Preparation of Mouthwash Using Clove and Cinnamon Ethanolic Extract and its Anti-Cariogenic Activity Against Oral Bacteria

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**Abstract: Aim:** To study the anti-cariogenic activity of a prepared clove and cinnamon ethanolic extract formulated by herbal mouthwash against oral bacteria. **Materials and methods:** The well diffusion method was used to evaluate the antibacterial activity of prepared mouthwash against oral bacteria. After incubation in an appropriate culture medium, the diameter of the zone of inhibition was measured to assess the anti-cariogenic efficacy of prepared clove and cinnamon ethanolic extract mouthwash. **Results and discussion:** The study proved that when the concentration of the extract in mouthwash increased the zone of inhibition, that is, the anti-cariogenic effect of the cinnamon and clove ethanolic extract mouthwash increased. **Conclusion:** Clove and cinnamon ethanolic extract mouthwash were successful as an anti-cariogenic agent. It significantly reduced the total bacteria (S.mutans, S.aureus, C.albicans, E.faecalis) in the culture plate.

**Keywords:** Herbal, Rinses, Anti-microbial, Culture plates.

# INTRODUCTION

Dental hygiene is essential for maintaining good health. It has been proven that more than half of children and practically all adults have dental caries all around the world. Dental decay can be caused by a variety of microorganisms prevalent in our mouth. According to studies, Streptococcus mutans (S.mutans) make up around a third of the germs found in dental cavities caused by caries. The presence of S.mutans in dental caries is critical for determining its susceptibility to antibacterial agents. Antibiotics are not recommended for usage as an anti-caries medication on a daily basis, so mouthwashes are a good option[(Ginting et al., 2021)](https://paperpile.com/c/VEgGmR/QtIt)[(Indumathi et al., n.d.; Sangavi et al., 2019)](https://paperpile.com/c/VEgGmR/JcIf+ODsB).

Since chlorhexidine has a bactericidal impact on a wide range of microorganisms, it is the most commonly prescribed antibacterial mouthwash. However, it has a number of undesirable side effects, including changes in taste, alteration of teeth, restorations, and tongue color, as well as the creation of calculus. Because toothbrushes and other mechanical aids can only reach about half of the teeth surfaces, mouth rinses should be used in addition to mechanical cleaning approaches to achieve the best results[(Chokkattu et al., 2022; Ramamurthy et al., 2022; Shama et al., 2018)](https://paperpile.com/c/VEgGmR/qxjm+QtXR+9A4y).

As a result, researchers are becoming more interested in traditional pharmaceuticals in order to find new materials that can fight bacteria. Plants have long been thought to be a source of novel antibacterial agents; so, research has focused on discovering antimicrobial drugs from plants[(Moon et al., 2011; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/VEgGmR/M3Rv+9f3r+kbtJ). Cinnamons and cloves are one of many plants utilized in the medical profession that can minimize the adhesion forces between bacteria and enamel, hence assisting in plaque deposition inhibition[(Chokkattu et al., 2022; Dhanvanth & Maheswari, 2022; Widi et al., 2018)](https://paperpile.com/c/VEgGmR/Z1cv+jEcT+QtXR).

Cinnamon is an ancient plant that contains volatile oils as well as chemical components such as trans cinnamaldehyde, eugenol, and cinnamyl acetate. Cinnamon was traditionally used to treat tooth pain and eliminate bad breath[(Julianti et al., 2017; Pandiyan et al., 2022; Poornima et al., 2021)](https://paperpile.com/c/VEgGmR/k4Q0+7y3J+9f3r). Cinnamon bark contains essential oils that have been used in medicine for their antimicrobial, antioxidant, and anti-inflammatory properties. Because cinnamon has antimicrobial properties, it was assumed that rinsing with cinnamon-containing mouthwash would reduce dental plaque and decay[(Jain & Verma, 2022; Marya et al., 2022; “Preparation and Characterisation of Gold Nanoparticles Using Cinnamon Oil and Its Antimicrobial Activity against Oral Pathogens,” 2020)](https://paperpile.com/c/VEgGmR/dWre+5LSY+ZrSk). Thus, the current study sought to determine the anti-cariogenic activity of a prepared clove and cinnamon ethanolic extract formulated by herbal mouthwash on streptococcus mutans bacteria in subjects of rinsing.

# MATERIALS AND METHODS

## Mouthwash preparation

Clove and cinnamon were collected and boiled for 15 mins and the extracts were filtered. The filtered extract was kept in a shaker for 3 days to synthesize nanoparticles.

After 3 days, 14ml of extract was measured and transferred to 6 tubes and centrifuged at 8000 rpm for 10 mins to collect pellets. Then the nanoparticles were seen settling in the bottom of the tube. Macconkey agar was used for this activity to define the zone of inhibition. The agar was sterilized for 15 mins at 121 C.

