

# **Comparative study on the efficacy of commercially available chemical and herbal mouthwash against oral microorganisms**

## **Study:Original research**

### **Abstract**

**Aim:** The present study was done to determine the activity of Naturally prepared licorice mouthwash in comparison to chlorhexidine mouthwash.

**Introduction:** Maintenance of oral hygiene is very important in preventing the growth of a sticky film of bacteria and food particles that accumulates on teeth and hence Mouthwashes are prescribed in dentistry for treatment of several oral conditions and for prevention against various microorganisms. *Licorice* is derived from "liquiritiae" which is shrub or herb and the yellow colour of it is due to the flavonoids content of the plant, which includes liquiritin, isoliquiritin and other compounds. Herbal Mouthwashes (mouth rinses) are solutions or liquids intended to reduce the microbial load in the oral cavity.

**Materials and methods:** This study was conducted in Saveetha Dental Hospital, Chennai, Tamilnadu, India. The liquorice was extracted and formulated as mouth wash. Figure 1 denotes the salivary samples were obtained from 20 volunteers (10 test group, 10 control group) and then, the participants were asked to rinse their mouth with freshly prepared licorice mouthwash (test group), chlorhexidine mouthwash (control group) and then paired saliva samples were obtained immediately after and before the mouthwash and finally the Colony forming units were recorded for pre and post usage of both the herbal and commercially available mouthwash.

**Results:** Study showed that there was a mean reduction in the colony-forming units with herbal mouthwash and Chlorhexidine mouthwashes. Herbal mouthwash showed reduction in colony forming growth after usage of herbal mouthwash.

**Keywords:** *Glycyrrhiza glabra*; Chlorhexidine; Dental caries; licorice mouthwash, innovative technique, energy green synthesis.

**Running title: Effectiveness of chemical and herbal mouthwash against oral microorganisms.**

## **INTRODUCTION**

Mouthwashes are prescribed in dentistry for treatment of several oral conditions and for prevention against various microorganisms[1]. Maintenance of oral hygiene is very important in preventing the growth of sticky film of bacteria and food particles that accumulates on teeth. Oral hygiene measures include mechanical aids like toothbrushes, floss, interdental cleansers and chemotherapeutic agents like mouthwashes, dentifrices and chewing gums. Mouthwashes (mouth rinses) are solutions or liquids intended to reduce the microbial load in the oral cavity.[2,3] *Glycyrrhiza glabra*: The genus includes nearly 20 species native to North , South America, Australia, Europe, Asia etc. The English name *licorice* is derived from "liquiritiae" which is shrub or herb and the

yellow colour of it is due to the flavonoids content of the plant, which includes liquiritin, isoliquiritin and other compounds. *Glycyrrhiza glabra* is a commonly used herb in Ayurvedic medicine. Various Studies indicate that *Glycyrrhiza glabra* Linn possesses vast properties like antimalarial, antispasmodic, antibacterial, antioxidant, anti-inflammatory and anti hyperglycemic properties. Various others properties includes antiulcer, antiviral, antihepatotoxic, antifungal and herpes simplex have also been studied.[4,5]

Chlorhexidine is regarded as the "gold standard" anti-plaque agent[6] However, it is not a "Magic Bullet" due to certain side effects like tooth staining, taste disturbance, etc[1,6,7] Most of the mouthwashes available in the market contain alcohol and other chemicals such as chlorhexidine gluconate and triclosan. These chemicals cause various side effects ranging from taste disturbance to allergic contact stomatitis. To overcome such side effects, nontoxic herbal mouthwashes using various herbs and plant extracts are introduced. Natural herbs like *Glycyrrhiza glabra*, *triphala*, *tulsi patra*, *jyeshthamadh*, *neem*, *clove oil*, *pudina*, *ajwain*, and lots of more used either alone or together are scientifically proven to be safe and effective against various oral health problems like bleeding gums, halitosis, mouth ulcers, and preventing cavity. Our team has extensive knowledge and research experience that has translate into high quality publications[8–12]

The major strength of those natural herbs is the absence of any side effects and they do not contain alcohol and sugar found in their counter products. The microorganisms prey on these ingredients, releasing by-products and causing halitosis. Thus, herbal mouth rinses promote better oral hygiene and health[7] The present study compared the effectiveness of an herbal mouthwash with different brands of commercially available mouthwashes in reducing the oral bacterial count.

