Antimicrobial, Antioxidant and Anti-Inflammatory Effect of Vitex Altissma Leaf Extract

Vinay Gautham1 ,J.shar1,a)

1Vinay Medical Diagnostics, Chennai, Tamil Nadu, India

Corresponding Author: a)[jinishar52@gmail.com](mailto:jinishar52@gmail.com)

**Abstract:** Traditional medicinal practice has been used since time immemorial for treating many disease ailments like cancer, diabetes, tuberculosis and leprosy. Indian Ayurvedic medicine used traditional herbs to treat various diseases. *Vitex altissma* is an Indian herb used as anti-inflammatory, antipyretic and anti-toxici and anti-malarial. Using in vitro techniques, the antibacterial, antioxidant, and anti-inflammatory properties of V. altissma's crude leaf extract were evaluated. The results revealed that *V.altissma* leaf extract showed significant antimicrobial effect against tested bacterial pathogens *E.coli*, *S.mutants* and *K.pneumoniae*. In the same way the *V.altissma* extract exhibited potent antioxidant effects in terms of DPPH, Nitrix oxide and reducing power assay. The anti-inflammatory effect of *V.altissma*  was significant to that of diclofenac drug. As a concluding remark *V.altissma* has immense pharmacological properties which can be taken up for further clinical trials.

**Key words:** Novel drug development, Ayurvedic, Anti-inflammatory, Good health and well being.

# Introduction

Traditional medicine and global ethnomedicine have long made use of medicinal plants. [(Aparna et al., 2021; Ganapathy 2021)](https://paperpile.com/c/xP0i1y/D8KW+h70A). Many valuable chemicals and/or medications are derived mostly from medicinal plants. Ninety percent of the more than 1300 medicinal plants utilized in European nations come from natural sources [(Chen et al., 2016)](https://paperpile.com/c/xP0i1y/t4aJc).The World Wildlife Fund and the International Union for Conservation of Nature estimate that between 50,000 and 80,000 blooming plants are employed for their therapeutic properties [(Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/xP0i1y/VpcZ). These dynamic fields, at the crossroads of phytochemistry and plant biology, deal with evolution mechanisms and systematics of medicinal plant genomes, origin and evolution of plant genotype and metabolic phenotype, interaction between medicinal plant genomes and environment, and correlation between genomic diversity and metabolite diversity, etc.[(Bauer & Brönstrup, 2014)](https://paperpile.com/c/xP0i1y/qObii).The genus Vitex comprises flowering plants that belong to the Lamiaceae family of sages. There are roughly 250 species [(Poornima et al., 2021)](https://paperpile.com/c/xP0i1y/5fPk). Common names include chaste tree, traditionally referring to V. agnus-castus, but often applied to other species, as well. Vitex altissma is a plant used in herbal medicine. Often used to treat women's health issues, it is also referred to as chaste tree or chasteberry [(Li et al., 2016)](https://paperpile.com/c/xP0i1y/FDm4N) [(Rani & Sharma, 2013)](https://paperpile.com/c/xP0i1y/qzJy1). Plant leaf and/or seed extracts are commonly seen in Vitex supplements. Although the full mechanism of action of Vitex is unknown, it may indirectly affect a number of hormones, including estrogen, prolactin, and progesterone [(Ganapathy 2021; Pandiyan et al., 2022)](https://paperpile.com/c/xP0i1y/6xmt+N6qP). Plant leaf and/or seed extracts are commonly seen in Vitex supplements. Although the full mechanism of action of Vitex is unknown, it may indirectly affect a number of hormones, including estrogen, prolactin, and progesterone [(Kamal et al., 2022)](https://paperpile.com/c/xP0i1y/ey0vn) Because of the pharmacological potentials provided by the phytochemicals found in the plant matrix, the Vitex genus has drawn interest from the health sector market [(Chokkattu et al., 2022; Poornima et al., 2021)](https://paperpile.com/c/xP0i1y/5fPk+7xJq). They can be used as complementary medicine in addition to other conventional treatments, or they can be marketed as a range of supplements to help treat and improve various ailments. Many researchers are currently interested in finding novel chemicals from the Vitex plant that have pharmacological activity. Using in vitro techniques, the antibacterial, antioxidant, and anti-inflammatory properties of Vitex altissma leaf extract are assessed in this work.

