HIGH SCHOOL EDITION
Journal of Student Research
Volume 14 Issue 1 (2025)

Minimum Inhibitory Concentration of Oral Bacteria: A Comparison Between Cloves and Cinnamon

Hannah Wang[1] and Lauren Lucas[#]

[1]Newport High School, USA
[#]Advisor

ABSTRACT

Clove essential oil (EO), used as an antiseptic for oral health, is effective against cavities and is added to products like mouthwash and toothpaste (Agrawal et al., n.d.), (Nuñez & Aquino, 2012). Cinnamon EO contains anti-inflammatory and antibacterial properties that can help with gum disease and tooth pain (Yanakiev, 2020). The minimum inhibitory concentration (MIC) of both cinnamon and clove EO was studied in this paper. Oral bacteria from the cheek were sampled for this experiment and allowed to grow on LB plates containing either clove or cinnamon essential oils diluted to 12.25%, 56.125% and 64.9% concentrations. Cinnamon EO had a lower MIC than clove EO. Cinnamon EO's MIC was less than 12.25% while clove EO's MIC was between 12.25% and 56.125%. Both EO's were able to reduce bacterial growth and can be used as an antimicrobial agent for external use or for those with limited access to modern oral care.

Introduction

Around 80% of the world relies on herbal medicines for some aspects of their health care (Zhang et al., 2015). Spices have long been valued for their culinary and medicinal properties for centuries (Institute, n.d.). Beyond their ability to enhance flavor, many spices possess antimicrobial properties that can be used to benefit oral health (Nuñez & Aquino, 2012), (Agrawal et al., n.d.). Commensal oral bacteria, which are naturally present in the mouth, can maintain and protect the mouth by reducing the growth of pathogenic microbes. However, with poor oral care, bacteria can cause many different dental problems including cavities, gingivitis, or periodontal disease (*24.2*, 2016). Incorporating spices such as cloves and cinnamon into the oral care routine can help manage these bacteria, promoting better oral health. The use of spices as antimicrobials in oral health can be used for people who do not have access to modern oral hygienic practices.

Essential oils infused with cloves are commonly used in traditional Indian and Chinese medicine as an antiseptic for oral infections because they inhibit Gram-negative and Gram-positive bacteria (Nuñez & Aquino, 2012), (Ginting et al., 2021). Clove oil has antibacterial, antifungal, insecticidal, and antioxidant properties, which protects cells from the damage caused by free radicals/unstable molecules (Nuñez & Aquino, 2012), (Nirmala et al., 2019). For these reasons, it is commonly used to fight cavities and is often added to oral products such as mouthwash or toothpaste (Marya et al., 2012). Eugenol is the chemical present in clove essential oils, commonly found in perfumes, mouthwash, and cosmetic products, that is responsible for its strong antimicrobial activities (Nuñez & Aquino, 2012). Eugenol phenolic compounds are able to alter proteins and react with cell membrane phospholipids to change their permeability and inhibit Gram-negative and Gram-positive bacteria (Nuñez & Aquino, 2012). Given its diverse range of effectiveness in combating pathogens, the use of cloves is a simple method for maintaining oral health.

The term cinnamon commonly refers to the dried bark of *Cinnamomum zeylanicum* and *Ctenium aromaticum*, which are plants used to make different types of chocolate, beverages, spiced candies and liquors (Nabavi et al., 2015), (Rao & Gan, 2014). However, cinnamon has also been used in various cultures of traditional medicine, such as embalming by the ancient Egyptian people, or in Ayurvedic medicine as an antiemetic, anti-diarrheal, and stimulant

(*Cinnamon | Plant, Spice, History, & Uses | Britannica*, n.d.). Cinnamon has also been used as a health-promoting agent in Asian and Near East countries for inflammation, gastrointestinal disorders and urinary infections (*Cinnamon*, n.d.), (Nabavi et al., 2015). Some studies show that cinnamon essential oils can be active against oral cavity infections, such as those caused by *Streptococcus mutans* (Nabavi et al., 2015). The cinnamaldehyde and eugenol contained in cinnamon play a role in the antimicrobial effects relating to oral health (Rao & Gan, 2014), (Worreth et al., 2022). Cinnamaldehyde has been reported to be among the most active components against Gram-positive and Gram-negative bacteria, including *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Escherichia coli*, and *Pseudomonas aeruginosa* (Usai & Di Sotto, 2023), (Worreth et al., 2022). Cinnamon essential oil has proven to be a versatile, natural antibacterial agent with potential in multiple fields, making it valuable in both scientific and practical contexts (Nabavi et al., 2015).