## **MATERIALS AND METHODS**

**This study was conducted in Saveetha Dental Hospital, Chennai, Tamilnadu, India and 28g of freshly prepared *Glycyrrhiza glabra (athimathuram)* and was mixed with 450ml of distilled water and were boiled for 10 minutes. Then the solution was strained and the strained liquid was stored in a sterilised vessel.[13]**

### **TEST GROUP:**

**Saliva samples were obtained from 10 volunteers and then, the participants were asked to rinse their mouth with freshly prepared *licorice* mouthwash and then two saliva samples were obtained immediately after and before the herbal mouthwash.**

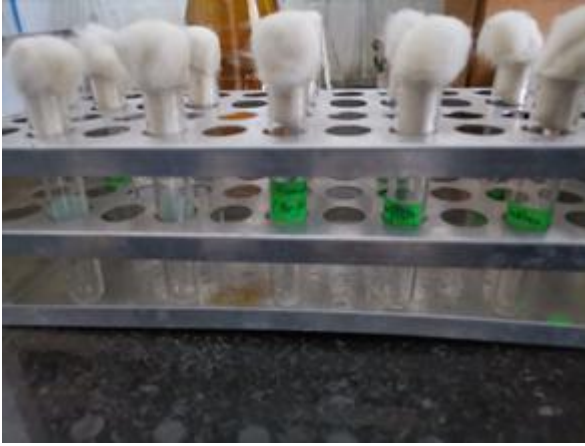
### **CONTROL GROUP:**

**Saliva samples were obtained from volunteers and then the participants were asked to rinse their mouth with chlorhexidine, the saliva samples were collected immediately after and before the mouthwash. After collecting saliva samples, the samples were sent to the microbiology laboratory immediately. Figure 1 denotes the collected samples before and after using mouthwash and saline was added in the test tubes and culture plates were used to see the reduction of oral pathogens before and after Licorice mouthwash and then Colony forming units were recorded for pre and post usage of the mouthwash with the help of colony counter app.**

