PREPARATION OF TRIDAX PROCUMBENS LEAVES MEDIATED SILVER NANOPARTICLES AND ITS ANTIMICROBIAL ACTIVITY AGAINST ORAL PATHOGENS

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Abstract

Introduction: The common name of Tridax procumbens used by practitioners of ayurveda is bhringraj. It has a number of pharmacological properties including hepatoprotective, wound healing and immunomodulating properties. The leaves of T. procumbens have always promised to be a great source of antimicrobial compounds. The nanoparticles synthesized in our study are silver nanoparticles which are known to be safe to mankind and have shown to have little to no allergic reaction when tested for curing various diseases. Due to their distinctive physical and chemical characteristics, silver nanoparticles are being employed more and more in a variety of industries, including medicine, food, health care, consumer goods, and manufacturing. The aim of this study is to prepare T. procumbens leaves mediated silver nanoparticles and analyze its antimicrobial properties.

Materials and Methods: T. procumbens leaf extract was prepared and then it is used to synthesize silver nanoparticles. Colour changes were observed at regular intervals during the synthesis process and characterisation was done using UV-vis spectroscopy, SEM, EDAX and FTIR. The antimicrobial activity was evaluated by well diffusion assay at different concentrations against more common oral pathogens such as S. aureus, P. auruginosa and compared with that of the standard drug.

Results: The silver nanoparticles synthesized using T. procumbens in this study were observed to show enhanced zones of inhibition against S. aureus (23±0.32mm) and P. auruginosa (20±0.32mm) at 100µL concentration when compared to standard.

Conclusion: T. procumbens mediated silver nanoparticles showed potent antimicrobial activity against common oral pathogens. Hence these nanoparticles can be used as antimicrobial agents in various nanoformulations for oral diseases.

Keywords: Antimicrobial activity, green synthesis, P. auruginosa, S. aureus, silver nanoparticles. Tridax procumbens

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1. Introduction

Nanotechnology or nanomedicine is a relatively new but growing field for research. Targeted medication delivery and medical imaging are two industries that are quickly adopting nanotechnology-based tools and methods. These developments pave the way for customized medicine and the possibility of early diagnoses, possibly even presymptomatic ones along with extremely successful tailored treatments. Dendrimers, liposomes, nanoshells, nanotubes, emulsions, and quantum dots are among the substances they use ('Nanotechnological applications in medicine', 2007). The silver nanoparticles allow for a wide variety of novel uses in many industrial sectors. There is ongoing interest in the creation of noble metal nanoparticles for use in a variety of fields, including catalysis, electronics, optics, environmental science, and biotechnology (Ali et al., 2020). The physical and chemical approaches are the two basic processes for producing silver nanoparticles (Abbasi et al., 2016). Plant extracts are being employed more frequently for the production of nanoparticles due to their widespread availability and range of bioactive reducing metabolites ('Green biosynthesis of silver nanoparticles using leaves extract of Artemisia vulgaris and their potential biomedical applications', 2017).

The perennial plant T. procumbens, sometimes referred to as "coat buttons," is a member of the Asteraceae family and is indigenous to Central and South America. This species has been utilized in Indian Ayurveda from ancient times (J et al., 2009; Jude, Catheri and Frank, 2010). A typical tropical grass is T. procumbens Linn. which has been used in the past to cure ailments like bronchial catarrh, dysentery, malaria, stomach aches, diarrhoea and high blood pressure as well as to arrest bleeding from wounds, bruises, and cuts and to stop hair loss. In addition to having obvious depressing effects on respiration, it has hepatoprotective, antiseptic, insecticidal, parasiticidal, and parasite-killing properties (J et al., 2009). T. procumbens is sold under the trade name "Bhringraj," which is well-known in Ayurvedic treatments for liver diseases.('Hepatoprotective activity of Tridax procumbens against d-galactosamine/lipopolysaccharide-induced hepatitis in rats', 2005; Andriana et al., 2019). Along with being an effective antimicrobial agent, T. procumbens is also used to treat conditions such as oxidative stress, chronic diseases and hyperuricemia (Andriana et al., 2019). In this study the leaf extract of this plant has been tested for antimicrobial activity in order to explore its uses in the field of dentistry.

