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# Antimicrobial, Anti-inflammatory activity and Cytotoxicity of mouthwash prepared from Red tea, Cinnamon and Neem Extract - An Invitro Study

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***Abstract:*** **-** The study investigates the cytotoxic, antibacterial, and anti-inflammatory qualities of mouthwash made using extracts of neem, cinnamon, and red tea. Poor oral hygiene is a common cause of dental disorders, including periodontal disease and gingivitis. Herbal medicines offer a potentially effective substitute for traditional treatments in this situation, especially when fighting germs that are resistant to drugs. The mouthwash was made by heating a concoction of neem, cinnamon, and red tea in distilled water, and then diluting it with different ingredients. Using in vitro cytotoxicity, antibacterial, and anti-inflammatory tests, the effectiveness of this herbal preparation was evaluated. When compared to conventional mouthwashes, the time-kill approach, which was used to assess the antimicrobial activity, showed higher rates of microbial mortality and indicated strong bactericidal effects, especially against Candida albicans and Enterococcus faecalis. The produced mouthwash, however, showed comparatively less efficacy against Staphylococcus aureus and Streptococcus mutans. An egg albumin denaturation experiment was used to examine the mouthwash's anti-inflammatory properties. The results indicated that it performed comparably to common anti-inflammatory medications like diclofenac sodium. Furthermore, the brine shrimp lethality assay used for cytotoxicity testing revealed minimal toxicity at lower dosages but minor toxicity at higher values. The mouthwash demonstrated strong antibacterial and anti-inflammatory characteristics while exhibiting low cytotoxicity, indicating its potential as a substitute for traditional chemical mouthwashes in preserving dental health. It is advised to do additional study and clinical trials to improve the formulation and assess long-term efficacy and safety.

***Keywords****: Red tea mouthwash, Cinnamon, Neem, Herbal mouthwash, Rooibos*

## 1. Introduction

The first step in preventing many dental problems is maintaining proper oral hygiene. When dental hygiene is neglected, germs in the mouth might grow larger than the body can handle. This causes a shift in the level of the dominant microbiota away from those linked to oral health; this kind of shift scans a location for more serious oral health issues such as periodontal diseases, dental caries, and gingivitis (1). In addition to causing redness and irritation of the gingiva (gums), gingivitis is a fairly common, non-destructive inflammatory gum disease that can potentially result in tooth loss. Plaque-induced gingivitis is the term used to describe the condition that is typically caused by bacterial plaque, a naturally occurring sticky film that forms on tooth shells (2). Keeping your teeth clean and getting regular checkups might help treat the illness. Gums that are swollen, receding, puffy, and occasionally sensitive or prone to bleeding are signs of gingivitis. Treatment options include dental floss with eucalyptol/menthol/salicylic acid/thymol, fluoride/triclosan, professional cleaning, mouth rinses, and oral hygiene practices (3).

The mainstay of treating and preventing periodontal disease is mechanical debridement, which involves scaling and root planning (SRP). Chemical plaque control techniques can also be applied as adjuvants to support

long-term results. Non-surgical methods can be useful for mild-to-moderate periodontitis, but surgical periodontal therapy is necessary in situations of progressing disease (4). In controlling periodontitis, irrigation solutions, various drug delivery strategies, long-term drug delivery mechanisms, and mouthwashes are commonly used as substitutes for non-surgical debridement. Tetracycline, azithromycin, amoxicillin, and metronidazole are the most often utilised supplementary therapy for instances of periodontitis in clinical interventions. Since multidrug-resistant (MDR) bacteria are the main cause of treatment failure, the exponential rise in MDR bacteria in response to modern antibiotics is a serious issue. Consequently, the development of antimicrobial drugs that prevent drug resistance from emerging and enhance the effectiveness of treating infectious diseases is imperative (5)(6).

Plants and their extracts have been used for medicinal purposes from the beginning of time. These substances have demonstrated promising outcomes in halting the development of antibiotic resistance in bacterial pathogens. The significant natural action, wider biological activity, significant safety, and cheaper cost of herbal pharmaceuticals make them appropriate alternatives to synthetic treatments in the prevention and treatment of periodontal diseases. Plant-derived chemicals are a broad range of chemical substances found naturally in plants (7).

These chemicals are widely distributed and have been shown to have beneficial anti-inflammatory, antibacterial, and antioxidant properties. Plant species include a wide range of antibacterial chemicals, and knowledge of ethnobotany can help identify plant extracts that can be used to create new antibacterials (8). Furthermore, the addition of herbal extracts to older antibiotics might improve their effectiveness and restore their clinical use, hence minimising the establishment of resistance (9). Aspalathus linearis (Fabaceae), a plant native to South Africa, is used to make Rooibos tea, which is available both fermented and unfermented. Owing to oxidative processes, fermented rooibos has less aspalathin and other flavonoids overall, which reduces its antioxidant activity (10). The dihydrochalcone C-glucoside aspalathin is primarily and only found in rooibos when it comes to the aqueous extract of unfermented rooibos (11). Native to the Indian subcontinent, Azadirachta indica is an evergreen tropical tree that goes by the name "neem". The main ingredients in this plant that have antibacterial properties include nimbnin, nimbidin, and azadirachtin (12).

Neem's antiviral, antibacterial, antifungal, antipyretic, antiparasitic, antacid, antidiabetic, anticancer, contraceptive, anti-inflammatory, antidermatitic, anti-inflammatory, antioxidant, dental, and other healing and protective qualities have led to the use of various parts of Neem in traditional Indian medicine (13). The amount of bacteria and dental plaque were greatly decreased by this plant extract. Two studies have also suggested that toothpaste and oral gel containing A. indica may have the same anti-plaque and anti-gingivitis effects as gels containing chlorhexidine while also reducing the amount of S. mutans in the mouth (14). In addition to being a popular culinary herb, cinnamon has long been utilised in medicine. Studies have been conducted on the effects of cinnamon on gynaecological issues, diabetes control, and pregnancy. Research has also been done on its antibacterial, anti-inflammatory, cardioprotective, and antioxidant qualities (15). Because of their antimicrobial, antifungal, and other qualities, cinnamon EO, extracts, and pure components may find application in toothpastes, mouthwashes, and root canal irrigants, among other dental products. Aim of the present study is to prepare mouthwash using red tea, Cinnamon & Neem and to evaluate its antimicrobial, anti- inflammatory and cytotoxicity.