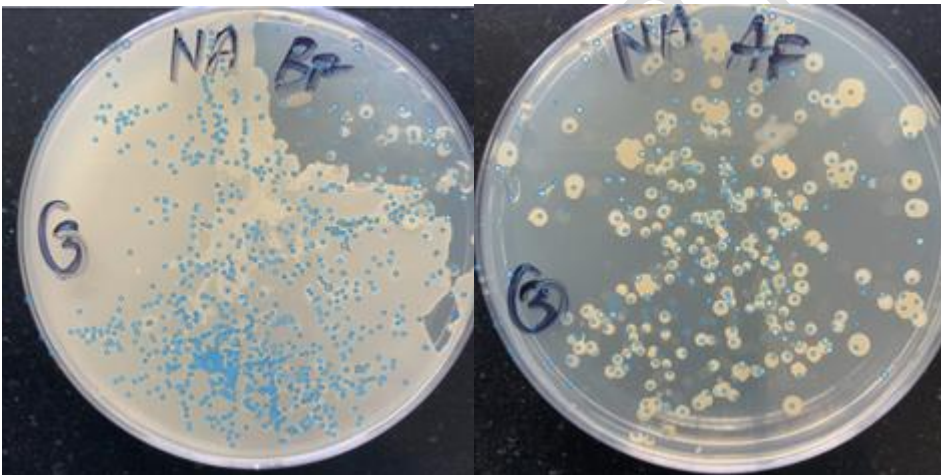


**Figure 1: Represents the procedure of inoculating collected salivary samples from sterile container to nutrient agar**

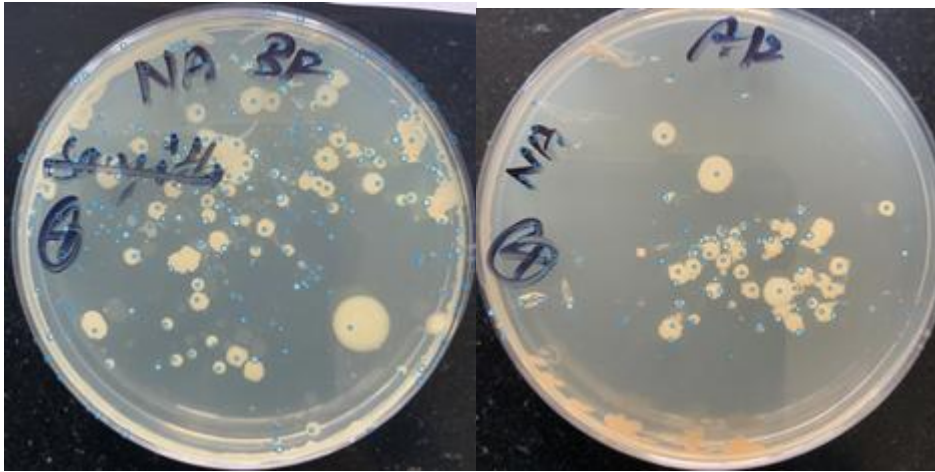
**RESULTS AND DISCUSSION:**



**FIGURE 2:Represents the collected salivary samples with saline before and after using mouthwash.**



**FIGURE 3:Depicts the Colony forming units of the given salivary samples before and after using mouthwash in Nutrient Agar for Sample 3**



**Figure 4:** Depicts the Colony forming units of the given salivary samples before and after using mouthwash in Nutrient Agar for Sample 4

PARTICIPANTS	CFU BEFORE USING MOUTHWASH	CFU AFTER USING MOUTHWASH
PARTICIPANT 1	225	101
PARTICIPANT 2	418	256

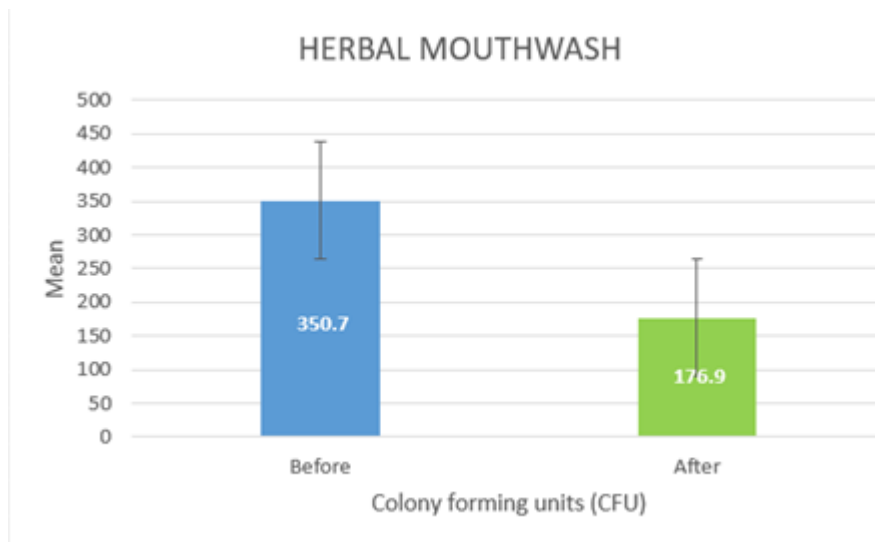
<b>PARTICIPANT 3</b>	<b>860</b>	<b>307</b>
<b>PARTICIPANT 4</b>	<b>246</b>	<b>89</b>
<b>PARTICIPANT 5</b>	<b>362</b>	<b>214</b>
<b>PARTICIPANT 6</b>	<b>240</b>	<b>150</b>
<b>PARTICIPANT 7</b>	<b>180</b>	<b>78</b>
<b>PARTICIPANT 8</b>	<b>420</b>	<b>230</b>
<b>PARTICIPANT 9</b>	<b>356</b>	<b>214</b>
<b>PARTICIPANT 10</b>	<b>200</b>	<b>130</b>
<b>MEAN VALUES</b>	<b>350.7</b>	<b>176.9</b>

