agriculture Technology*. doi: 10.29369/ijrbat.2018.01.i.0055.
29. Nasim, I., Kamath, K. and Rajeshkumar, S. (2020) 'Evaluation of the re-mineralization capacity of a gold nanoparticle-based dental varnish: An in vitro study', *Journal of Conservative Dentistry*, p. 390. doi: 10.4103/jcd.jcd_315_20.
30. Papageorgiou, V. P. *et al.* (1999) 'The Chemistry and Biology of Alkannin, Shikonin, and Related Naphthazarin Natural Products', *Angewandte Chemie International Edition*, pp. 270–301. doi: 10.1002/(sici)1521-3773(19990201)38:3<270::aid-anie270>3.0.co;2-0.
31. Pauline, C.R., Akshita, Pavithra, T., Kannan, K. and Sivaperumal, P., 2024. Characterization and Biological Activity of Silver Nanoparticles from (Rhizophora Mucronata) Mangrove Extract. Nano LIFE, p.2450018.
32. Ponnulakshmi, R. *et al.* (2019) 'In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats', *Toxicology mechanisms and methods*, 29(4), pp. 276–290.
33. PradeepKumar, A. R. *et al.* (2016) 'Diagnosis of Vertical Root Fractures in Restored Endodontically Treated Teeth: A Time-dependent Retrospective Cohort Study', *Journal of endodontia*, 42(8), pp. 1175–1180.
34. Princeton, B., Santhakumar, P. and Prathap, L. (2020) 'Awareness on Preventive Measures taken by Health Care Professionals Attending COVID-19 Patients among Dental Students', *European journal of dentistry*, 14(S 01), pp. S105–S109.
35. Prithiviraj, N. *et al.* (2020) 'Anticancer Compounds From Starfish Regenerating Tissues and Their Antioxidant Properties on Human Oral Epidermoid Carcinoma KB Cells', in *PANCREAS*. LIPPINCOTT WILLIAMS & WILKINS TWO COMMERCE SQ, 2001 MARKET ST, PHILADELPHIA …, pp. 155–156.
36. Rajakumari, R. *et al.* (2020) 'Grape seed extract-soluplus dispersion and its antioxidant activity', *Drug Development and Industrial Pharmacy*, pp. 1219–1229. doi: 10.1080/03639045.2020.1788059.
37. Raj Preeth, D. *et al.* (2021) 'Bioactive Zinc(II) complex incorporated PCL/gelatin electrospun nanofiber enhanced bone tissue regeneration', *European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences*, 160, p. 105768.
38. R, H. *et al.* (2020) 'CYP2 C9 polymorphism among patients with oral squamous cell

carcinoma and its role in altering the metabolism of benzo[a]pyrene', *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, pp. 306–312. doi: 10.1016/j.oooo.2020.06.021.

39. Santos, V. E. *et al.* (2014) 'Antimicrobial activity of silver nanoparticles in treating dental caries', *Revista da Faculdade de Odontologia - UPF*. doi: 10.5335/rfo.v18i3.3568.
40. Santhosh, K., Kamala, K., Ramasamy, P., Musthafa, M.S., Almujri, S.S., Asdaq, S.M.B. and Sivaperumal, P., 2024. Unveiling the silent threat: Heavy metal toxicity devastating impact on aquatic organisms and DNA damage. Marine Pollution Bulletin, 200, p.116139.
41. Sarode, S. C., Gondivkar, S., Sarode, G. S., *et al.* (2021) 'Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis', *Oral oncology*, p. 105390.
42. Sarode, S. C., Gondivkar, S., Gadbail, A., *et al.* (2021) 'Oral submucous fibrosis and heterogeneity in outcome measures: a critical viewpoint', *Future oncology* , 17(17), pp. 2123–2126.
43. Sharma, R. K., Goel, A. and Bhatia, A. K. (2016) 'Lawsonia Inermis Linn: A Plant with Cosmetic and Medical Benefits', *International Journal of Applied Sciences and Biotechnology*, pp. 15–20. doi: 10.3126/ijasbt.v4i1.14728.
44. Shree, K. H. *et al.* (2019) 'Saliva as a Diagnostic Tool in Oral Squamous Cell Carcinoma – a Systematic Review with Meta Analysis', *Pathology & Oncology Research*, pp. 447–453. doi: 10.1007/s12253-019-00588-2.
45. Soh, C. L. and Narayanan, V. (2013) 'Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery—A systematic review', *International journal of oral and maxillofacial surgery*, 42(8), pp. 974–980.
46. Sridharan, G. *et al.* (2019) 'Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma', *Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, 48(4), pp. 299–306.
47. S, S. *et al.* (2020) 'Antioxidant activity of silver nanoparticles synthesis using Cinnamomum verum and Phyllanthus emblica formulation', *International Journal of Research in Pharmaceutical Sciences*, pp. 6918–6921. doi: 10.26452/ijrps.v11i4.3682.
48. Subramanyam, D. *et al.* (2018) 'Comparative evaluation of salivary malondialdehyde levels as a marker of lipid peroxidation in early childhood caries', *European journal of dentistry*, 12(1), pp. 67–70.
49. Sundaram, R., Nandhakumar, E. and Haseena Banu, H. (2019) 'Hesperidin, a citrus flavonoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats', *Toxicology mechanisms and methods*, 29(9), pp. 644–653.
50. Swain, A. K. (2016) 'Review on Green Synthesis of Silver Nanoparticles by Physical, Chemical and Biological Methods', *International Journal of Scientific & Engineering Research*, pp. 551–554. doi: 10.14299/ijser.2016.10.008.
51. Veeresham, C. (2012) 'Natural products derived from plants as a source of drugs', *Journal*

of Advanced Pharmaceutical Technology & Research, p. 200. doi: 10.4103/2231-4040.104709.

52. Wang, L., Hu, C. and Shao, L. (2017) 'The antimicrobial activity of nanoparticles: present situation and prospects for the future', *International journal of nanomedicine*, 12, pp. 1227–1249.
53. Yu, J. *et al.* (2020) 'Inhibitory effects of triterpenoid betulin on inflammatory mediators inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-alpha, interleukin-6, and proliferating cell nuclear antigen in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis', *Pharmacognosy magazine*, 16(72), p. 836.
54. Zafar, A. *et al.* (2020) 'Diagnostic utility of touch imprint cytology for intraoperative assessment of surgical margins and sentinel lymph nodes in oral squamous cell carcinoma patients using four different cytological stains', *Diagnostic cytopathology*, 48(2), pp. 101–110.