The goal of this experiment was to quantify clove and cinnamon essential oil's antibacterial effectiveness. To do so, this study sought to determine the minimum inhibitory concentration (MIC) for clove and cinnamon essential oils against oral bacteria. The experimental design assessed an overall reduction of bacterial growth and did not differentiate between beneficial and pathogenic bacteria. The MIC is the lowest concentration of an antimicrobial agent needed to inhibit the visible growth of bacteria. Studying MIC is important for finding the efficacy of both clove and cinnamon EO. In this study, the MIC was determined by diluting different concentrations of both cinnamon and clove essential oils to find the lowest concentration that can inhibit oral bacterial growth. These findings will facilitate future research to determine the lowest concentration needed for EO products to be effective, which will help people with limited access to prescribed antimicrobials and oral healthcare in general.

Methods

Bacterial Sampling

In this study, the antibacterial activity of cinnamon and clove essential oil (EO) was tested against a cocktail of oral bacteria by swabbing the cheek area of the mouth. The parotideomasseteric area was swabbed for 30 seconds, rotating the swab around. The bacteria were obtained from one participant, age 17. The participant consumed food 17 hours prior and brushed their teeth 12 hours prior to the swab.

Preparation

A total of 29 sterile agar plates and 23 sterile swabs were used. Eleven plates were labeled as controls, with three plates with no treatment for both spices, two plates with the carrier/hazelnut oil, two plates not swabbed with the carrier/hazelnut oil, and 2 not swabbed plates each, for both 100% cinnamon and 100% clove. The remaining 18 plates were labeled with a specified concentration of each spice. Swabs of oral bacteria were then added to each plate in a zigzag pattern with 8 streaks. 0.5 mL of each concentration of EO was then added to the appropriately labeled plate. Time measurements started after the 0.5 mL of EO was added to each plate.

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using different concentrations of cinnamon and clove EO. The EO were prepared in sterile containers, at a dilution of 12.25%, 56.125%, and 64.9%. The essential oils were diluted using hazelnut carrier oil. Carrier oil was used as a miscible solvent in order to dilute the essential oils to different concentrations. Hazelnut oil was chosen as the carrier oil for its low antibacterial properties (Barta et al., n.d.), which could have interfered with the experimental essential oils' potency.

Bacterial Growth

All agar plates were placed on a heating pad at 100° F to allow aerobic oral bacteria to grow. The heating pad was set to 100° F to mimic body temperature, assuming a small amount of temperature loss from the heating pad to the plates. A baking sheet was spread on top of the heating pad to allow for even heat distribution. The plates were monitored for growth about five times a day. Photos were taken for daily records. Time was measured based on hours from T0 (time when experimental setup was complete). Times when the heating pad was off were not included in the time calculations.

Data Analysis

Pictures were taken each day to measure the rate of growth at each concentration and EO. MIC was determined as the lowest concentration of each EO needed to inhibit all visible growth of bacteria. Graphs were plotted using Excel by taking the averages of all data sets, and standard deviation was used to calculate the error bars. A two-way ANOVA was also performed using comparisons to calculate statistical significance.

Results

Antibacterial Activity

Cinnamon oil was able to inhibit all bacteria tested at each of the tested concentrations, 12.25%, 56.125% and 64.9%. Clove oil was only able to inhibit bacterial growth at concentrations of 56.125% and 64.9%, while the 12.25% plates showed signs of bacterial growth (Figure 1, 1a-1c). The three untreated plates that were swabbed with oral bacteria had growth after 24 hours (Figure 1, 2a-2c). The carrier oil plates that were swabbed also took around 24 hours to show signs of bacterial growth (Figure 1, 3a-3b). At around 41 hours, the 12.25% clove EO plates also showed bacterial growth, making cinnamon EO a more potent antimicrobial than clove EO (Figure 2). The maximum number of colonies after 110 hours with the heating pad was 138 on the untreated plate with swab (Figure 2). All other plates that were not mentioned remained sterile for the entire experiment.