One of the most common infectious disease-related causes of morbidity and death globally is Staphylococcus aureus. This organism may cause a broad range of illnesses, from sepsis and pneumonia to fairly severe skin infections that can be deadly. Antibiotic resistance makes it difficult to treat S. aureus infections and a reliable vaccination is not yet available (Cheung, Bae and Otto, 2021). It is one of the primary organisms causing gingivitis. A widespread, gram-negative environmental bacterium is Pseudomonas aeruginosa. It may be a substantial pathogenic component of severe infections in people, particularly in those with cystic fibrosis and periodontitis. Infection with this bacterium can result in significant treatment issues because of its built-in resistance to antibiotics and its capacity to create biofilms.(Mielko et al., 2019; Cheung, Bae and Otto, 2021).

This study mainly focuses on the effect of silver nanoparticles on the T. procumbens leaf extract and their microbial activity with a potent use as a pharmacological drug in future with the focus of its use in the dental field. (Ramesh Kumar et al., 2011; Jain, Kumar and Manjula, 2014; Krishnan, Pandian and Kumar S, 2015; Keerthana and Thenmozhi, 2016; Sivamurthy and Sundari, 2016; Felicita, 2017a, 2017b; Kumar, 2017; Sekar et al., 2019; Johnson et al., 2020)(Jeevitha et al., 2022) (Begum, Jeevitha and Preetha, 2020; Prathap et al., 2021; Santhakumar et al., 2021)(Rajeshkumar and Jeevitha, 2021; Santhakumar et al., 2021). The present study aims to prepare T. procumbens leaf extract mediated silver nanoparticles and analyze its antimicrobial activity.

2. Materials And Methods

Biosynthesis of silver nanoparticles using leaf extract:
1g of T. procumbens leaf powder was weighed and dissolved in 100 mL of distilled water and boiled for 5-10 mins. The solution was filtered through whatman filter paper and the extract was obtained (Fig. 1).
For the biosynthesis of silver nanoparticles from the leaf extract, 0.001g of silver nitrate ($AgNO_3$) was first carefully dissolved in 70 mL of distilled water to obtain $AgNO_{3)}$ solution. To the solution obtained, about 30 mL of leaf extract was added and kept in an orbital shaker (Fig 2). Color changes were noted continuously at regular intervals.

Characterization of silver nanoparticles:
Characterization of nanoparticles were done using UV- vis spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray

Analysis (EDAX), Fourier-transform infrared spectroscopy (FTIR). Morphological classification was done through SEM. The elemental composition was further observed through EDAX and fourier transform infrared spectroscopy (FTIR) helps in analyzing the functional group of the nanoparticles that are synthesized.

Antimicrobial activity

The antimicrobial activity was evaluated using well diffusion assay against more common oral pathogens, S. aureus and P. aeruginosa. The test organisms employed in this study came from the culture collections at Saveetha Dental College's Nanomedicine Laboratory in Chennai. S. aureus and P. aeruginosa were the species employed in this study. For the assay, Muller hinton agar plates were utilized. The zone of incubation was measured for each of the organisms at different concentrations (25µL, 50µL, 100µL) and compared with that of the standard drug.