## 2. Aim

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## 3. Methods

This study was performed in Gold Lab of Saveetha Dental College, Chennai. The study was performed during February 2023 and March 2023.

**Preparation of Mouthwash:**

1 gram of Red Tea, Neem powder and Cinnamon powder were taken and made up to a solution of 100 ml using distilled water. The solution was heated to boiling point for 10 mins, cooled down and filtered to abstain from the mouthwash. One ml solution extracts with 9 ml distilled water along with Sucrose (0.3 grams), Sodium Benzoate

(0.001 gram), and Sodium lauryl sulphate 0.01 gram was taken and mixed in a Vortex mixer for 10 Minutes. The prepared Mouthwash was collected in a collection tube. Different concentrations of mouthwash were prepared by diluting the concentrated mixture with distilled water (16).

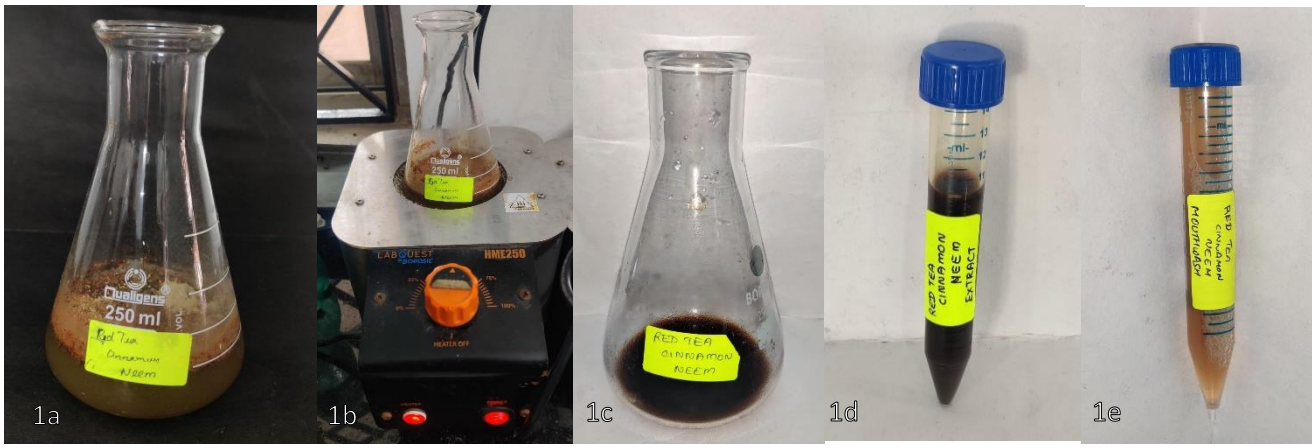



**Figure 1a. 1 gram of Red Tea + 1 gram of Neem + 1 gram of Cinnamon + 100 ml distilled water mixture**

**Figure 1b. The mixture was heated to boiling point for 10 minutes**

**Figure 1c. Solution extracts were filtered (50ml filtered solution)**

**Figure 1d. Diluted solution extract (1ml)**

**Figure 1e. Prepared mouthwash**

**Antimicrobial Activity:**

**Time-Kill Method:**

A specific bacterial strain is tested for the bactericidal activity of one or more antimicrobial substances using the time-kill method. The process involves determining the potential for survival of numerous bacterial strains at various time points. The 96-well microtiter plate would be used to conduct the experiment. The bacterial strains growth rate must be counted at various time intervals, ranging from 0 hours to 24 hours, and these time intervals are plotted as a semi-log plot or graph, in order to generate the time-kill curve (17).

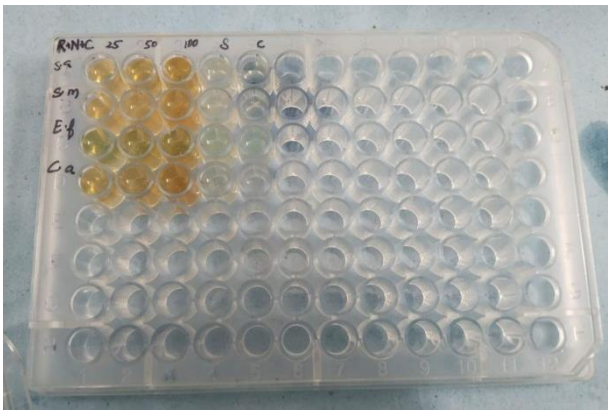


**Figure 2. Time-Kill Curve Assay**

**Anti Inflammatory Activity:**

**Egg Albumin Denaturation Assay**

A mixture of 2.8 mL of phosphate buffer and 0.2 mL of fresh egg albumin was prepared. The reaction mixture was supplemented with prepared mouthwash at various concentrations (10–50 µg/mL). A pH of 6.3 was achieved. After being left at room temperature for ten minutes, it was incubated for thirty minutes at 55°C in a water bath. The standard group was diclofenac sodium, and the control group was dimethyl sulphoxide. The samples were then subjected to spectrophotometric measurements at 660 nm (18).