# Materials and methods

## Sample collection and extraction

*Vitex altissma* leaf samples were collected from the Kalvarayan hills (11.7043° N, 78.7358° E). Collected leaf samples were washed extensively with fresh water and then air-dried for a week. The dried leaf (1 kg) was extracted by exhaustive percolation for three days in methanol with intermittent stirring every 18 hours at room temperature. The extract was concentrated in a rotary vacuum evaporator. The concentrated extract of 2.0 grams was used for biological screening.



**Figure.1:** *Vitex altissma* leaf sample

## Antimicrobial screening

For antimicrobial screening, human pathogenic bacteria such as Klebsilla pneumoniae (ATCC 10231), Escherichia coli (ATCC 25922), and Streptococcus mutans (ATCC 25175) were employed. The agar well diffusion method was used to assess the antibacterial activity of the Vitex altissma methanol extract. On bacterial nutrient agar plates, an inoculum comprising 106 CFU/ml of each bacterial culture under test was applied. After that, 100 μl (25 mg/ml) of ascidia extract was added to wells that were punched into the agar medium and left to diffuse for two hours at room temperature. After that, the plates were incubated for 24 hours at 37° in the upright position. Standard antibiotics such as Ampicillin (10 μg/mL) for bacteria were used as positive controls, whereas wells with the same volume of methanol were utilized as negative controls. The growth inhibition zones' widths were measured in millimeters following incubation. For every extract, three duplicates were tested against every test organism. The mean±standard deviation was used to express the data [(Balouiri et al., 2016)](https://paperpile.com/c/xP0i1y/1Csm)

## Antioxidant assay

## DPPH Assay

0.25 mL of 0.5 mM DPPH in ethanol was combined with 1.0 mg of Vitex altissma leaf extract. The absorbance at 517 nm was measured after 20 minutes of standing at room temperature. The absorbance at 517 nm decreased when the sample was added in comparison to the control, and this was used to compute the DPPH radical scavenging activity (%) [(Kedare & Singh, 2011)](https://paperpile.com/c/xP0i1y/BlIWs)

## Nitric oxide scavenging activity

When sodium nitroprusside and nitric oxide react, oxygen molecules interact to produce nitric ions. The Griess reagent was used to quantify this reaction. With minor adjustments, the nitric oxide scavenging assay was carried out [(Naresh et al., 2015)](https://paperpile.com/c/xP0i1y/Va3UN). After adding 1.0 mg of Vitex altissma leaf extract to 3 ml of phosphate-buffered saline containing 10 mM sodium nitroprusside, the mixture was incubated for 150 minutes at 25 °C. After removing 0.5 ml of an aliquot of the incubated sample every 30 minutes, 0.5 ml of Griess reagent was added. At 546 nm, the color absorption was measured. Quercetin served as the reference medication.

## Ferrous ion chelating activity

The ferrous ion chelating action of Vitex altissma leaf extract was investigated using the approach of Dinis et al. (1994). 2 mM FeCl2 (0.05 ml) was mixed with 1 mg of Vitex altissma extract. After adding 0.2 ml of 5 mM ferrozine to start the reaction, the mixture was agitated briskly and allowed to stand at room temperature for ten minutes. After some time, the solution's absorbance at 562 nm was measured using spectrophotometry. A control run with just ferrozine and Fecl2 was carried out. Every analysis and test was performed three times and averaged. The following formula provided the percentage of inhibition of the development of the ferrozine-Fe2+ complex:

## % Inhibition = [(A0 – A1) / A0] x 100

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of the seahorse sample and standard.

## Reducing power assay

Extracts from Vitex altissma leaves were tested for reductive capacity using the methodology of Güder & Korkmaz (2012). Potassium ferricyanide [K3 Fe (CN) 6] (1%), phosphate buffer [2.5 ml, 0.2M (pH 6.6)], and 1 mg of Vitex altissma extract were combined with 1 ml of distilled water. For 20 minutes, the reaction mixture was incubated at 50°C. After adding 2.5 milliliters of 10% trichloroacetic acid to the reaction mixture, it was centrifuged at 1000 grams for ten minutes. A spectrophotometer was used to measure the absorbance at 700 nm when the top layer of solution (2.5 ml) was combined with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%).