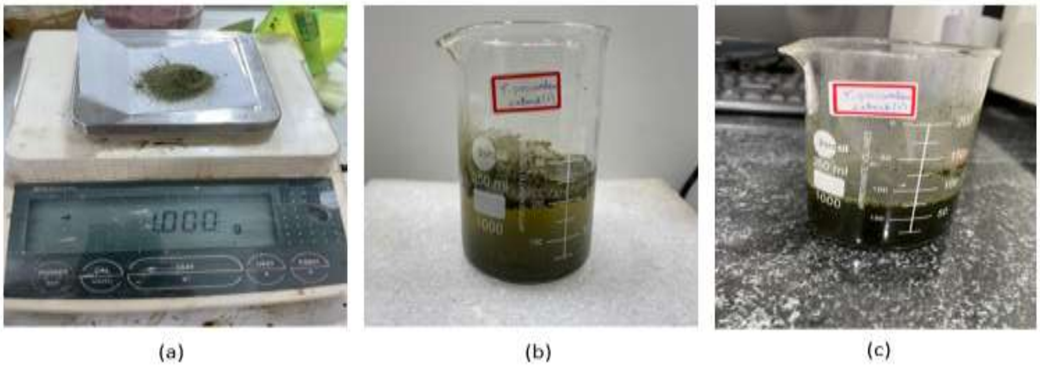

Figure 1: Preparation of T. procumbens leaf extract (a) Weighing the T. procumbens leaves powder (b) T. procumbens dissolved in distilled water (c) T. procumbens leaves extract after boiling and filtering process

Figure 2: Synthesis of silver nanoparticles from T. procumbens leaves extract (a) $AgNO_3$ solution (b) T. procumbens leaves extract dissolved in $AgNO_3$ solution

3. Results and Discussion

Visual observation of color changes

The colour changes during the synthesis process of silver nanoparticles at various time periods were visually observed. After about 74 h of the incubation period, the colour of the mixture does not change a lot in the course of time except for getting slightly darker (Fig. 3). In a similar study synthesizing silver nanoparticles mediated by T. procumbens flower extract, the color changes were observed to shift from light yellow to dark brown in 24 h without any changes later on illustrating that the synthesis of AgNPs was complete (J.soni , Jeevitha M 2022).

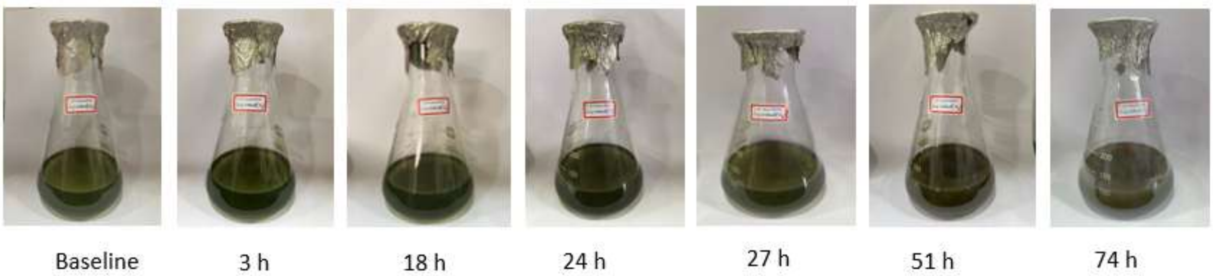

Figure 3: Reduction of silver ions to silver nanoparticles visually identified by color change at various periods of incubation time

UV-vis spectrophotometer analysis:
UV-vis spectroscopy was used to determine the optical property of silver nanoparticles in 250-650 nm range.The absorbance peak of the T. procumbens leaves extract mediated silver nanoparticles was observed at 430 nm similar to previous studies (J. Soni et al., 2022). Figure 4 illustrates the UV absorption spectra of silver nanoparticles prepared from the T. procumbens leaf extract on reaction with AgNO3. In a previous study of synthesizing silver nanoparticles, a large absorption peak between 400 and 460 nm was observed which is similar to the observation of the present study (Vastrad and Goudar, 2016).