The medium was poured into plates and left to solidify. The alveoli were excised using a sterile 9mm polystyrene bronchoscope and the test organisms were swabbed. Nanoparticles with different concentrations were loaded and in the fourth well standard was kept.

After 24 hours, the culture plates were observed and noted for the range of zones of inhibition with respect to the increase in concentration of the prepared extract mouthwash. The range of inhibition was noted using a measuring scale.

## Antibacterial Activity

Antibacterial activity of respective nanoparticles against the strain *Staphylococcus aureus, Bacillus, and E.coli.* Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller Hinton agar was prepared and sterilized for 15 minutes at 121oC. Media was poured into the sterilized plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The nanoparticles with different concentrations (25µL, 50 µL,100 µL )were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hours at 37 °C. After the incubation time, the zones of inhibition were measured.

## Antifungal activity

*Candida albicans* is used as a test pathogen by agar well diffusion assay. Rose Bengal Agar is used to prepare the fungal medium. The prepared and sterilized medium was swabbed with test organisms and nanoparticles with different concentrations (25µL, 50 µL,100 µL )were added to the wells and in the fourth well standard antibiotic fluconazole was loaded. The plates were incubated at 37°C for 48-72 hours. After the incubation time ,the zone of inhibition was measured.

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1. (b) (c)

Figure 1: (a)(b)(c) Antifungal activity

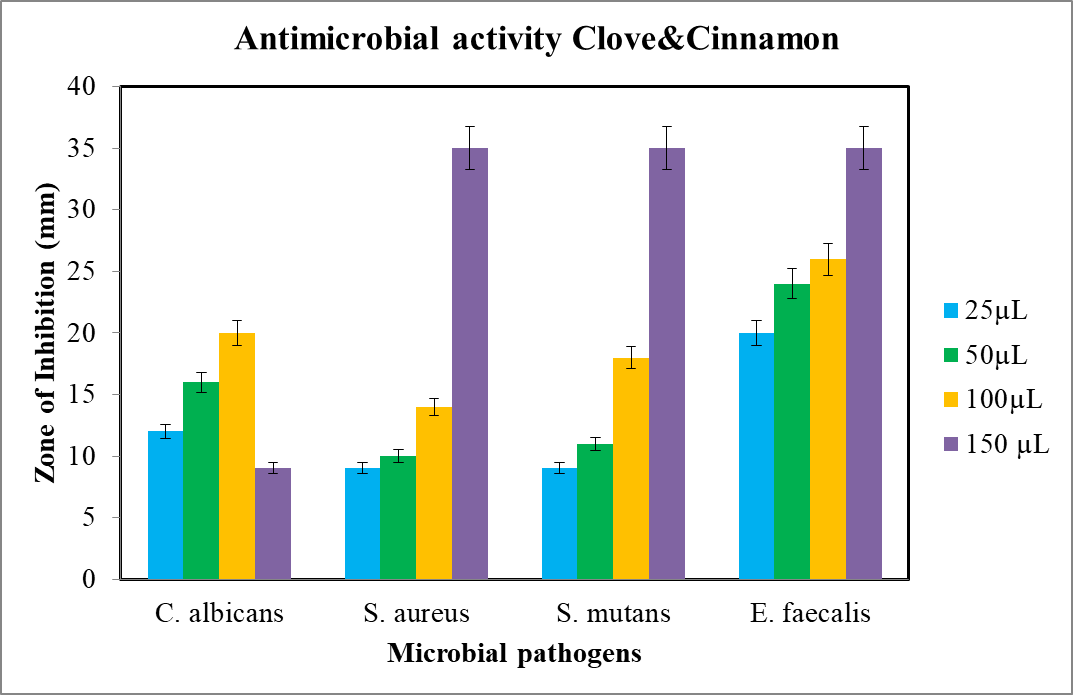
# RESULTS

The results of the study state that when the concentration of the mouthwash is increased the anticariogenic effect of the cinnamon and clove ethanolic extract mouthwash increases. Clove and cinnamon-formulated rinse were found to be a much better antagonistic agent, exhibiting a broad range of antimicrobial activity against the microbes causing dental caries.

Table 1: results of study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **25 μL** | **50μL** | **100μL** | **Ab μL** |
| **S.mutans** | **9** | **11** | **18** | **43** |
| **S.aureus** | **9** | **10** | **14** | **35** |
| **E.faecalis** | **20** | **24** | **26** | **35** |
| **C.albicans** | **12** | **16** | **20** | **9** |

**Figure 1:** Maximum zone of inhibition was found with a concentration of 100μL against each bacteria ( 18mm in S.mutans, 14mm in S.aureus, 26mm in E.faecalis and 20mm in C.albicans ). This showed that there is a noticeable increase in the zone of inhibition with increase in concentration of extract.