**TABLE 1: Depicts the number of colony forming units before and after using herbal mouthwash.**

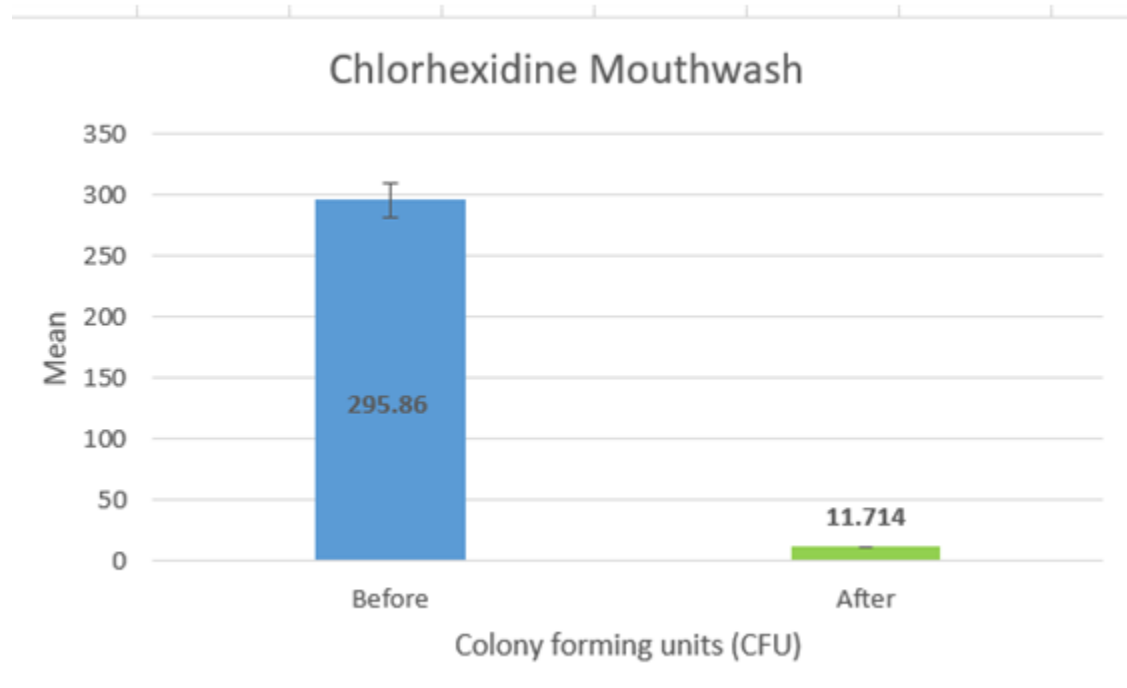
<b>SAMPLE</b>	<b>CFU BEFORE USING MOUTHWASH</b>	<b>CFU AFTER USING MOUTHWASH</b>
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<b>SAMPLE 1</b>	<b>330</b>	<b>25</b>
<b>SAMPLE 2</b>	<b>420</b>	<b>1</b>
<b>SAMPLE 3</b>	<b>190</b>	<b>5</b>
<b>SAMPLE 4</b>	<b>185</b>	<b>6</b>
<b>SAMPLE 5</b>	<b>140</b>	<b>15</b>
<b>SAMPLE 6</b>	<b>700</b>	<b>28</b>
<b>SAMPLE 7</b>	<b>106</b>	<b>2</b>
<b>MEAN VALUES</b>	<b>295.86</b>	<b>11.714</b>