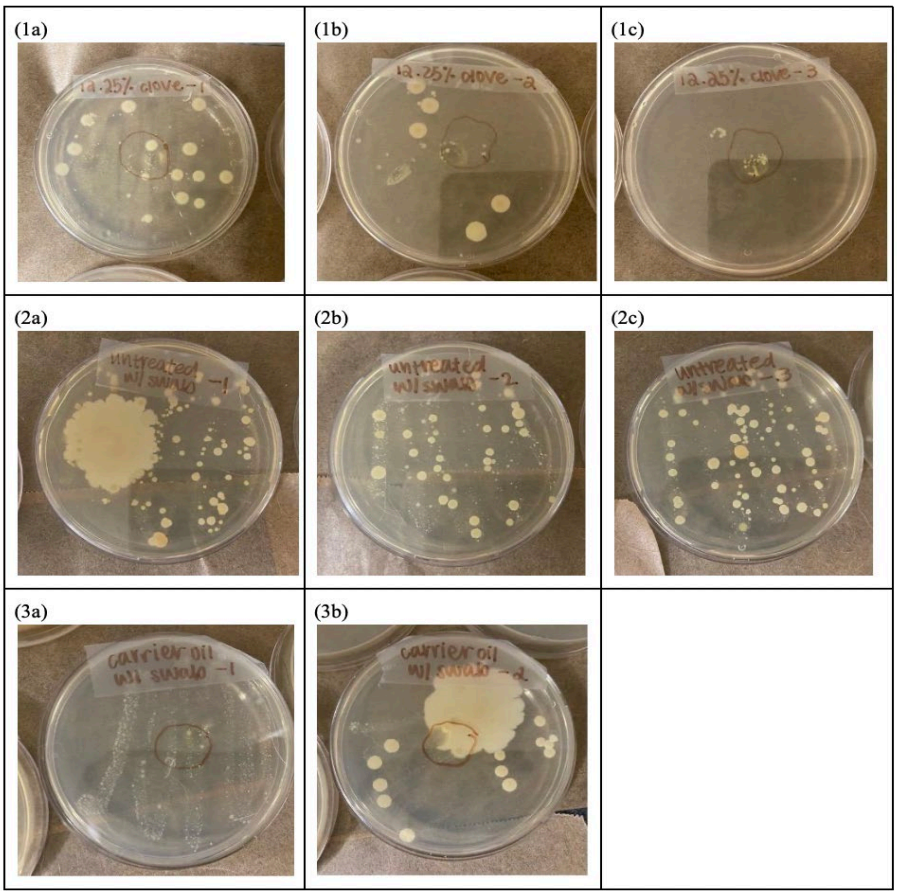

Figure 1. Colony Growth at 110 Hours. 1: 12.25% clove condition, replicates 1a-c. 2: Untreated plates with swab, replicates 2a-c. 3: Carrier oil plates with swab, replicates 3a-c.

Growth Over Time

The untreated plates and carrier oil plates took around 24 hours to show signs of bacteria, while the 12.25% clove took around 41 hours to show bacterial growth (Figure 2). After 110 hours, there were an average of 126 colonies of untreated plates, 84 colonies for carrier oil plates, and 32 colonies of 12.25% clove plates (Figure 2).