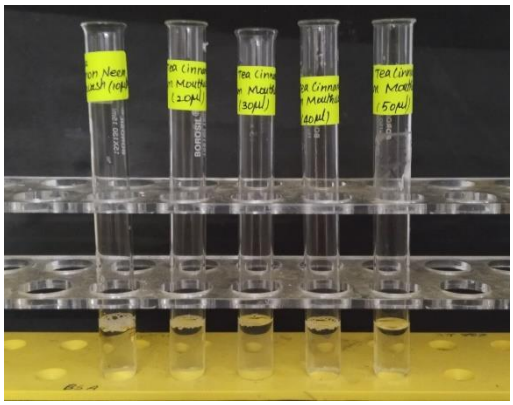


**Figure 3. Egg Albumin Denaturation Assay**

**Cytotoxicity:**

**Brine Shrimp Lethality Assay**

A weight of two grammes of iodine-free salt was dissolved in two hundred millilitres of distilled water. Ten to twelve millilitres of saline water were added to six well ELISA plates. Ten nauplii were gradually introduced to each well (5µL, 10µL, 20µL, 40µL, and 80µL). Next, the mouthwash was added based on the desired amount of concentration. For a whole day, the plates were incubated. The ELISA plates were examined and the quantity of live nauplii was recorded after 24 hours (19).

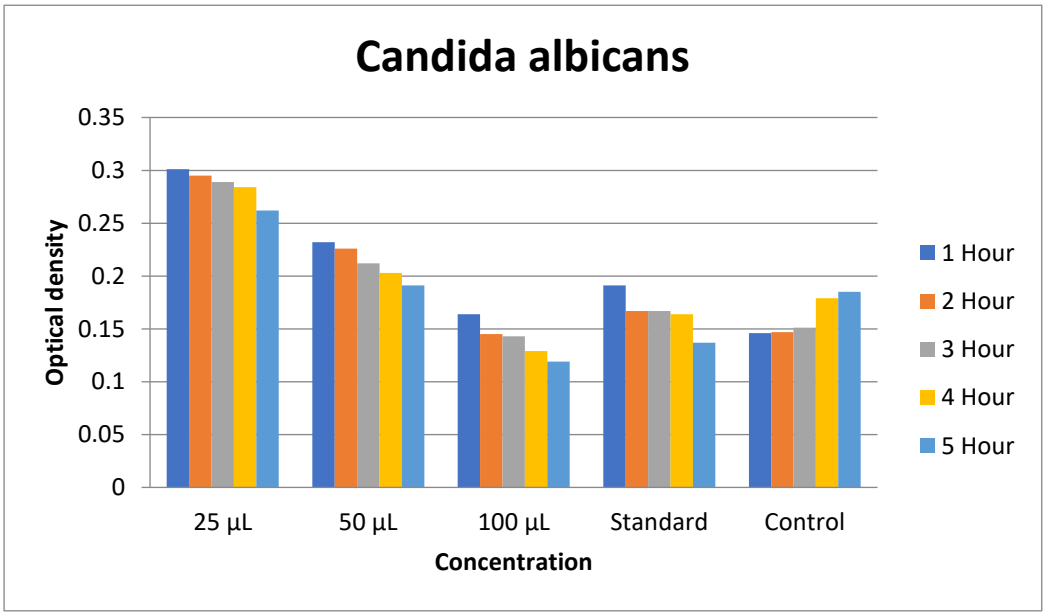

**Figure 4. Brine Shrimp Lethality Assay**

## 4. Results

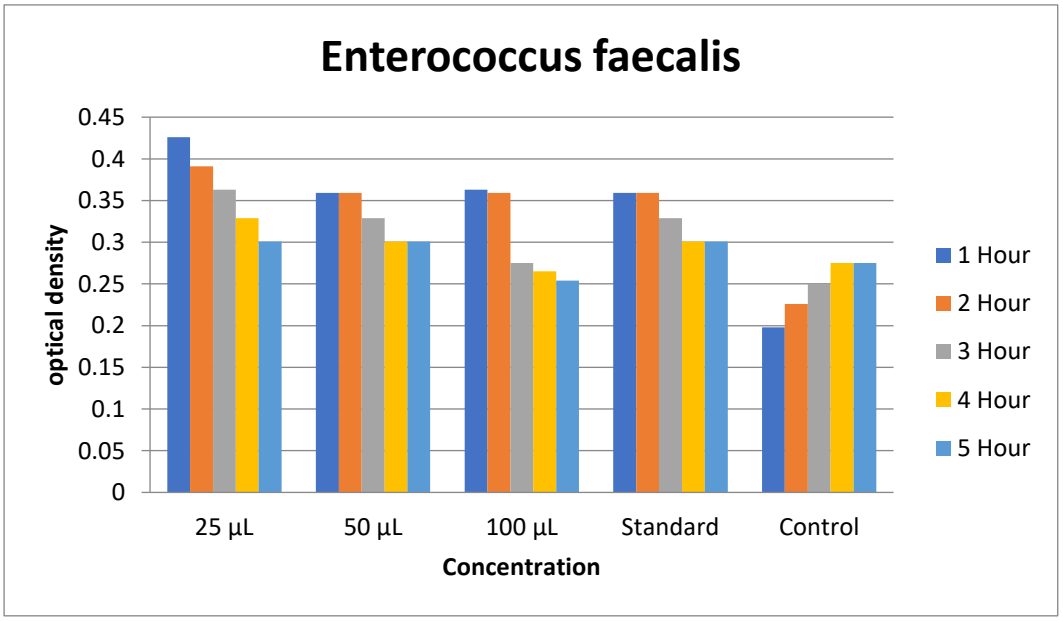
The present study's findings suggested that mouthwash prepared using red tea, Neem and Cinnamon could be a potential source of antibacterial and anti-inflammatory activity that were almost similar to all the organisms used in the study. The antimicrobial activity by time-kill method uses optical density values to calculate the bactericidal

effect of the given solution, optical density values and bacterial growth are inversely proportional, with decreasing OD value, bacterial death increases. After 5 hours, the maximum death of candida albicans (Graph 1) was evident in 100 µL concentration of mouthwash than the standard solution, the same pattern was seen in Enterococcus faecalis (Graph 2) the differences were statistically significant $p<0.05$ (Table 1). In Streptococcus mutans and Staphylococcus aureus maximum death of microbes after 5 hours was more in the standard solution than in the prepared mouthwash.