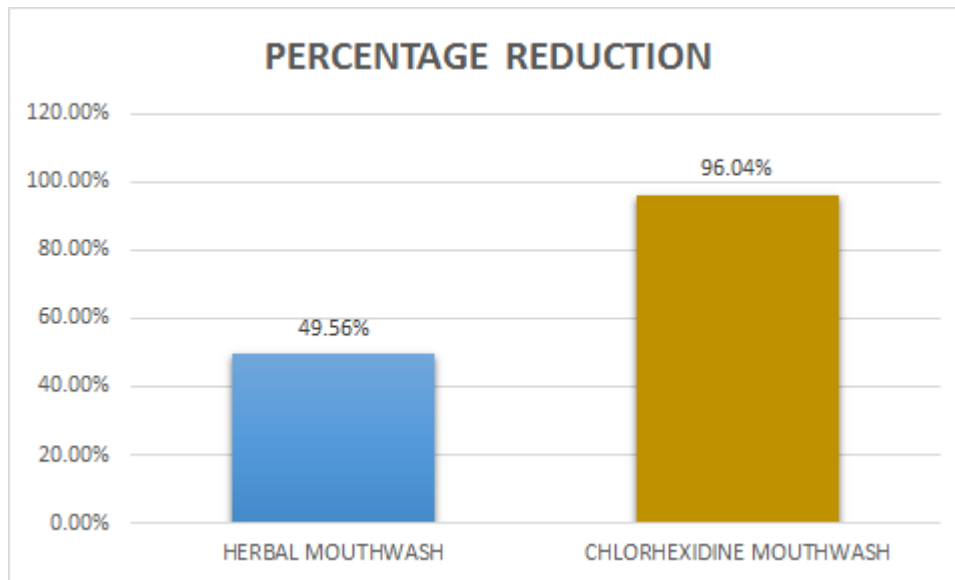
**TABLE 2:** Depicts the Mean values of CFU before and after Chlorhexidine mouthwash



**FIGURE 5 :** Error graph depicts the CFU before and after usage of Herbal mouthwash. X axis represents the number of participants involved in this study, Y axis represents the CFU. Blue colour denotes the CFU before using mouthwash, green colour depicts the CFU after usage of mouthwash. From this it is inferred that the majority of the participants showed more CFU before mouthwash when compared to CFU after herbal mouthwash.



**FIGURE 6 :**Bar graph depicts the CFU of before and after the Chlorhexidine mouthwash.X axis represents the number of samples taken in this study, Y axis represents the CFU.Blue colour denotes the CFU before using mouthwash, green color depicts the CFU after usage of mouthwash. From this it is inferred that the majority of the participants showed more CFU before mouthwash with the least in CFU after mouthwash.



**FIGURE 7:** Bar graph Depicts the mean percentage value reduction of herbal and chlorhexidine mouthwash. Blue represents the herbal mouthwash and yellow represents the chlorhexidine mouthwash. From this it is shown that chlorhexidine mouthwash showed maximum reduction compared to herbal mouthwash.