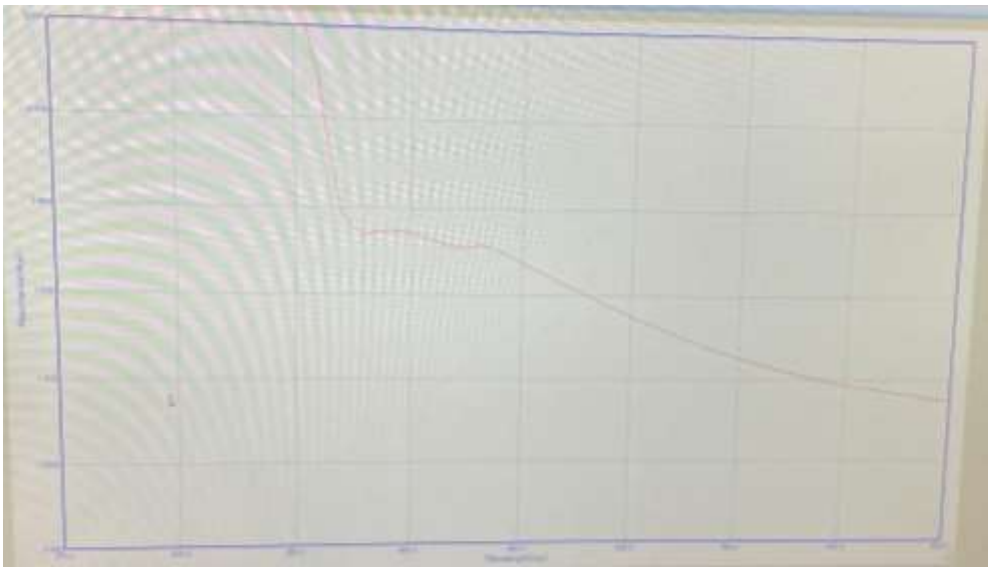

Figure 4: UV Spectroscopic analyses of silver nanoparticles synthesized from the leaf extract of T. procumbens recorded as function of time

Scanning electron microscopy (SEM):
The scanning electron microscopy is used to analyze the dimensions of the silver nanoparticles. SEM further is used to categorize nanostructure of the nanomaterials as well. Silver nanoparticles micrographs show that they were spherical, had some aggregates, and had a high-density crystalline structure. The nanoparticles were spherical and somewhat truncated form may be the result of the chemical components in T. procumbens leaf extract capping the nanoparticles. The scale on the particles indicated a 65–100 nm size range, pointing to polydispersity (Fig. 5). Previous study infer the same and hence assuring the consistency of our research (Sharma, Yngard and Lin, 2009)

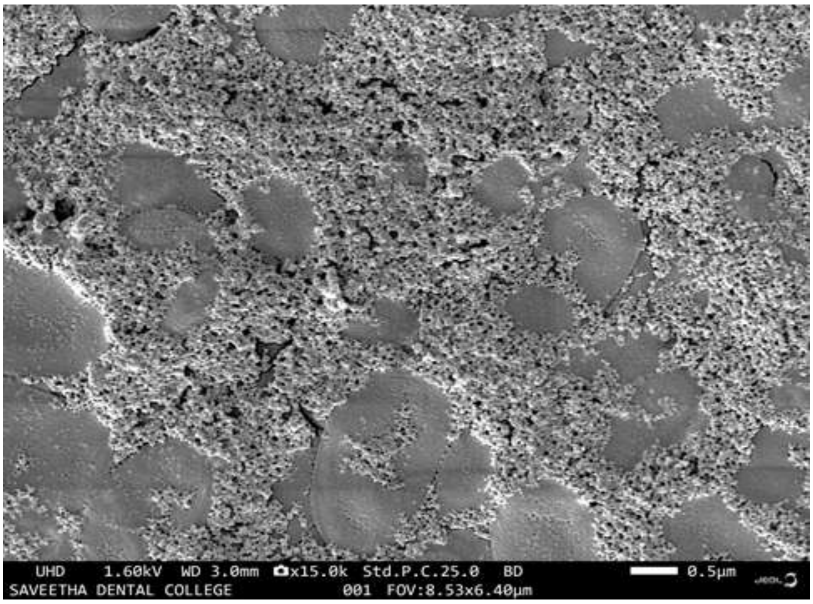

Figure 5: SEM image of of silver nanoparticles synthesized from the leaf extract of T. procumbens