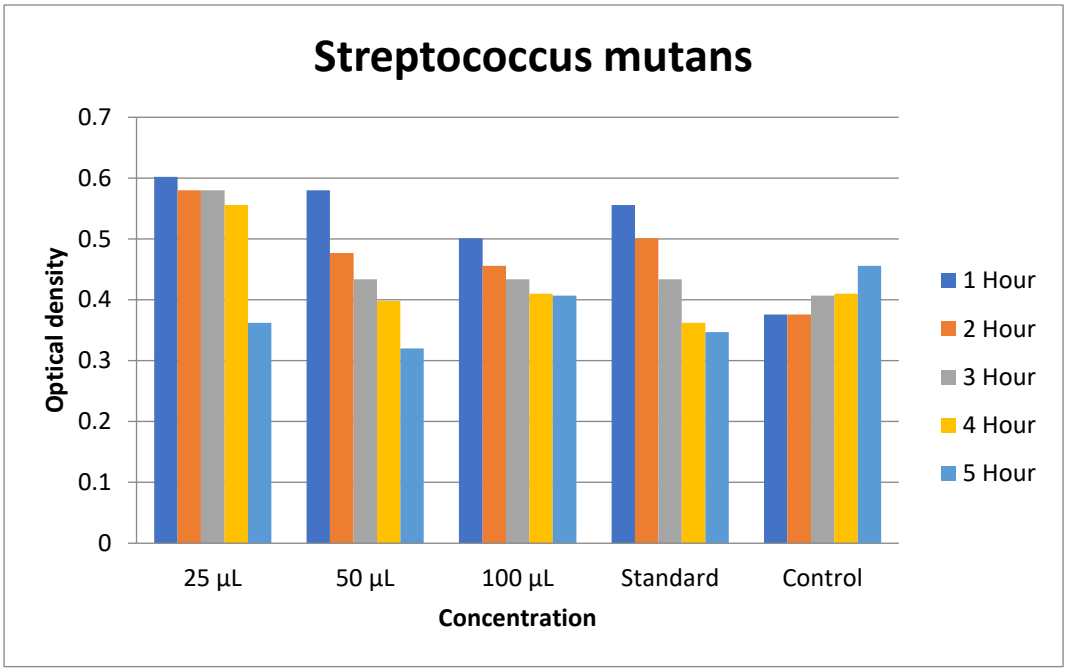
The anti-inflammatory activity when compared to Diclofenac sodium and Dimethyl sulphoxide reveals the prepared mouthwash was similar to the standard in different concentrations (10µL, 20µL, 30µL, 40µL and 50µL) (Graph 5). Cytotoxicity was tested using Brine Shrimp Lethality assay revealed that at 5% and 10 % concentration there was no mortality, 10 % mortality at 20% and 40 % concentration and 20% at 80% concentration (Graph 6).