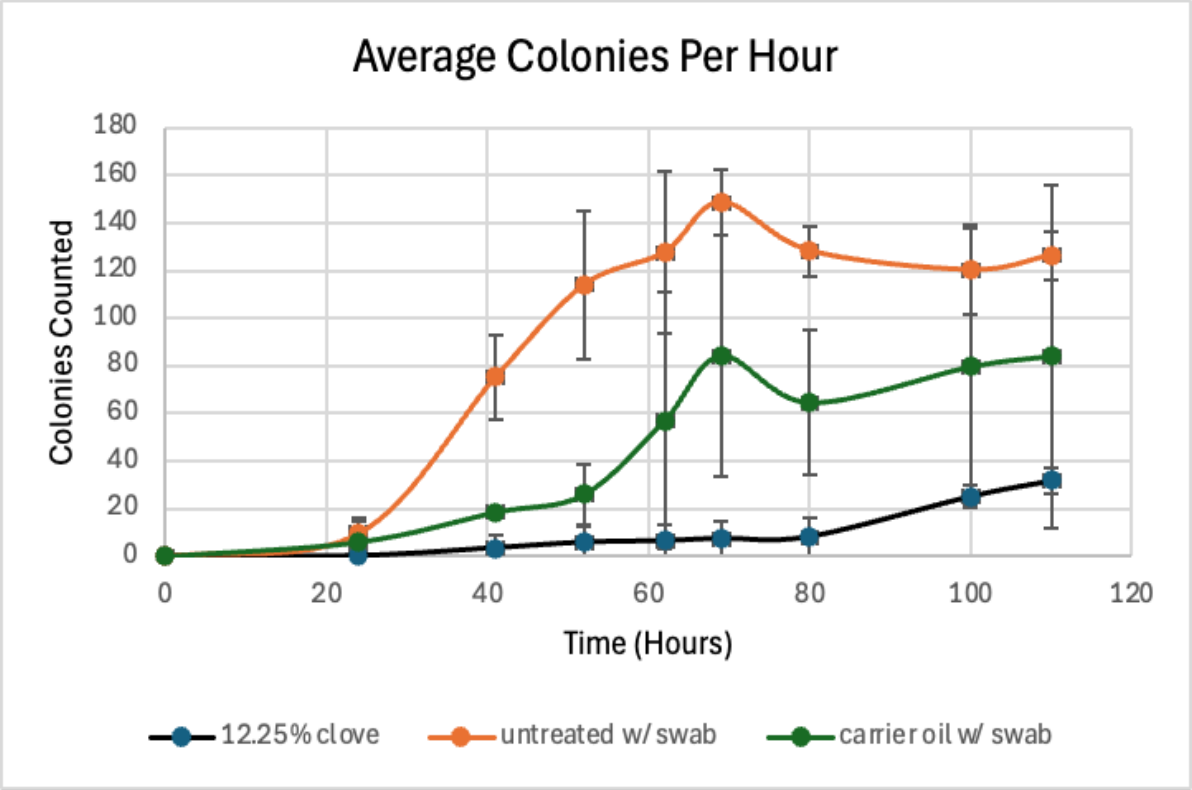

Figure 2. Average number of bacterial colonies per hour. Bacterial colonies were counted on each plate at each time point measured in hours. Replicate values were averaged and standard deviation was included as error bars. Orange line indicates untreated plates with swab. Green line indicates plates with carrier oil and with swab. Black line with blue dot indicates 12.25% clove plates with swab. Both the orange and green lines were positive controls.

Table 1. Raw Data and Statistical Analysis.

	Average			Standard Deviation			p-value		
Time (hours)	**12.25% clove**	**untreated w/ swab**	**carrier oil w/ swab**	**12.25% clove**	**untreated w/ swab**	**carrier oil w/ swab**	**12.25% clove vs. untreated w/swab**	**12.25% clove vs. carrier oil w/swab**	**untreated w/swab vs. carrier oil w/swab**
0	0.00	0.00	0.00	0.00	0.00	0.00	>0.9999	>0.9999	>0.9999
24	0.00	9.67	6.00	0.00	6.66	8.49	0.857	0.953	0.982
41	3.33	75.33	18.50	5.77	17.79	2.12	0.001	0.739	0.021
52	5.67	114.00	26.00	6.66	31.18	12.73	<0.0001	0.582	0.000
62	6.33	127.67	57.00	6.51	34.36	53.74	<0.0001	0.043	0.003
69	7.33	148.67	84.00	7.02	13.87	50.91	<0.0001	0.001	0.008
80	8.00	128.33	64.50	8.00	10.50	30.41	<0.0001	0.022	0.008

100	25.00	120.33	79.50	4.58	19.01	58.69	<0.0001	0.028	0.123
110	31.67	126.33	84.00	5.13	10.21	72.12	<0.0001	0.036	0.106