**In our present study Figure 3 represents Nutrient agar which is used in procedures commonly performed for the cultivation of microbes and supporting growth of a wide range of non-fastidious organisms and its popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth and in our present study ,the efficacy of the herbal mouthwash and Chlorhexidine mouthwash in reducing oral pathogens were assessed. In our present study Table 1 and figure 5 explains the total Colony forming unit before and after using herbal mouthwash where the mean value before using herbal mouthwash was 350.7 and the mean after using herbal mouthwash was 176.9.**

In Figure 6 the mean reduction percentage is estimated to be 49.56%. From our study Table 2 and figure 5 explains the total Colony forming unit before and after using Chlorhexidine mouthwash which was considered as a negative control in our study where the mean value before using Chlorhexidine mouthwash was 295.86 and the mean value after using Chlorhexidine mouthwash was 11.714, in figure 5 mean reduction percentage is estimated to be 96.04%. While in a study Decker et al. improved antiplaque strategies by using Chlorhexidine mouthwash in combination with plaque. Chlorhexidine (0.1%) was used as the positive control, saline was the negative control, and two CHT derivatives were used against streptococcus sanguis. Their results proved that Chlorhexidine and CHT combination was more stronger than Chlorhexidine alone because of the bioadhesive properties of CHT and the antibacterial activity of Chlorhexidine.[14]

Andre et al. demonstrated the bactericidal activity against *Streptococcus mutans* growth on dentures as a biofilm by using cepacol. On the other hand a product named pearl drops were also tested in this study which showed negative results in accordance with the standard[15] Al Bayati, Sumaiman's et al demonstrated with Listerine® mouthwash for 30 s which results in decrease of bacterial counts in saliva.. They reported that the aqueous extract of *Salvadora persica* showed strongest antibacterial activity against *Streptococcus faecalis*[16] AM Khalessi et al in 2004 demonstrated the efficacy of herbal mouth wash in controlling the plaque formation[17] Limitation of the study is small sample size and in vitro. Our team has extensive knowledge and research experience that has translate into high quality publications[18–29],[30–34]. In future similar study in large scale productions for targeted drug delivery to treat and prevent a wide array of oral microbial infections.

## CONCLUSION

The results of our study and also figure 6 shows a mean reduction in the colony-forming units with herbal mouthwash and Chlorhexidine mouthwashes and showed that both mouthwashes gave reduction in colony forming growth after its usage. Though chlorhexidine shows higher percentage reduction (98 %) the herbal mouthwash has reduced the count upto 50 percent.

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## **CONFLICTS OF INTEREST**

**There are no conflicts of interest**

## **REFERENCE**

- [1] Manipal S, Hussain S, Wadgave U, Duraiswamy P, Ravi K. The Mouthwash War - Chlorhexidine vs. Herbal Mouth Rinses: A Meta-Analysis. *J Clin Diagn Res* 2016;10:ZC81–3.
- [2] Jeddy N, Ravi S, Radhika T, Sai Lakshmi LJ. Comparison of the efficacy of herbal mouth rinse with commercially available mouth rinses: A clinical trial. *J Oral Maxillofac Pathol* 2018;22:332–4.
- [3] Akande OO, Alada ARA, Aderinokun GA, Ige AO. Efficacy of different brands of mouth rinses on oral bacterial load count in healthy adults. *African Journal of Biomedical Research* 2004;7. <https://doi.org/10.4314/ajbr.v7i3.54160>.
- [4] Jatav VS, Singh SK, Khatri P, Sharma AK. Recent pharmacological trends of *Glycyrrhiza glabra* Linn. *Unani Res* 2011;1:1–11.
- [5] Namita P, Mukesh R. Medicinal plants used as antimicrobial agents: a review. *Int Res J Pharm* 2012;3:31–40.
- [6] Prasad KARV, John S, Deepika V, Dwijendra KS, Reddy BR, Chincholi S. Anti-Plaque Efficacy of Herbal and 0.2% Chlorhexidine Gluconate Mouthwash: A Comparative Study. *J Int Oral Health* 2015;7:98–102.
- [7] Malhotra R, Grover V, Kapoor A. Comparison of the effectiveness of a commercially available herbal mouthrinse with chlorhexidine gluconate at the clinical and patient level. *Journal of Indian Society* 2011.
- [8] PradeepKumar AR, Shemesh H, Jothilatha S, Vijayabharathi R, Jayalakshmi S, Kishen A. Diagnosis of Vertical Root Fractures in Restored Endodontically Treated Teeth: A Time-dependent Retrospective Cohort Study. *J Endod* 2016;42:1175–80.
- [9] Dhinesh B, Isaac JoshuaRamesh Lalvani J, Parthasarathy M, Annamalai K. An assessment on performance, emission and combustion characteristics of single cylinder diesel engine powered by *Cymbopogon flexuosus* biofuel. *Energy Convers Manage* 2016;117:466–74.

