



## Comparative Cytotoxic Effect of Commercial Mouthwash and Clove and Cinnamon Ethanolic Extract-Based Mouthwash

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### **ABSTRACT:**

#### **Introduction:**

Mouthwashes also referred to as oral rinses, are commonly employed to diminish oral microbial populations and combat halitosis. They can act by directly targeting volatile sulfur compounds, thus reducing their presence in the oral cavity, or by impeding microbial growth and enzymatic processes. Notably, the potential cytotoxicity of these oral rinses on oral cells, due to their putative antibacterial activity, is an area of concern.

#### **Aim:**

This study aims to compare the cytotoxic effects of a commercially available mouthwash with a mouthwash formulated from ethanolic extracts of clove and cinnamon.

#### **Materials and Methods:**

Clove and cinnamon powder (5 g) were mixed with 100 ml of water, heated, and subsequently cooled before filtration to create the ethanolic extract. The mouthwash was prepared by mixing 1 ml of the concentrated extract with 0.3 grams of sucrose, 0.001 grams of a preservative, and 0.01



grams of sodium lauryl sulfate (SLS), followed by dilution with 10 ml of distilled water. Cytotoxicity was assessed using the brine shrimp lethality assay.

### Results:

The findings reveal that as the concentration of the mouthwash increases, the cytotoxic effects of the cinnamon and clove ethanolic extract-based mouthwash become more pronounced when compared to the commercial mouthwash.

### Conclusion:

In conclusion, this study demonstrated the cytotoxic potential of clove and cinnamon ethanolic extracts in mouthwash formulations, especially at higher concentrations, alongside their well-established antimicrobial properties when compared to commercial mouthwash.

**KEYWORDS:** Cytotoxicity, Mouthwash, Clove, Cinnamon, Good health and Wellbeing.

### INTRODUCTION:

Biofilm formation on tooth surfaces is predominantly influenced by factors such as wettability, surface tension, surface roughness, and surface free energy, all of which impact the adhesion of salivary proteins (Goldberg, 2016). The initial attachment of bacterial biofilms to tooth surfaces is well-established to occur through hydrophobic and electrostatic interactions (Marsh, 1993; Merchant et al., 2022). The sustained interplay of these factors eventually leads to the formation of carious lesions and subsequent tooth structure loss.

The prevention and control of dental biofilm formation are primarily achieved through the use of toothpaste formulations that exhibit antibacterial and anti-inflammatory properties, complemented by adjunctive measures such as mouthwash and floss (Adel et al., 2023; Elgamily et al., 2018). Among mouthwash options, chlorhexidine (CHX) digluconate has long been the preferred antibacterial agent and is considered the gold standard (Buakaew et al., 2021; *Cytotoxic and Antimicrobial Effects of Herbal Formulation (Ficus Benghalensis, Azadirachta Indica and Menthapiperita) Based Mouthwash*, n.d.). However, patient compliance with prolonged CHX use, especially for extended therapy, can be hindered by its adverse effects, necessitating judicious use primarily in high-risk patients and for shorter durations. In recent years, there has been a growing interest in the utilization of naturally occurring products in dental care, including mouthwash formulations (Buakaew et al., 2021; Sreevarun et al., 2023; Wadhvani et al., 2022).

Cinnamon, a well-known culinary herb with historical medicinal applications, has been the subject of various studies. It has been investigated for its effects during pregnancy, in managing diabetes, and for addressing gynecological concerns (Hili et al., 1997; Poornima et al., 2021). Research has also explored its anti-inflammatory, cardioprotective, antioxidative, and antibacterial properties. These characteristics make cinnamon extracts a promising candidate for incorporation into mouthwashes, toothpaste formulations, or even root canal irrigants, owing to their antimicrobial attributes, particularly in the field of dentistry (Budri et al., 2015; Jain & Verma, 2022; Marya et al., 2022).



Clove, renowned for its impeccable safety record, has a historical tradition of use as a breath freshener, dating back to the third century B.C. Chinese emperors and ancient Hindu writings have mentioned its dental applications (Chokkattu et al., 2022; Kammon, 2019; Pandiyan et al., 2022). Avicenna, a prominent historical figure in medicine and teacher of Hippocrates, advocated the use of clove oil capsules for treating decayed teeth and gum issues. The germ-killing properties of clove have been harnessed for root canal therapy and various dental procedures since the 19th century (Chaieb et al., 2007; Chokkattu et al., 2022; Ramamurthy et al., 2022). Clove essential oil contains eugenol, a compound known for its analgesic and antiseptic qualities, inhibiting the growth of most pathogenic bacteria while safeguarding beneficial microflora. Consequently, this study aims to assess and compare the cytotoxic effects of commercial mouthwash against a mouthwash formulation containing ethanolic extracts of cinnamon and clove.