## In vitro anti-inflammatory activity

The anti-inflammatory activity was assessed using the HRBC membrane stabilization technique (Saleem et al., 2011). In short, fresh chicken blood was drawn from the slaughterhouse and combined with the same amount of sterilized Alsever medium (0.42% sodium chloride in water, 0.8% sodium citrate, 0.5% citric acid, and 2% (w/v) dextrose). After centrifuging the blood for 10 minutes at 3000 rpm, the packed cells were cleaned with isosaline (0.85%, pH 7.2), and then 10% (v/v) suspension was created using isosaline(Rafi et al., 2024). The assay combination included 1 mL of phosphate buffer (0.15 M, pH 7.4), 2 mL of hyposaline (0.36%), 0.5 mL of HRBC solution, and the secondary metabolite from the plant extract of 25 mg, 50 mg, 100, and 200 mg/mL. The reference medication utilized was diclofenac. The control was 2 mL of distilled water rather than hyposaline. After 30 minutes of incubation at 37 °C, the test mixtures were centrifuged for 10 minutes at 3000 rpm. A UV-visible spectrophotometer set to 560 nm was used to quantify the amount of hemoglobin in the supernatant. The following formula was used to determine the % hemolysis:

Protection (%) 100−[(Optical density of test sample/Optical density of control)]×100

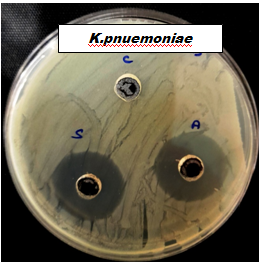
## Statistical analysis

The experiments were done in triplicate assay to obtain standard error mean ± values. One-Way ANOVA was performed to validate the p value of significance where p>0.5 was considered significant. SPSS package was used for One way ANOVA.

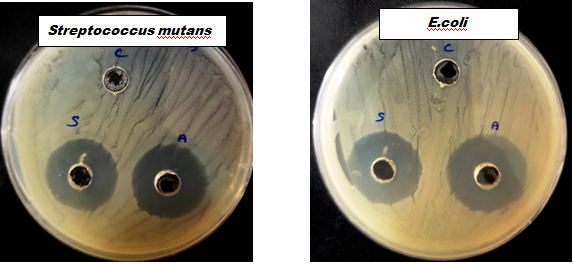
# Results

## Antimicrobial effect

*Vitex altissma* leaves extract showed a significant antimicrobial effect against all the three bacteria tested. The extract showed a 4.9 mm zone of inhibition against *Streptococcus mutans* bacteria and a 4.7 mm zone of inhibition against *E.coli* bacteria. It showed a 5.5 mm zone of inhibition against *K.pneumoniae* (Figure.2).



(a)

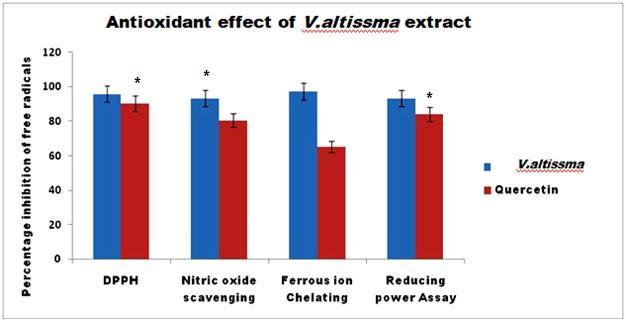


(b) (c)