Growth Pattern

On all untreated plates, bacterial colonies grew along the swab pattern. The carrier oil plates also grew bacteria along the zigzag pattern, but it was more spread out. For the carrier plates, the zigzag pattern was not as noticeable as with the untreated plates (Figure 1). With the 12.25% clove plates, the bacterial growth was more sporadic, with bacteria located close to the oil as well as farther away. On all untreated plates, there were three types of colonies: large yellow colonies, small yellow colonies, and small white colonies (Figure 1). This variation in morphology may be indicative of three different species of bacteria. Cinnamon EO was effective in inhibiting all bacteria; clove EO was effective in inhibiting the smaller yellow and larger yellow colonies but was less effective in inhibiting the white colonies.

Controls

Positive controls used in this experiment were untreated plates swabbed with oral bacteria and carrier oil plates swabbed with oral bacteria. Negative controls were the carrier oil plates without swabs of oral bacteria, 100% clove without swabs of oral bacteria, and 100% cinnamon without swabs of oral bacteria. Untreated plates were included to show that bacteria could be grown under these conditions. Carrier oil plates were used to check for antibacterial properties of the carrier oil itself, which was used as a diluent for the essential oils. The carrier oil plates with a swab of oral bacteria contained less bacterial growth than the untreated plates with a swab of oral bacteria, suggesting that the hazelnut carrier oil contained some antimicrobial properties (Figure 2). Carrier oil plates were used to ensure the oil was not contaminated and did not contain bacteria that would interfere with the experiment. Hazelnut oil plates without swabs did not show bacterial growth. Both 100% clove without swab and 100% cinnamon without swab plates were also used as controls to ensure the essential oils were not contaminated with bacteria that could interfere with the experiment. Both 100% clove and 100% cinnamon plates did not exhibit bacterial growth.

Discussion

This experiment demonstrates the effectiveness of spices as antimicrobials. Both cinnamon and clove contain strong antimicrobial properties that are effective against oral bacteria. This experiment found that cinnamon EO has a lower minimum inhibitory concentration than clove EO. The minimum inhibitory concentration (MIC) for clove EO is between 12.25% and 56.125%, while the MIC for cinnamon EO is less than 12.25%. A reason cinnamon was a more potent antimicrobial agent may be due to its chemical composition, containing both cinnamaldehyde and eugenol, whereas clove only contains eugenol.

Through literature review, similar experiments used 0.025%, 0.125%, and 0.25% dilutions (Ginting et al., 2021). Consequently, we adopted these concentrations to ensure consistency and comparability with past research. However, an error in the dilution process led to the actual concentrations to be 12.25%, 56.125%, and 64.9%, which may explain why a majority of the experimental plates did not show bacterial growth, even at the lower concentrations tested. The fact that any bacteria grew at the tested concentrations suggests the essential oils used may have had lower concentrations of the active ingredients than in the literature reviewed. If the clove essential oil's active ingredient concentration was similar to the literature reviews', the 12.25% clove plate would not have exhibited bacterial growth. Strengths of this experiment were that trials were replicated for each concentration of spice and comprehensive controls were included to ensure the validity of results. These data were consistent and could be assessed for statistical significance. From 62-80 hours, all groups were statistically significant. When comparing the 12.25% clove to

untreated plates, they were significant from 41-110 hours. This observation means that even though there was growth on the clove plates, the treatment still worked better than no treatment. Comparing 12.25% clove and carrier oil plates, the data was significant from 62-110 hours. This observation means that the treatment plates exhibited similar colony growth as the carrier oil plates until 62 hours, after which the 12.25% clove treatment was more effective than the carrier oil. Comparing untreated plates to the carrier oil plates, the data was significant from 41-80 hours. This observation shows that the carrier oil had significantly reduced colony growth until 80 hours, and after, there was no difference between the carrier oil plate and untreated plates. Carrier oil plates may have slowed bacterial growth but were as ineffective as the no treatment plates at inhibiting bacterial growth after 80 hours. Pictures were taken each day for accurate record keeping of bacterial growth over time.