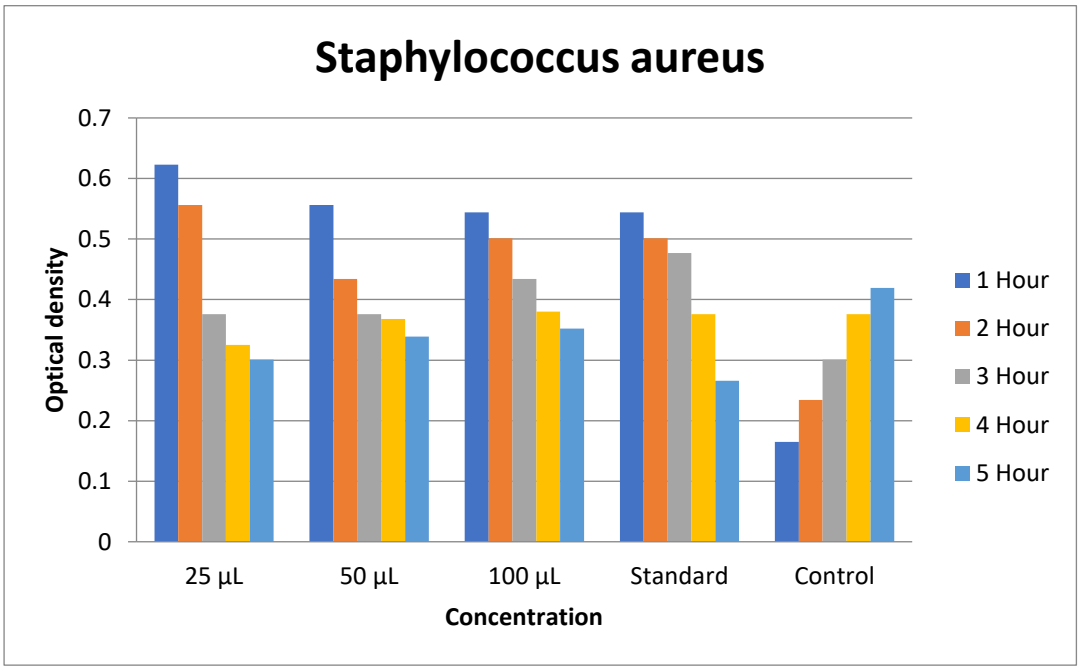

**Graph 1: Time-kill graph of Candida albicans in different concentrations during different time**




**Graph 2: Time-kill graph of Enterococcus faecalis in different concentrations during different time**




**Graph 3: Time-kill graph of Streptococcus mutans in different concentrations during different time**

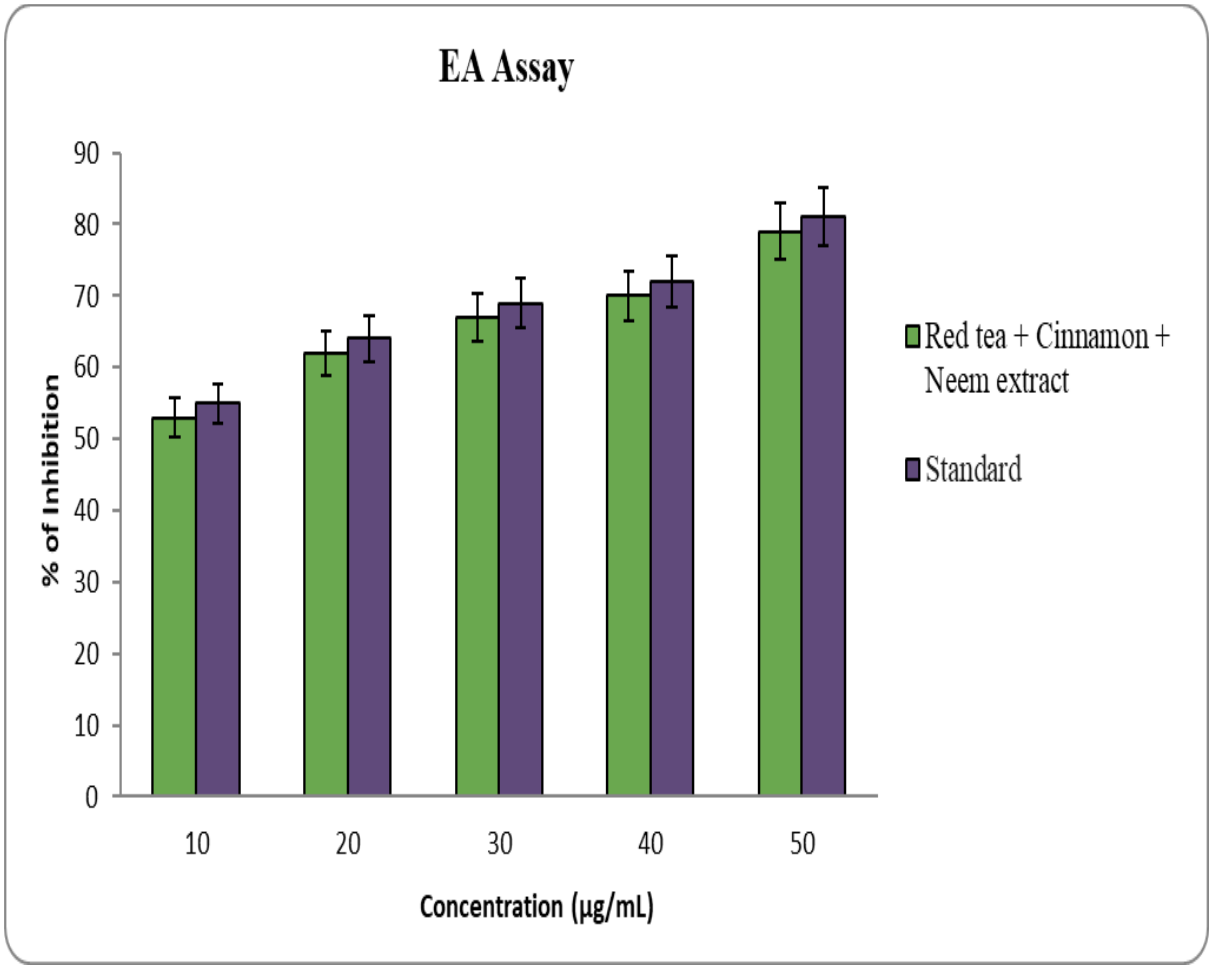



**Graph 4: Time-kill graph of Staphylococcus aureus in different concentrations during different time**

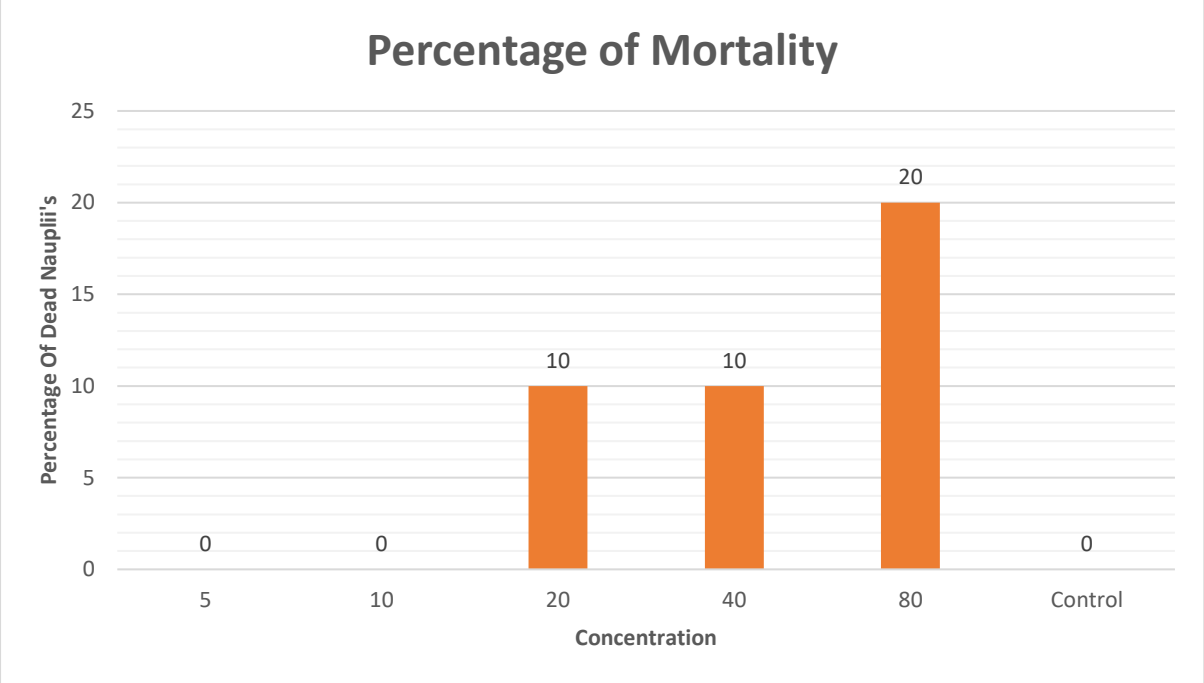
**Table 1: The table presents the results of a one-way ANOVA analysis conducted to evaluate the antibacterial activity of red tea, cinnamon, and neem mouthwash to that of conventional mouthwash at different concentrations.**