Energy Dispersive X-Ray Analysis (EDAX):
EDAX analysis can be used to map out the lateral distribution of elements from the photographed region or to ascertain the elemental makeup of specific spots. In a previous analysis it was shown that the existence of certain elements was confirmed by the EDAX attachment on the SEM, which offered chemical analysis of the field of view and spot studies of tiny particles. EDAX analysis proved that there was elemental silver in the reaction mixture (Fig. 6). On sample stubs, dried silver nanoparticles were put for examination using a Philips XL-30. The optical absorption band of the silver nanoparticle peaked at 3 keV, which is characteristic of the absorption of metallic silver nanoparticles (Fernandez, Thomas and Shailaja, 2016).

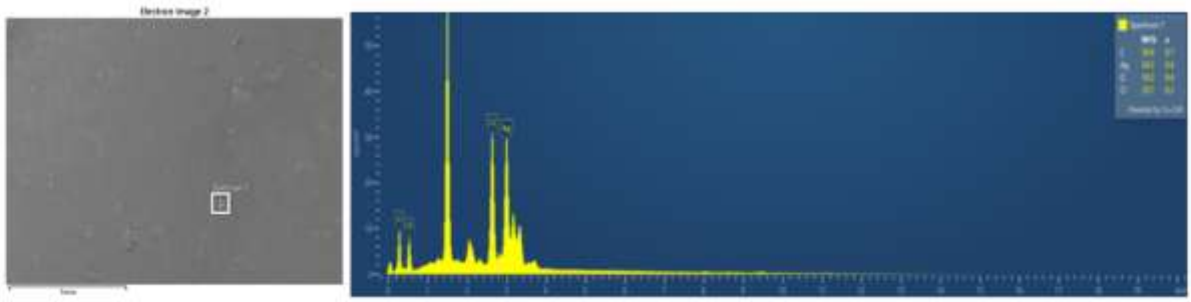

Figure 6: EDAX analysis of of silver nanoparticles synthesized from the leaf extract of T. procumbens

Fourier-transform infrared spectroscopy (FTIR)
The biomolecules in T. procumbens that are in charge of reducing silver ions were identified using Fourier transform infrared spectroscopy. The functional groups were identified as hydrogen bonded O-H stretch of phenols, alcohols and carboxylic acids, amines and amides (N-H stretch) corresponding to peak at 3329.18, alkynes (C≡C) corresponding to peak at 2100.46, amines (N-H bend) and amides (C=O stretch and N-H bend) corresponding to peak at 1632.10 (Fig. 7). An earlier investigation into silver nanoparticles synthesized from S. asperum revealed that the FTIR peaked at 3409.53, indicating that the functional groups were primarily hydrogen bonded O-H stretch of phenols, alcohols and carboxylic acids (Jeevitha and Rajeshkumar, 2019). Silver nanoparticles synthesized from fucoidan seaweed Padina tetrastromatica had weak bands at 2982, 2653, and 2334 indicating the presence of an O-H stretching carboxylic acid group (Rajeshkumar, 2017).

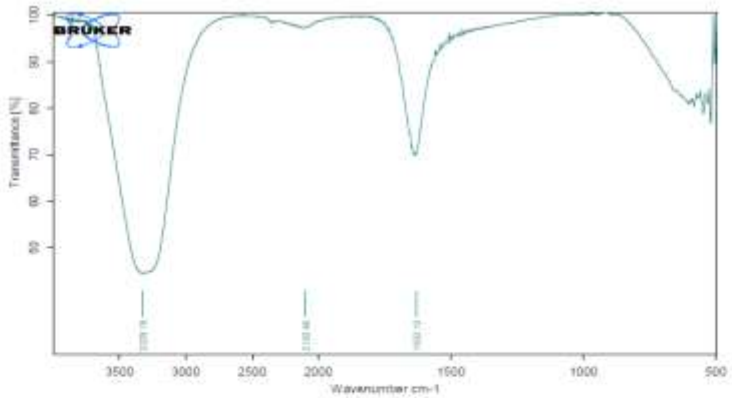

Figure 7: FTIR showing the functional groups of silver nanoparticles synthesized from the leaf extract of T. procumbens