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**Figure 2:** The above bar graph more clearly shows the increase in antimicrobial activity with the increase in concentration of the extract against all the oral pathogens.

# DISCUSSIONS

The majority of oral health disorders can be treated when they are young and are mainly avoidable. Dental caries, periodontal disorders, tooth loss, and oral malignancies account for the majority of occurrences. [(Sreevarun et al., 2023; Wadhwani et al., 2022)](https://paperpile.com/c/VEgGmR/XDPW+DKD9) According to the WHO Global Oral Health Status Report (2022), about 3.5 billion people worldwide suffer from oral diseases, with three out of every four of these individuals residing in middle-income nations (Chehelgerdi et al., 2023). 514 million children worldwide suffer from primary tooth decay, while 2 billion adults are thought to have permanent tooth decay [(Virdi, 2015; Zohoori & Duckworth, 2019)](https://paperpile.com/c/VEgGmR/6Viu+PDUF). Because of increased urbanization and modifications in living conditions, the prevalence of the major oral disease is continuing to rise globally.

The best way to remove harmful bacteria from teeth is to brush properly and frequently. When you brush your teeth with toothpaste, you may prevent decay, polish off stains, and get rid of bacterial forms (Saadh et al., 2024). More mechanical and chemical bacterial management techniques are being employed to prevent tooth decay and periodontal disease.[(Solanki et al., 2023; Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/VEgGmR/y2jW+P7vk) Using mouthwash, floss, and proper and frequent tooth brushing are a few of the methods. There are several chemical mouthwashes available, however they have shortcomings such as toxicity, staining of the teeth, and quick hypersensitivity reactions. Alternative drugs can be prepared using medicinal herbs.[(Ganapathy 2022)](https://paperpile.com/c/VEgGmR/Aboc+vrEM)

According to a study on cinnamon extract, the compound has significant anticariogenic, cytotoxic and antioxidant effects on cells. According to the recommendations for caries treatment by risk assessment, antimicrobial mouthwash is an essential caries preventive drug for the prevention or control of dental caries in high-risk individuals. In addition to well-known mouthwashes like chlorhexidine, natural items are increasingly being employed as oral care therapies[(Chokkattu et al., 2023; G et al., 2021)](https://paperpile.com/c/VEgGmR/9qH5+paQr). The hypothesis established in this study was based on the antibacterial effects of glycosides generated from plant products on microbes.

Mouthwash made from natural extracts is known as a herbal mouthwash. Due to its non-irritating, non-staining, and lack of alcohol ingredients, herbal mouthwash has gained popularity over chemical mouthwashes.[(*Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study*, n.d.)](https://paperpile.com/c/VEgGmR/V0Lc) Benefits of using mouthwashes include lowering the risk of plaque development, preventing gum disease, reducing tooth decay, removing debris while eradicating bacteria, and aiding in the treatment of aphthous ulcers[(Muthuswamy Pandian et al., 2022; Nazliniwaty et al., 2019)](https://paperpile.com/c/VEgGmR/afav+CgvH).

According to earlier research by the scientists, cinnamon and clove's natural inflammatory properties are more effective at killing oral infections. This cure has been used for many years and is safer than the current chemical formulation[(Julianti et al., n.d.; Laghari et al., 2023)](https://paperpile.com/c/VEgGmR/NkvV+iyl6).

The results of the zone of inhibition test in the current study further supported the findings that this herbal mouthwash is a potent caries inhibitor. Patients appreciated it for its taste, ease of use, and test duration in the mouth after rinsing. In order to treat caries-induced gingivitis, they can be used as a supplement to mechanical therapy. The current study has a significant impact on efforts to develop a herbal oral health intervention for low socioeconomic populations that is both efficient and affordable. Long-term studies with bigger sample sizes are needed because this study was short-term in nature. Medical research has shown that the natural herbs utilized in the current formulation can prevent oral hygiene issues and foul breath.[(Adel et al., 2023)](https://paperpile.com/c/VEgGmR/ZGGk)

Numerous studies have shown that these plants have a long history of being used successfully. This herbal mouthwash makes it simple for a person to rinse his mouth and prevent a variety of oral health problems.

# CONCLUSION

Clove and cinnamon ethanolic extract mouthwash was successful as an anticariogenic agent. It significantly reduced the total bacteria (S.mutans, S.aureus, C.albicans, E.faecalis) in the culture plate. Hence it represents an alternative source of natural antimicrobial substances for use in oral hygiene.

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