| Dependent variable | Concentration (I) | Concentration (J) | Significance (p<0.05) |
|---|---|---|---|
| Candida albicans | 25µL | 50µL | 0.512 |
| | | 100µL | 0.024* |
| | | Standard | 0.021* |

| | | | |
|---|---|---|---|
| | 50µL | 100µL | 0.031* |
| | | Standard | 0.472 |
| | 100µL | Standard | 0.001* |
| Enterococcus faecalis | 25µL | 50µL | 0.002* |
| | | 100µL | 0.000* |
| | | Standard | 0.000* |
| | 50µL | 100µL | 0.002* |
| | | Standard | 0.042* |
| | 100µL | Standard | 0.009* |
| Streptococcus mutans | 25µL | 50µL | 0.155 |
| | | 100µL | 0.254 |
| | | Standard | 0.122 |
| | 50µL | 100µL | 0.214 |
| | | Standard | 0.121 |
| | 100µL | Standard | 0.211 |
| Staphylococcus aureus | 25µL | 50µL | 0.001* |
| | | 100µL | 0.652 |
| | | Standard | 0.007* |
| | 50µL | 100µL | 0.101 |
| | | Standard | 0.621 |
| | 100µL | Standard | 0.844 |




**Graph 5: Anti-inflammatory properties of prepared mouthwash with standard in different concentrations. (p-value < 0.05)**




**Graph 6: Cytotoxicity of prepared mouthwash at different concentrations.**

## 5. Discussion

The study on the antimicrobial, anti-inflammatory, and cytotoxic properties of the mouthwash prepared from red tea, neem, and cinnamon demonstrated promising results, particularly against specific pathogens such as *Candida albicans* and *Enterococcus faecalis*. These findings are consistent with a growing body of literature emphasizing the potential of plant-based mouthwashes as effective alternatives to conventional chemical mouth rinses. In comparing these results with similar studies, it becomes evident that natural extracts like neem and cinnamon are gaining traction due to their broad-spectrum antimicrobial and anti-inflammatory effects (20).

Study by Ahmad, S et al, Formulation and Evaluation of Antibacterial Herbal Mouthwash Against Oral Disorders using neem, clove and cinnamon also exhibited efficient antimicrobial activity and efficacy of natural herbs used in mouthwash to prevent bad breath and oral hygiene problems (21). Study by Casilda Sushanthi.L et al on Assessment of Antimicrobial Activity And Cytotoxicity Of Green Tea, Stevia And Fresh Coriander Mouthwash - A Herbal Formulation, reveals in the long run, mouthwashes that include harmful compounds tend to irritate the oral mucosa. The toxic levels can be lowered by replacing these with the naturally acquired extract. As the extract's antibacterial activity was tested, positive outcomes were obtained. Herbal gargles are harmless because they have no negative effects due to their systemic availability in trace amounts (22). When paired with regular brushing and flossing, antimicrobial ingredients present in mouthwashes, including as neem, clove, and other important plant extracts, have been shown to reduce plaque and gingivitis. The main cause of foul mouth odour is volatile sulphur compounds. They originate from several sources, including microorganisms linked to oral illness, dental plaque, and food breakdown. These results showed that the herbal mouthwash has significant antibacterial, and anti-inflammatory activity and the present preparation can inhibit bacterial growth in the oral cavity (23).

**Antimicrobial Activity**: The study's findings revealed that the prepared mouthwash significantly reduced microbial growth in *Candida albicans* and *Enterococcus faecalis* at higher concentrations, outperforming the standard mouthwash. These results are in line with the work of Syahdiana et al. (2018), who explored the antimicrobial activity of cinnamon essential oil in mouthwashes and found that it exhibited potent effects against gram-positive bacteria such as *Staphylococcus aureus* and fungi like *Candida albicans*. The antimicrobial properties of cinnamon are attributed to its key bioactive compounds such as cinnamaldehyde, which disrupts the bacterial cell wall, leading to cell death. This mechanism was likely at play in the current study as well, especially in the context of *Candida albicans* (24)(25).

Similarly, neem has long been recognized for its antimicrobial properties. A study by Sharma et al. (2014) showed that neem mouthwash was as effective as chlorhexidine in reducing plaque and gingivitis (26). This aligns with the results of the current study, where neem in combination with red tea and cinnamon demonstrated significant antimicrobial effects, particularly against *Enterococcus faecalis*. The bioactive compounds in neem, such as nimbidin and azadirachtin, are known to inhibit bacterial growth by targeting cell membranes and inhibiting biofilm formation, which is crucial in oral infections. However, in contrast to the present study, which focused on the time-kill method, Sharma et al. used plaque indices and microbiological counts, indicating the need for further studies using different methods to evaluate neem's effectiveness (27).

While the prepared mouthwash was highly effective against *Candida albicans* and *Enterococcus faecalis*, it demonstrated weaker activity against *Streptococcus mutans* and *Staphylococcus aureus*, with the standard solution proving more effective in this regard. This discrepancy could be due to the difference in the cell wall structure of these bacteria. A similar pattern was observed in a study by Yanakiev S et al. (2020), where essential oils like cinnamon exhibited stronger antimicrobial effects against fungal species than gram-positive bacteria like *Staphylococcus aureus*. The authors noted that the thick peptidoglycan layer in gram-positive bacteria could limit the penetration of essential oil components, thereby reducing their efficacy (28).