Antimicrobial activity

The antimicrobial activity of T. procumbens silver nanoparticles were assayed by well diffusion method against the common oral pathogens, S. aureus and P. aeruginosa (Fig. 8). At the concentration of 25 µL, the zone of inhibition was observed to be 21±0.42mm (S.aureus) and 20±0.32mm (P. aeruginosa) respectively. At the concentration of 50 µl, the zone of inhibition was observed to be 23±0.24mm (S.aureus) and 20±0.24mm (P. aeruginosa) respectively. At the concentration of 100 µL, the zone of inhibition was observed to be 23±0.32mm (S.aureus) and 20±0.32mm (P. aeruginosa) respectively (Table 1). As the concentration increases the zone of inhibition was observed to increase steadily. The standard drug was observed to have a lower range of zone of inhibition compared to T. procumbens leaf extract mediated silver nanoparticles when treated with the two oral pathogens (Fig. 9). These findings therefore show that the antimicrobial activity of the silver nanoparticles prepared in this study has proved to have a potent antimicrobial activity and hence can be effective in developing new pharmacological drugs for oral diseases. Studies have demonstrated potent antimicrobial activity of T. procumbens leaves based chitosan gel against common oral pathogens (Rieshy V., 2022). The study paves way for the future scope of developing nanostructured products which are eco-friendly and easily available without much side effects.

Figure 8: Antimicrobial activity of T. procumbens leaves mediated silver nanoparticles against S. aureus and P. auruginosa by well diffusion assay.

Table 1: Antimicrobial activity of T. procumbens leaves mediated silver nanoparticles

	Zone of Inhibition (mm)			
Pathogen	Concentration			
	25µL	50µL	100µL	Ab
S. aureus	21±0.42	23±0.24	23±0.32	13±0.14
P. aeruginosa	20±0.32	20±0.24	20±0.32	10±0.24

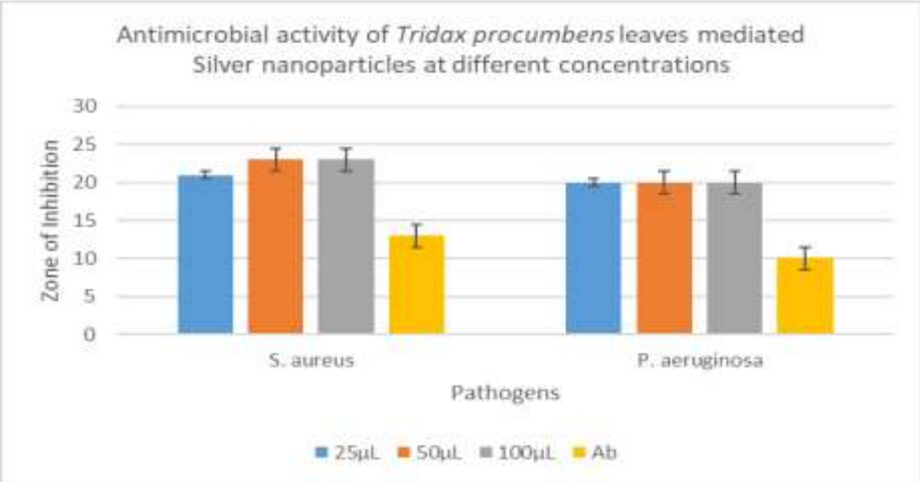

Figure 9: Bar chart depicting the comparison of antimicrobial activity of T. procumbens leaves mediated silver nanoparticles and standard drug (Ab) against S. aureus and P. aeruginosa

4. Conclusion

T. procumbens leaves mediated silver nanoparticles have a higher antimicrobial activity against oral pathogens (S.aureus, P.auriginosa) assuring their therapeutic nature and nontoxic application against various oral infectious diseases. Silver nanoparticles synthesis were initially identified by the dark green colour and then confirmed by UV-vis spectroscopy. This study enlightens the cost-effective synthesis process of nanoparticles and the better use of traditional medicine as nanoparticles providing its own added advantage.