## **MATERIALS AND METHODS:**

### **Preparation of Cinnamon and Clove Extract:**

Five grams of powdered cinnamon and clove were combined with 100 milliliters of water and brought to a boil. After reaching a boiling point, the mixture was allowed to cool to room temperature. Subsequently, the solution was carefully filtered to obtain the cinnamon and clove extract. This process is visually represented in Figure 1.

### **Preparation of Mouthwash:**

To formulate the mouthwash, several components were added. Specifically, 0.3 grams of sucrose served as the sweetening agent, 0.001 grams of a preservative was incorporated for stability, and 0.01 grams of sodium lauryl sulfate (SLS) was included as a foaming agent. In addition, 1 milliliter of the concentrated cinnamon and clove extract was mixed with 10 milliliters of distilled water to create the final mouthwash solution.

### **Brine Shrimp Lethality Assay:**

Commercially acquired brine shrimp eggs were utilized for this assay. The eggs were incubated in a small water tank filled with brine or seawater for a period of 48 hours to facilitate hatching. After the initial 24 hours, the resulting larvae, known as nauplii, were employed for the experimental procedures.

Six-well ELISA plates were prepared by adding 10–12 milliliters of saltwater solution to each well. To conduct the assay, varying volumes of the mouthwash solution (5  $\mu$ L, 10  $\mu$ L, 20  $\mu$ L, 40  $\mu$ L, and 80  $\mu$ L for the control group) were added to separate wells, with each well receiving 10 nauplii. The plates were then incubated for a duration of 24 hours under controlled conditions. This entire procedure was repeated three times to ensure the robustness of the data, and values were obtained in triplicate. The number of deceased nauplii was determined using the formula: (number of dead nauplii / number of live nauplii) x 100, after the ELISA plates had been incubated for the specified 24-hour period. This has been visually represented in Figure 2.

## **RESULTS:**

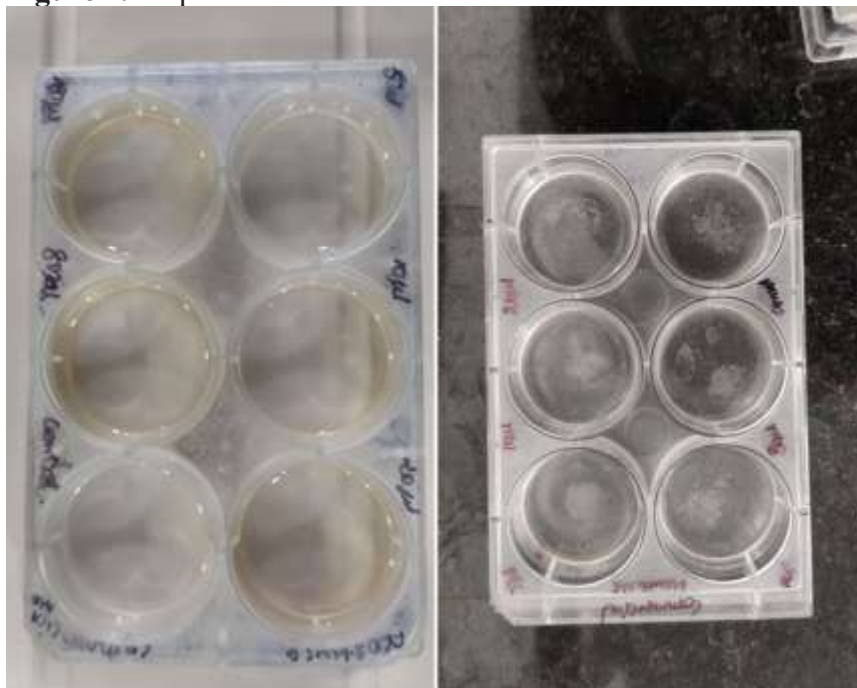


The outcomes of the investigation revealed a noteworthy relationship between mouthwash concentration and cytotoxic effects. As the concentration of the mouthwash increased, the cytotoxic impact of the cinnamon and clove ethanolic extract-based mouthwash became more pronounced when compared to the commercial mouthwash. This observed trend underscores the importance of mouthwash concentration in influencing its cytotoxic potential.