**Anti-Inflammatory Activity**: The anti-inflammatory effects of the prepared mouthwash were found to be comparable to Diclofenac sodium, which is a widely used non-steroidal anti-inflammatory drug (NSAID). This finding is consistent with studies by Sandra et al. (2023), who demonstrated that herbal mouthwashes containing neem exhibited strong anti-inflammatory properties, comparable to chemical anti-inflammatory agents. The anti-inflammatory activity of neem is primarily due to its ability to inhibit cyclooxygenase and lipoxygenase pathways, which are involved in the inflammatory response. Similarly, cinnamon has been shown to suppress the production of inflammatory mediators such as prostaglandins and interleukins, making it an effective anti-inflammatory agent in oral care products (29).

Interestingly, the present study also highlighted the efficacy of red tea, or rooibos, in contributing to the anti-inflammatory effects of the mouthwash. Rooibos contains aspalathin, a unique flavonoid with potent antioxidant and anti-inflammatory properties. A study by Jagadheeswari et al. (2021) supports the notion that rooibos tea can inhibit inflammation by scavenging free radicals and reducing oxidative stress, which is a key factor in developing oral inflammatory conditions like gingivitis. While the anti-inflammatory effects of rooibos have been studied extensively in systemic conditions, the current study is among the few to explore its application in oral health, making it a novel contribution to the field (30).

**Cytotoxicity**: The cytotoxicity of the prepared mouthwash was assessed using the Brine Shrimp Lethality assay, which revealed minimal toxicity at lower concentrations, with a slight increase in mortality at higher concentrations. Neem's non-toxic nature, combined with its antimicrobial and anti-inflammatory effects, has made it a popular choice in the development of natural oral health products.

However, the slight increase in cytotoxicity at higher concentrations (80%) may be attributed to the combined effects of multiple plant extracts in the mouthwash. Similar results were found in a study by Banglao et al. (2020), where higher concentrations of cinnamon oil showed mild cytotoxic effects. The authors speculated that while cinnamon and other essential oils are generally safe at low concentrations, higher doses could disrupt cellular integrity. This is particularly relevant in oral products, where the mucosal tissues are highly permeable and can absorb higher concentrations of active compounds more rapidly (31).

**Comparison with Conventional Mouthwashes**: When compared to conventional chemical mouthwashes, such as chlorhexidine, the prepared mouthwash showed comparable, if not superior, results in some cases. Chlorhexidine is often regarded as the gold standard for oral antimicrobial therapy, but it is not without side effects, including staining of the teeth, altered taste sensation, and mucosal irritation. In contrast, herbal mouthwashes like the one prepared in this study offer a safer alternative with fewer side effects. A study by Sharma et al. (2014) comparing herbal and chemical mouthwashes found that while both were effective in reducing microbial load, herbal mouthwashes had the added benefit of being less cytotoxic and more acceptable to patients due to their pleasant taste and natural origin (26).

The findings of this study, when compared with similar research, indicate that the mouthwash prepared from red tea, neem, and cinnamon is a viable alternative to conventional chemical mouthwashes. Its potent antimicrobial and anti-inflammatory properties, combined with minimal cytotoxicity, make it suitable for regular use in maintaining oral hygiene and preventing oral infections. However, further studies are necessary to optimize the formulation for better efficacy against gram-positive bacteria like *Streptococcus mutans* and *Staphylococcus aureus*. Additionally, clinical trials are needed to evaluate the long-term safety and effectiveness of this mouthwash in real-world settings.

**Refrences**

[1] Fiorillo L. Oral Health: The First Step to Well-Being. *Medicina (Kaunas)*. 2019;55(10):676. Published 2019 Oct 7. doi:10.3390/medicina55100676

[2] Valkenburg C, Van der Weijden FA, Slot DE. Plaque control and reduction of gingivitis: The evidence for dentifrices. *Periodontol 2000*. 2019;79(1):221-232. doi:10.1111/prd.12257

[3] Chapple IL, Van der Weijden F, Doerfer C, et al. Primary prevention of periodontitis: managing gingivitis. *J Clin Periodontol*. 2015;42 Suppl 16:S71-S76. doi:10.1111/jcpe.12366

[4] Tanwar J, Hungund SA, Dodani K. Nonsurgicalperiodontal therapy: A review. J Oral Res Rev 2016;8:39-44

[5] H R, Rajeshwari, Dhamecha, Dinesh, Jagwani, Local drug delivery systems in the management of periodontitis: A scientific review. Journal of Controlled Release. 2019. 307. 10.1016/j.jconrel.2019.06.038.

[6] Hirschfeld, J., Higham, J., Chatzistavrianou, D. *et al.* Systemic disease or periodontal disease? Distinguishing causes of gingival inflammation: a guide for dental practitioners. Part 1: immune-mediated, autoinflammatory, and hereditary lesions. *Br Dent J* 227, 961–966 (2019).

[7] Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. *Nucleus (Calcutta)*. 2022;65(3):399-411. doi:10.1007/s13237-022-00405-3

[8] Alharbi WS, Almughem FA, Almehmady AM, Jarallah SJ, Alsharif WK, Alzahrani NM, Alshehri AA. Phytosomes as an emerging nanotechnology platform for the topical delivery of bioactive phytochemicals. *Pharmaceutics.* 2021;13(9):1475. doi: 10.3390/pharmaceutics13091475.

[9] Barkat MA, Das SS, Beg S, Ahmad FJ. Nanotechnology-based phytotherapeutics: current status and challenges. In: Beg S, Barkat M, Ahmad F (eds) Nanophytomedicine. Springer, Singapore 2020. pp. 1–7. 10.1007/978-981-15-4909-0_1.

[10] Erlwanger, Kennedy & Ibrahim, Kasimu. (2017). Aspalathin a unique phytochemical from the South African rooibos plant (Aspalathus linearis): A mini Review. Journal of African Association of Physiological Sciences. 5. 1-6.