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Conflict of Interest:
The author declares that there were no conflicts of interests in the present study.

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5. References

Abbasi, E. et al. (2016) 'Silver nanoparticles: Synthesis methods, bio-applications and properties', Critical reviews in microbiology, 42(2), pp. 173–180.

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. Available at: https://doi.org/10.14260/jemds/2020/533.

Andriana, Y. et al. (2019) 'Antihyperuricemia, Antioxidant, and Antibacterial Activities of Tridax procumbens L', Foods, 8(1), p. 21.

Begum, A., Jeevitha, M. and Preetha, S. (2020) 'Knowledge and Awareness on Vitamin D Deficiency among IT Employees', Journal of Pharmaceutical Research International, pp. 6–12. Available at:

https://doi.org/10.9734/jpri/2020/v32i1830683.

Cheung, G.Y.C., Bae, J.S. and Otto, M. (2021) 'Pathogenicity and virulence of', Virulence, 12(1), pp. 547–569.

Felicita, A.S. (2017a) 'Orthodontic management of a dilacerated central incisor and partially impacted canine with unilateral extraction - A case report', The Saudi dental journal, 29(4), pp. 185–193.

Felicita, A.S. (2017b) 'Quantification of intrusive/retraction force and moment generated during en-masse retraction of maxillary anterior teeth using mini-implants: A conceptual approach', Dental press journal of orthodontics, 22(5), pp. 47–55.

Fernandez, C., Thomas, A. and Shailaja, R.M. (2016) 'Green synthesis of silver oxide nanoparticle and its antimicrobial activity against organisms causing Dental plaques', International Journal of Pharma and Bio Sciences. Available at: https://doi.org/10.22376/ijpbs.2016.7.4.b14-19.

'Green biosynthesis of silver nanoparticles using leaves extract of Artemisia vulgaris and their potential biomedical applications' (2017) Colloids and surfaces. B, Biointerfaces, 158, pp. 408–415.

'Hepatoprotective activity of Tridax procumbens against d-galactosamine/lipopolysaccharide-induced hepatitis in rats' (2005) Journal of ethnopharmacology, 101(1-3), pp. 55–60.

Jain, R.K., Kumar, S.P. and Manjula, W.S. (2014) 'Comparison of intrusion effects on maxillary incisors among mini implant anchorage, j-hook headgear and utility arch', Journal of clinical and diagnostic research: JCDR, 8(7), pp. ZC21–4.

J, C.I. et al. (2009) 'Chemical Profile of Tridax procumbens Linn', Pakistan Journal of Nutrition, pp. 548–550. Available at: https://doi.org/10.3923/pjn.2009.548.550.

Jeevitha, M. et al. (2022) 'Clinical Evaluation of Lateral Pedicle Flap Stabilized with Cyanoacrylate Tissue Adhesive: A Randomized Controlled Clinical Trial', Contemporary clinical dentistry, 13(1), pp. 24–29.

Jeevitha, M. and Rajeshkumar, S. (2019) 'Antimicrobial activity of silver nanoparticles synthesized using marine brown seaweed Spatoglossum asperum against oral pathogens', Indian journal of public health research and development, 10(11), p. 3568.

Johnson, J. et al. (2020) 'Computational identification of MiRNA-7110 from pulmonary arterial hypertension (PAH) ESTs: a new microRNA that links diabetes and PAH', Hypertension research: official journal of the Japanese Society of Hypertension, 43(4), pp. 360–362.