**Figure.2:** Antimicrobial effect of *Vitex altissma* leaves extractagainst bacteria.

## Antioxidant effect

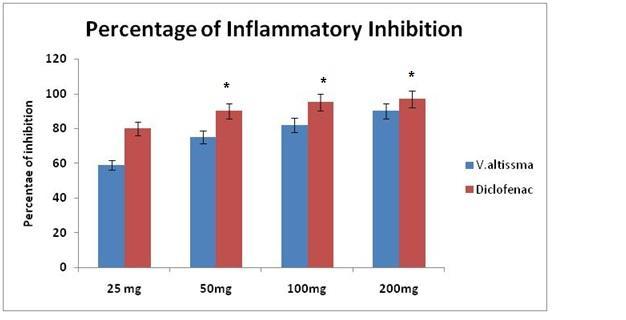
Vitex altissma leaves extract exhibited a strong in-vitro antioxidant effect in terms of scavenging free radicals and reducing agents. The extract showed a 95.77% scavenging effect against DPPH radicals alongside a 93.29% scavenging effect against nitric oxide and a 97.37% ferrous ion chelating effect. Almost 93.48% of the reduction percentage of radicals is observed in the reducing power assay (Tuluwengjiang et al., 2024). Quercetin was used as a positive control antioxidant standard. \*p>0.5 value of significance (Figure.3).



**Figure.3:** Antioxidant effect of *Vitex altissma* leaves extract.

## Anti-inflammatory effect

At a concentration of 200 mg/mL in the hypotonic solution, the leaf extract demonstrated the highest protection of the HRBC (82%, respectively). When compared to the normal diclofenac, the results demonstrated a protection of less than 91.18% (Figure.4). \*p>0.5 is a significant value.



**Figure.4:** Anti-inflammatory effect of *Vitex altissma* leaves extract

# Discussion

In the present research the *V.altissma* leaf extract was tested for antimicrobial, antioxidant and anti-inflammatory effect through in-vitro method. The results revealed that *V.altissma* leaf extract exhibited an antibacterial effect of 4.9 mm zone of inhibition against *Streptococcus mutans* bacteria and a 4.7 mm zone of inhibition against *E.coli* bacteria [(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/xP0i1y/N6qP+sb4q). It showed a 5.5 mm zone of inhibition against *K.pneumoniae.* The *V.altissma* leaf extract exhibits a significant antioxidant effect of 95.77% scavenging effect against DPPH radicals alongside a 93.29% scavenging effect against nitric oxide and a 97.37% ferrous ion chelating effect [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/xP0i1y/7xJq+mWVD). Almost 93.48% of the reduction percentage of radicals is observed in the reducing power assay [(Adel et al., 2023; *Anti-Inflammatory Potential Mouthwash Formulated Using Clove Ginger Mediated Zinc Oxide Nanoparticles: Vitro Study*, n.d.; Laghari et al., 2023)](https://paperpile.com/c/xP0i1y/IqM2+jlaT+JTFE). In the same way *V.altissma* leaf extract showed strong anti-inflammatory effect by maximum protection of the HRBC (82%, respectively) at a concentration of 200 mg/mL in hypotonic solution. The results were compared with the standard diclofenac, which showed a <91.18% protection [(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/xP0i1y/PuTH).

The Vitex genus contains a variety of secondary metabolites, including phenolic chemicals, flavonoids, lignans, terpenes, and steroids [(Chokkattu et al., 2023; Solanki et al., 2023; Yao et al., 2016)](https://paperpile.com/c/xP0i1y/KmW5+wVRE+2UYi). Pedunculariside and iridoid are two of Vitex's bioactive components that have been linked to its anti-inflammatory properties. They were shown to have a minimal inhibitory effect on COX-1 and preferential inhibition of COX-2 [(dos Santos et al., 2001; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/xP0i1y/bObt+qECY). The plant's antifungal, trypanocidal, and anti-filarial qualities were also aided by flavonoids that were extracted from Vitex. Additionally, diterpenoid and triterpenoid are some of the constituents in the Vitex genus that have been shown to have cytotoxic activity, anti-proliferative qualities, and a dopaminergic effect [(Marya et al., 2022; Nwodo et al., 2015)](https://paperpile.com/c/xP0i1y/oF59+ubbz).The majority of Vitex's commercial product is used to treat menstrual issues, such as irregular menstruation, mastodynia, and alleviated symptoms related to premenstrual syndrome, despite the numerous discoveries of Vitex metabolites and their biological effects [(Jain & Verma, 2022; Sreevarun et al., 2023; Wadhwani et al., 2022)](https://paperpile.com/c/xP0i1y/xDF3+vR8K+sjp3).