These findings emphasize the diverse cytotoxic profiles that oral rinses can exhibit. This variability in cytotoxicity underscores the need for a comprehensive understanding of the composition and concentration of mouthwash formulations, particularly in their potential effects on oral cell health.

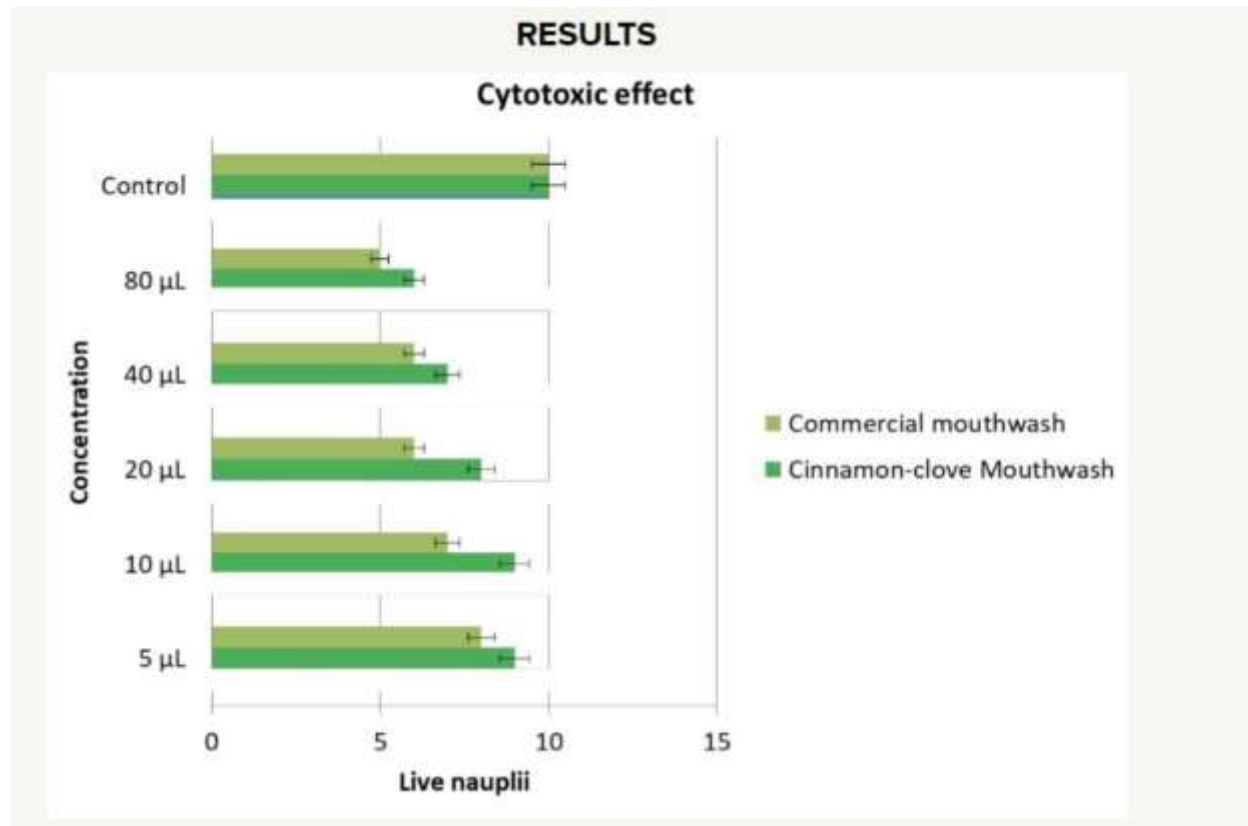


**Figure 1.** Preparation of cinnamon and clove extract and mouthwash.





**Figure 2:** Isolation of brine shrimp eggs and incorporation of cinnamon and clove mouthwash and commercial mouthwash.



**Figure 3:** Bar graph shows the cytotoxic activity of cinnamon-clove mouthwash and commercial mouthwash control against brine shrimp at various concentrations. The X axis represents the various concentrations of commercial and cinnamon-clove extract in units of µl and the Y axis represents the number of brine shrimps.