- [10] Lekha L, Kanmani Raja K, Rajagopal G, Easwaramoorthy D. Schiff base complexes of rare earth metal ions: Synthesis, characterization and catalytic activity for the oxidation of aniline and substituted anilines. *J Organomet Chem* 2014;753:72–80.
- [11] Soh CL, Narayanan V. Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery—A systematic review. *Int J Oral Maxillofac Surg* 2013;42:974–80.
- [12] Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. *J Adv Pharm Technol Res* 2013;4:78–83.
- [13] Hassan KA, Khalil S. Liquorice mouth wash as treatment for mouth ulcer. *Int J Adv Pharm Sci* 2013;4:335.
- [14] Decker E-M, Weiger R, von Ohle C, Wiech I, Brex M. Susceptibility of planktonic versus attached *Streptococcus sanguinis* cells to chlorhexidine. *Clin Oral Investig* 2003;7:98–102.
- [15] André RFG, de Andrade IM, Silva-Lovato CH, de Freitas Oliveira Paranhos H, Pimenta FC, Ito IY. Prevalence of mutans streptococci isolated from complete dentures and their susceptibility to mouthrinses. *Brazilian Dental Journal* 2011;22:62–7. <https://doi.org/10.1590/s0103-64402011000100011>.
- [16] Al-Bayati FA, Sulaiman KD. In vitro antimicrobial activity of *Salvadora persica* L. extracts against some isolated oral pathogens in Iraq. *Turk J Biol* 2008;32:57–62.
- [17] Khalessi AM, Pack ARC, Thomson WM, Tompkins GR. An in vivo study of the plaque control efficacy of Persica™: a commercially available herbal mouthwash containing extracts of *Salvadora persica*. *Int Dent J* 2004;54:279–83.
- [18] Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen *A. baumannii* and related species. *Archives of Oral Biology* 2018;94:93–8. <https://doi.org/10.1016/j.archoralbio.2018.07.001>.
- [19] Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. *J Periodontol* 2019;90:1441–8.
- [20] Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. *Hypertens Res* 2020;43:153–4.
- [21] Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of *Acinetobacter baumannii* as an oro-dental pathogen and its drug resistance gene profile - An in silico approach. *Heliyon* 2018;4:e01051.
- [22] Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. *Cell Mol Immunol* 2020;17:668–9.
- [23] Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. *Cell Mol Immunol* 2020;17:550–1.
- [24] Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced Hyperinflammation Magnify the Severity of Coronavirus Disease (CoViD-19) Leading to Acute Respiratory Distress Syndrome? *Front Immunol* 2020;11:1206.
- [25] Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. *Hypertens Res* 2020;43:74–5.
- [26] Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. *Nat Prod Res* 2021;35:1893–8.
- [27] Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from *Porphyromonas gingivalis* with the bioactive compounds from *Rosmarinus officinalis*. *Asian Biomed* 2019;13:197–203.

- [28] Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. *Pharmaceutical-Sciences* 2020;82. <https://doi.org/10.36468/pharmaceutical-sciences.650>.
- [29] Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koengii* bio-compounds: An in-silico approach. *Acta Virol* 2020;64:93–9.
- [30] Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent* 2021;31:436–41.
- [31] Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent* 2021;31:285–6.
- [32] Barma MD, Muthupandiyam I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol* 2021;126:105132.
- [33] Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? *J Dent Sci* 2020;15:562–3.
- [34] Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. *Oral Health Prev Dent* 2020;18:379–86.