# Conclusion

The present study indicates that *V.altissma* showed profound antimicrobial, antioxidant and anti-inflammatory effects by in-vitro analysis. *Vitex altissma* leaf extract has significant antimicrobial, antioxidant and anti-inflammatory efficacy through preliminary experimental results. Thus, the goal of this study is to provide a summary of the secondary metabolites, pharmacological effects, and traditional medicinal uses of Vitex species that can be used as a guide for further study and application of the species.

# References

1. [Adel, S. M., El-Harouni, N., & Vaid, N. R. (2023). White Spot lesions: State of the art biomaterials and workflows used in prevention, progression and treatment. Seminars in Orthodontics. https://doi.org/](http://paperpile.com/b/xP0i1y/JTFE)[10.1053/j.sodo.2023.01.002](http://dx.doi.org/10.1053/j.sodo.2023.01.002)
2. [Anti-inflammatory Potential Mouthwash Formulated Using Clove Ginger Mediated Zinc Oxide Nanoparticles: Vitro Study. (n.d.).](http://paperpile.com/b/xP0i1y/IqM2)
3. [Aparna, J., Maiti, S., & Jessy, P. (2021). Polyether ether ketone - As an alternative biomaterial for Metal Richmond crown-3-dimensional finite element analysis. Journal of Conservative Dentistry: JCD, 24(6), 553–557.](http://paperpile.com/b/xP0i1y/D8KW)
4. [Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71–79.](http://paperpile.com/b/xP0i1y/1Csm)
5. [Bauer, A., & Brönstrup, M. (2014). Industrial natural product chemistry for drug discovery and development. Natural Product Reports, 31(1), 35–60.](http://paperpile.com/b/xP0i1y/qObii)
6. [Chen, S.-L., Yu, H., Luo, H.-M., Wu, Q., Li, C.-F., & Steinmetz, A. (2016). Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chinese Medicine, 11, 37.](http://paperpile.com/b/xP0i1y/t4aJc)
7. [Chokkattu, J. J., Mary, D. J., Shanmugam, R., & Neeharika, S. (2022). Embryonic toxicology evaluation of ginger- and clove-mediated titanium oxide nanoparticles-based dental varnish with zebrafish. The Journal of Contemporary Dental Practice, 23(11), 1157–1162.](http://paperpile.com/b/xP0i1y/7xJq)
8. [Chokkattu, J. J., Neeharika, S., & Rameshkrishnan, M. (2023). Applications of nanomaterials in dentistry: A review. Journal of International Society of Preventive & Community Dentistry, 13(1), 32–41.](http://paperpile.com/b/xP0i1y/2UYi)
9. [Dinis, T. C., Maderia, V. M., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics, 315(1), 161–169.](http://paperpile.com/b/xP0i1y/mavua)
10. [dos Santos, T. C., Delle Monache, F., & Leitão, S. G. (2001). Ecdysteroids from two Brazilian Vitex species. Fitoterapia, 72(3), 215–220.](http://paperpile.com/b/xP0i1y/bObt)
11. [Ganapathy, D., (2021). Health benefits of Annona muricata - A review. International Journal of Dentistry and Oral Science, 2965–2967.](http://paperpile.com/b/xP0i1y/h70A)
12. [Ganapathy, D., (2021). Awareness of hazards caused by long-term usage of polyethylene terephthalate (PET) bottles. International Journal of Dentistry and Oral Science, 2976–2980.](http://paperpile.com/b/xP0i1y/6xmt)
13. [Güder, A., & Korkmaz, H. (2012). Evaluation of in-vitro Antioxidant Properties of Hydroalcoholic Solution Extracts Urtica dioica L., Malva neglecta Wallr. and Their Mixture. Iranian Journal of Pharmaceutical Research : IJPR, 11(3), 913–923.](http://paperpile.com/b/xP0i1y/oy87s)