## DISCUSSION:

This study was undertaken to evaluate the cytotoxic activity of a mouthwash formulated with cinnamon-clove ethanolic extracts in comparison to a commercially available mouthwash. Our results revealed that the highest mortality rate was observed in the concentration range between 10µL and 20µL, as compared to the commercial mouthwash. These findings suggest that the cinnamon-clove ethanolic extract-based mouthwash may possess heightened cytotoxic properties, particularly within this concentration range.

It is well-recognized that the development of dental plaque at the tooth/gingiva interface plays a pivotal role in the onset of gingival irritation and caries. Effective plaque removal is essential in preventing these oral health issues, and regular and proper tooth brushing stands as the cornerstone of this preventive approach. Toothpaste, when used for brushing, not only helps prevent decay but



also aids in stain removal and the elimination of plaque. The quest to counteract tooth caries and periodontal disease has prompted the exploration of a diverse array of mechanical and chemical plaque management techniques. (*Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study*, n.d.; Ganapathy & Professor and Head of Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Chennai, 2021; Laghari et al., 2023) These include flossing, the use of mouthwash, and the importance of correct and frequent tooth brushing. While chemical mouthwashes are readily available on the market, they are not without their drawbacks, including the potential for toxicity, teeth discoloration, and the risk of rapid hypersensitivity reactions. (Chokkattu et al., 2023; Muthuswamy Pandian et al., 2022) As an alternative approach, medicinal plants offer the potential for creating alternative oral care remedies.

Our study results are in alignment with previous research, which has highlighted the cytotoxic effects of compounds on cells. It is crucial to acknowledge that the presence of cytotoxic compounds in cells can give rise to a range of cell fates, including necrosis, which occurs when cells lose their membrane integrity and subsequently undergo cell death (Fackler & Grosse, 2008; Verma & Muthuswamy Pandian, 2021). It's important to note that our study primarily focused on cytotoxic activity, which should be recognized as a limitation. Future research should aim to explore a broader spectrum of parameters to provide a more comprehensive understanding of the potential benefits and limitations associated with the use of cinnamon and clove ethanolic extract-based mouthwashes.

In concordance with our findings, previous studies have suggested substantial cytotoxic effects of cinnamon extract on cells, in addition to its antioxidant properties. Antimicrobial mouthwash is a critical element in caries prevention, particularly for individuals at high risk, in line with caries management guidelines (Seelan et al., 2015; Subramanian & Harikrishnan, 2023; Verma & Muthuswamy Pandian, 2021). Natural products are increasingly gaining attention as oral care therapies alongside well-established mouthwashes such as chlorhexidine (CHX). The antibacterial activities of glycosides derived from plant products on microorganisms served as the foundational premise for this investigation (Aparna et al., 2021; Bugaj et al., 2013; Solanki et al., 2023) Analyzing the cytotoxicity of solutions that come into contact with the oral mucosa, complementing the results on antimicrobial effectiveness, is of paramount importance (Dhanvanth & Maheswari, 2022; Fackler & Grosse, 2008; Nivedha et al., 2021).

It is essential to acknowledge that this study focused exclusively on the antimicrobial and cytotoxic activity of the mouthwash. Further research is warranted to explore the diverse characteristics of cinnamon and clove ethanolic extract-based mouthwashes. Potential sources of error, such as those associated with counting live nauplii, should be addressed. Additionally, more in vitro and in vivo research is needed to gain a deeper understanding of the potential applications of this extract in the treatment of various illnesses, particularly as a traditional medicine that may mitigate systemic toxicity and other adverse effects. These avenues of investigation will contribute to a more comprehensive assessment of the extract's therapeutic potential.

## CONCLUSION:



The cytotoxic activity of cinnamon and clove mouthwash exhibits a notable increase as the concentration is elevated, outperforming the commercial mouthwash in this regard. This observation underscores the potential of cinnamon and clove extracts as valuable components in the formulation of oral care products with enhanced cytotoxic properties. The scope was primarily focused on cytotoxicity activity, thus leaving avenues for further exploration in terms of other potential attributes and applications of this extract-based mouthwash.

In summary, the study's findings pave the way for further research and development in the field of oral care, presenting an opportunity to harness the potential of natural extracts, such as cinnamon and clove, in the formulation of mouthwashes that offer a new dimension of cytotoxicity. Through ongoing investigations and clinical trials, we aim to advance our knowledge and expand the applications of these extracts in the realm of oral health, promising safer and more effective solutions for the benefit of patients and practitioners alike.

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