Jude, I.C., Catheri, I.C. and Frank, O.C. (2010) 'Effect of Aqueous Extract of Tridax procumbens Linn on Plasma Electrolytes of Salt-Loaded Rats', Pakistan Journal of Nutrition, pp. 103–105. Available at: https://doi.org/10.3923/pjn.2010.103.105.

Keerthana, B. and Thenmozhi, M.S. (2016) 'Occurrence of foramen of huschke and its clinical significance', Research Journal of Pharmacy and Technology, 9(11), pp. 1835–1836.

Krishnan, S., Pandian, S. and Kumar S, A. (2015) 'Effect of bisphosphonates on orthodontic tooth movement-an update', Journal of clinical and diagnostic research: JCDR, 9(4), pp. ZE01–5.

Kumar, S. (2017) 'The emerging role of botulinum toxin in the treatment of orofacial disorders: Literature update', Asian journal of pharmaceutical and clinical research, 10(9), p. 21.

Mielko, K.A. et al. (2019) 'Metabolomic studies of Pseudomonas aeruginosa', World journal of microbiology & biotechnology, 35(11), p. 178.

'Nanotechnological applications in medicine' (2007) Current opinion in biotechnology, 18(1), pp. 26–30.

Prathap, L. et al. (2021) 'Molecular docking analysis of stachydrine and sakuranetin with IL-6 and TNF-α in the context of inflammation', Bioinformation, 17(2), pp. 363–368.

Rajeshkumar, S. (2017) 'Phytochemical constituents of fucoidan () and its assisted AgNPs for enhanced antibacterial activity', IET nanobiotechnology / IET, 11(3), pp. 292–299.

Rajeshkumar, S. and Jeevitha, M. (2021) 'Plant-mediated biosynthesis and characterization of zinc oxide nanoparticles', Zinc-Based Nanostructures for Environmental and Agricultural Applications, pp. 37–51. Available at: https://doi.org/10.1016/b978-0-12-822836-4.00023-9.

Ramesh Kumar, K.R. et al. (2011) 'Depth of resin penetration into enamel with 3 types of enamel conditioning methods: a confocal microscopic study', American journal of orthodontics and dentofacial orthopedics: official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics, 140(4), pp. 479–485.

Rieshy, V et al (2022) 'Preparation of Tridax procumbens leaves based chitosan gel and its antimicrobial activity', Journal of Pharmaceutical Negative Results, 13(7), pp. 2593-2599.

Santhakumar, P. et al. (2021) 'Molecular docking analysis of furfural and isoginkgetin with heme oxygenase I and PPARγ', Bioinformation, 17(2), pp. 356–362.

Sekar, D. et al. (2019) 'Methylation-dependent circulating microRNA 510 in preeclampsia patients', Hypertension research: official journal of the Japanese Society of Hypertension, 42(10), pp. 1647–1648.

Sharma, V.K., Yngard, R.A. and Lin, Y. (2009) 'Silver nanoparticles: Green synthesis and their antimicrobial activities', Advances in Colloid and Interface Science, pp. 83–96. Available at: https://doi.org/10.1016/j.cis.2008.09.002.

Sivamurthy, G. and Sundari, S. (2016) 'Stress distribution patterns at mini-implant site during retraction and intrusion—a three-dimensional finite element study', Progress in orthodontics, 17(1), pp. 1–11.

Soni J, Jeevitha M (2022). Cytotoxicity of Tridax procumbens flower mediated silver nanoparticles. Journal of Pharmaceutical Negative Results, 13(7), pp. 2600-2608.

Vastrad, J.V. and Goudar, G. (2016) 'Green Synthesis and Characterization of Silver Nanoparticles Using Leaf Extract of Tridax Procumbens', Oriental Journal of Chemistry, pp. 1525–1530. Available at: https://doi.org/10.13005/ojc/320327.