[11] Gouws, L., Janse van Rensburg, H.D., Terre'Blanche, G. *et al.* Evaluation of chemical constituents of Rooibos (*Aspalathus linearis*) and Honeybush (*Cyclopia intermedia*) as adenosine $A_1/A_{2A}$ receptor ligands. *Med Chem Res* 32, 2535–2548 (2023).

[12] Wylie MR, Merrell DS. The Antimicrobial Potential of the Neem Tree *Azadirachta indica*. *Front Pharmacol*. 2022;13:891535. Published 2022 May 30. doi:10.3389/fphar.2022.891535

[13] Anand P. J., Athira S., Chandramohan S., Ranjith K., Raj V. V., Manjula V. D. (2016). Comparison of Efficacy of Herbal Disinfectants with Chlorhexidine Mouthwash on Decontamination of Toothbrushes: An Experimental Trial. *J. Int. Soc. Prev. Community Dent.* 6 (1), 22–27. 10.4103/2231-0762.175406

[14] Janakiram C, Venkitachalam R, Fontelo P, Iafolla TJ, Dye BA. Effectiveness of herbal oral care products in reducing dental plaque & gingivitis - a systematic review and meta-analysis. *BMC Complement Med Ther*. 2020;20(1):43. Published 2020 Feb 11. doi:10.1186/s12906-020-2812-1

[15] Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. *Evid Based Complement Alternat Med*. 2014;2014:642942. doi:10.1155/2014/642942

[16] Gandhi, Hinal & Dodiya, Tanvi & Patel, Vishal. (2020). A SYSTEMATIC REVIEW: HERBAL MOUTHWASH FOR EFFECTIVE ORAL CARE. 7. 126-131.

[17] Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. *J Pharm Anal*. 2016;6(2):71-79. doi:10.1016/j.jpha.2015.11.005

[18] Tharindu Madhuranga, Hewa Dikkumburage & Samarakoon, Nirmani. (2023). In vitro Anti-Inflammatory Egg Albumin Denaturation Assay: An Enhanced Approach. Journal of Natural & Ayurvedic Medicine. 7. 000411.

[19] Banti CN, Hadjikakou SK. Evaluation of Toxicity with Brine Shrimp Assay. *Bio Protoc*. 2021;11(2):e3895. Published 2021 Jan 20. doi:10.21769/BioProtoc.3895

[20] Duane B, Yap T, Neelakantan P, et al. Mouthwashes: Alternatives and Future Directions. *Int Dent J*. 2023;73 Suppl 2(Suppl 2):S89-S97. doi:10.1016/j.identj.2023.08.011

[21] Ahmad, Shafi & Sinha, Saloni & Ojha, Smriti & Chadha, Hina & Aggarwal, Babita & Ajeet, Ajeet & Jain, SeemaMahor & Bhati, Meenu. (2018). Formulation and Evaluation of Antibacterial Herbal Mouthwash Against Oral Disorders. Indo Global Journal of Pharmaceutical Sciences. 08. 37-40. 10.35652/IGJPS.2018.3740.

[22] Sushanthi.L, Casilda. (2021). Assessment Of Antimicrobial Activity And Cytotoxicity Of Green Tea, Stevia And Fresh Coriander Mouthwash - A Herbal Formulation. International Journal of Dentistry and Oral Science. 3816-3819. 10.19070/2377-8075-21000782.

[23] Brookes Z, McGrath C, McCullough M. Antimicrobial Mouthwashes: An Overview of Mechanisms-What Do We Still Need to Know?. *Int Dent J*. 2023;73 Suppl 2(Suppl 2):S64-S68. doi:10.1016/j.identj.2023.08.009

[24] Syahdiana Waty et al 2018 IOP Conf. Ser.: Earth Environ. Sci. 130 012049

[25] de Araújo MRC, Maciel PP, Castellano LRC, et al. Efficacy of essential oil of cinnamon for the treatment of oral candidiasis: A randomized trial. *Spec Care Dentist*. 2021;41(3):349-357. doi:10.1111/scd.12570

[26] Sharma R, Hebbal M, Ankola AV, Murugaboopathy V, Shetty SJ. Effect of two herbal mouthwashes on gingival health of school children. *J Tradit Complement Med*. 2014;4(4):272-278. doi:10.4103/2225-4110.131373

[27] Jalaluddin, Mohammad & Rajasekaran, Ub & Paul, Sam & Dhanya, Rs & C B, Sudeep & Vj, Adarsh. (2017). Comparative Evaluation of Neem Mouthwash on Plaque and Gingivitis: A Double-blind Crossover Study Comparative Evaluation of Neem Mouthwash on Plaque and Gingivitis: A Double-blind Crossover Study 1. Journal of Contemporary Dental Practice. 18. 567-571. 10.5005/jp-journals-10024-2085.

[28] Yanakiev S. Effects of Cinnamon (*Cinnamomum* spp.) in Dentistry: A Review. *Molecules*. 2020;25(18):4184. Published 2020 Sep 12. doi:10.3390/molecules25184184

[29] Sagar, Sandra & Ramani, Pratibha & Ramasubramanian, Abilasha & Shanmugam, Rajeshkumar. Antioxidant and anti-inflammatory effects of herbal formulation (ficus benghalenis, azadirachta indica and mentha piperita) based mouthwash. International Journal of Medical Dentistry, 2023; 27(3) 458:462.

[30] Ramamoorthy, Jagadheeswari & Rajasekar, Arvina & Shanmugam, Rajeshkumar. (2021). Preparation of Mouthwash Using Red Tea and Its Antimicrobial and Antioxidant Activity. Journal of Pharmaceutical Research International. 287-296. 10.9734/jpri/2021/v33i61A35588.

[31] Banglao, Warunya. (2020). DETERMINATION OF ANTIOXIDANT, ANTI-AGING AND CYTOTOXICITY ACTIVITY OF THE ESSENTIAL OILS FROM CINNAMOMUM ZEYLANICUM. Journal of Microbiology, Biotechnology and Food Sciences. 10. 436-440. 10.15414/jmbfs.2020.10.3.436-440.