14. [Jain, R. K., & Verma, P. (2022). Visual assessment of extent of White Spot lesions in subjects treated with fixed orthodontic appliances: A retrospective study. World Journal of Dentistry, 13(3), 245–249.](http://paperpile.com/b/xP0i1y/xDF3)
15. [Kamal, N., Mio Asni, N. S., Rozlan, I. N. A., Mohd Azmi, M. A. H., Mazlan, N. W., Mediani, A., Baharum, S. N., Latip, J., Assaw, S., & Edrada-Ebel, R. A. (2022). Traditional Medicinal Uses, Phytochemistry, Biological Properties, and Health Applications of Vitex sp. Plants, 11(15). https://doi.org/](http://paperpile.com/b/xP0i1y/ey0vn)[10.3390/plants11151944](http://dx.doi.org/10.3390/plants11151944)
16. [Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology, 48(4), 412–422.](http://paperpile.com/b/xP0i1y/BlIWs)
17. [Laghari, I. A., Pandey, A. K., Samykano, M., Aljafari, B., Kadirgama, K., Sharma, K., & Tyagi, V. V. (2023). Thermal energy harvesting of highly conductive graphene-enhanced paraffin phase change material. Journal of Thermal Analysis and Calorimetry, 148(18), 9391–9402.](http://paperpile.com/b/xP0i1y/jlaT)
18. [Li, B., Cantino, P. D., Olmstead, R. G., Bramley, G. L. C., Xiang, C.-L., Ma, Z.-H., Tan, Y.-H., & Zhang, D.-X. (2016). A large-scale chloroplast phylogeny of the Lamiaceae sheds new light on its subfamilial classification. Scientific Reports, 6, 34343.](http://paperpile.com/b/xP0i1y/FDm4N)
19. [Marya, A., Venugopal, A., Karobari, M. I., & Rokaya, D. (2022). White Spot lesions: A serious but often ignored complication of orthodontic treatment. The Open Dentistry Journal, 16(1). https://doi.org/](http://paperpile.com/b/xP0i1y/ubbz)[10.2174/18742106-v16-e2202230](http://dx.doi.org/10.2174/18742106-v16-e2202230)
20. [Merchant, A., Ganapathy, D. M., & Maiti, S. (2022). Effectiveness of local and topical anesthesia during gingival retraction. Brazilian Dental Science, 25(1), e2591.](http://paperpile.com/b/xP0i1y/sb4q)
21. [Muthuswamy Pandian, S., Subramanian, A. K., Ravikumar, P. A., & Adel, S. M. (2022). Biomaterial testing in contemporary orthodontics: Scope, protocol and testing apparatus. Seminars in Orthodontics. https://doi.org/](http://paperpile.com/b/xP0i1y/qECY)[10.1053/j.sodo.2022.12.011](http://dx.doi.org/10.1053/j.sodo.2022.12.011)
22. [Naresh, K., Varakumar, S., Variyar, P. S., Sharma, A., & Reddy, O. V. S. (2015). Enhancing antioxidant activity, microbial and sensory quality of mango (Mangifera indica L.) juice by γ-irradiation and its in vitro radioprotective potential. Journal of Food Science and Technology, 52(7), 4054–4065.](http://paperpile.com/b/xP0i1y/Va3UN)
23. [Nwodo, N., Okoye, F., Lai, D., Debbab, A., Kaiser, M., Brun, R., & Proksch, P. (2015). Evaluation of the in vitro trypanocidal activity of methylated flavonoid constituents of Vitex simplicifolia leaves. BMC Complementary and Alternative Medicine, 15, 82.](http://paperpile.com/b/xP0i1y/oF59)
24. [Pandiyan, I., Sri, S. D., Indiran, M. A., Rathinavelu, P. K., Prabakar, J., & Rajeshkumar, S. (2022). Antioxidant, anti-inflammatory activity of Thymus vulgaris-mediated selenium nanoparticles: An in vitro study. Journal of Conservative Dentistry: JCD, 25(3), 241–245.](http://paperpile.com/b/xP0i1y/N6qP)
25. [Poornima, P., Krithikadatta, J., Ponraj, R. R., Velmurugan, N., & Kishen, A. (2021). Biofilm formation following chitosan-based varnish or chlorhexidine-fluoride varnish application in patients undergoing fixed orthodontic treatment: a double blinded randomised controlled trial. BMC Oral Health, 21(1), 465.](http://paperpile.com/b/xP0i1y/5fPk)
26. Rafi, D. M., Lakshmi, T. V., Shirley, C. P., Ravivarman, G., & Senthilkumar, G. (2024, April). Improving Prostate Cancer Diagnosis with Weakly Supervised Learning and Radiology-Confirmed Negative MRI Data. In 2024 International Conference on Inventive Computation Technologies (ICICT) (pp. 1183-1188). IEEE.
27. [Ramamurthy, S., Thiagarajan, K., Varghese, S., Kumar, R., Karthick, B. P., Varadarajan, S., & Balaji, T. M. (2022). Assessing the in vitro antioxidant and anti-inflammatory activity of Moringa oleifera crude extract. The Journal of Contemporary Dental Practice, 23(4), 437–442.](http://paperpile.com/b/xP0i1y/mWVD)
28. [Rani, A., & Sharma, A. (2013). The genus Vitex: A review. Pharmacognosy Reviews, 7(14), 188–198.](http://paperpile.com/b/xP0i1y/qzJy1)
29. [Saleem, T. K. M., Azeem, A. K., Dilip, C., Sankar, C., Prasanth, N. V., & Duraisami, R. (2011). Anti-inflammatory activity of the leaf extacts of Gendarussa vulgaris Nees. Asian Pacific Journal of Tropical Biomedicine, 1(2), 147–149.](http://paperpile.com/b/xP0i1y/PeEu6)
30. [Solanki, L. A., Dinesh, S. P. S., Jain, R. K., & Balasubramaniam, A. (2023). Effects of titanium oxide coating on the antimicrobial properties, surface characteristics, and cytotoxicity of orthodontic brackets - A systematic review and meta analysis of in-vitro studies. Journal of Oral Biology and Craniofacial Research, 13(5), 553–562.](http://paperpile.com/b/xP0i1y/wVRE)
31. [Sreevarun, M., Ajay, R., Suganya, G., Rakshagan, V., Bhanuchander, V., & Suma, K. (2023). Formulation, configuration, and physical properties of dental composite resin containing a novel 2π + 2π photodimerized crosslinker - cinnamyl methacrylate: An in vitro research. The Journal of Contemporary Dental Practice, 24(6), 364–371.](http://paperpile.com/b/xP0i1y/sjp3)
32. [Subramanian, A., & Harikrishnan, S. (2023). 3D printing in orthodontics: A narrative review. Journal of International Oral Health: JIOH, 15(1), 15.](http://paperpile.com/b/xP0i1y/PuTH)
33. Tuluwengjiang, G., Rasulova, I., Ahmed, S., Kiasari, B. A., Sârbu, I., Ciongradi, C. I., & Samaniego, S. S. C. (2024). Dendritic cell-derived exosomes (Dex): Underlying the role of exosomes derived from diverse DC subtypes in cancer pathogenesis. Pathology-Research and Practice, 254, 155097.
34. [Verma, P., & Muthuswamy Pandian, S. (2021). Bionic effects of nano hydroxyapatite dentifrice on demineralised surface of enamel post orthodontic debonding: in-vivo split mouth study. Progress in Orthodontics, 22(1), 39.](http://paperpile.com/b/xP0i1y/VpcZ)
35. [Wadhwani, V., Sivaswamy, V., & Rajaraman, V. (2022). Surface roughness and marginal adaptation of stereolithography versus digital light processing three-dimensional printed resins: An in-vitro study. Journal of Indian Prosthodontic Society, 22(4), 377–381.](http://paperpile.com/b/xP0i1y/vR8K)
36. [Yao, J.-L., Fang, S.-M., Liu, R., Oppong, M. B., Liu, E.-W., Fan, G.-W., & Zhang, H. (2016). A Review on the Terpenes from Genus Vitex. Molecules , 21(9). https://doi.org/](http://paperpile.com/b/xP0i1y/KmW5)[10.3390/molecules21091179](http://dx.doi.org/10.3390/molecules21091179)