In future experiments, more concentrations for clove EO between 12.25% and 56.125% and cinnamon EO with a concentration of less than 12.25% should be tested to narrow down the MIC. Future research can also benefit from assessing anaerobic bacteria for growth against spices due to their prevalence in the mouth.

Conclusion

Essential oils continue to play a crucial role in healthcare. Though clove EO was able to inhibit bacteria, cinnamon EO showed greater signs of antimicrobial properties. Eugenol and cinnamaldehyde were the main compounds with antimicrobial properties. This study has shown cinnamon EO as an effective agent against oral bacteria, indicated by the MIC value. The results of this study are able to show clove and cinnamon essential oils as good alternatives for those with limited access to oral healthcare during a bacterial infection. However, the daily use of essential oils may be unsuitable due to the healthy balance of one's oral bacteria. Because the oral cavity maintains a healthy balance of beneficial and pathogenic bacteria, cinnamon and clove EO may not be as helpful in a daily practice. However, in the case of a bacterial infection, these essential oils may serve as effective options. Cinnamon EO could also be used to treat infectious diseases. Because these essential oils are natural products, it would be an interesting and cheaper alternative to common antibiotics. Since some essential oils show adverse effects on human cells, we recommend further studies on the toxicity of cinnamon and clove essential oils and the possible side effects (Nabavi et al., 2015).

Limitations

Some limitations were that materials such as extra agar plates or essential oils were limited due to financial constraints. For this reason, not as many concentrations and spices were tested. It would be ideal to have included concentrations below 12.25%, as well as some between 12.25% and 56.125%. Additional spices could have included oregano or peppermint. It was also difficult to know the specific concentration of the active ingredients when using the essential oils. In addition, the heating pad had a time limit of 90 minutes. Due to this programming, the shutoff made it difficult to keep the temperature at 100 degrees consistently. Hours when the heating pad was off were subtracted from the total hours that had passed to calculate growth time. Anaerobic bacteria were unable to be measured in this study due to the experimental design. Research shows that anaerobic bacteria outnumber aerobic bacteria 10:1 in the mouth (*Anaerobic and Aerobic Culture » Pathology Laboratories » College of Medicine » University of Florida*, n.d.). Anaerobic bacteria may be an area for future research. This may include using controlled environments without oxygen. For example, chemical reactions can be used to generate carbon dioxide and remove oxygen, creating an anaerobic environment. Another limitation was condensation. At T0, all plates were originally set up with the oil facing upward. After heating the plates, condensation appeared on the lids of all plates. To address this issue, the plates were flipped upside down within 24 hours, causing some oil to drip down. Both cinnamon and clove essential oils were affected by the oil drips, however cinnamon appeared to drip more frequently than cloves. However, the concentration of the oils did not affect dripping. After flipping the plates, condensation went away after 24-30 hours.

Acknowledgments

This paper and the extensive research behind it would not have been possible without the exceptional support of my mentor, Lauren Lucas. Her guidance and expertise has been an inspiration in the completion of this research. Her insights and feedback challenged me to think critically and push the boundaries of my work. Additionally, she provided thorough feedback on the manuscript, offered key advice throughout the research process, and was willing to answer all of my questions, for which I am deeply grateful.

I would also like to express my sincere gratitude to my parents, who helped by providing the equipment that was necessary, without which I would not have been able to complete this project. They have supported and encouraged me throughout every step.

References

24.2: Microbial Diseases of the Mouth and Oral Cavity. (2016, July 10). *Biology LibreTexts*. https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(OpenStax)/24%3A_Digestive_System_Infections/24.02%3A_Microbial_Diseases_of_the_Mouth_and_Oral_Cavity

Agrawal, M., Agrawal, S., Rastogi, D. R., Singh, D. P., & Br, A. (2014). A review on uses of clove in oral and general health. *Indian Journal of Research in Pharmacy and Biotechnology 2*(4), 1321-1324. https://www.ijrpb.com/issues/Volume%202_Issue%204/ijrpb%202(4)%2016%20Mayank%20Agarwal%201321-1324.pdf

Anaerobic and Aerobic Culture *» Pathology Laboratories » College of Medicine » University of Florida*. (n.d.). https://pathlabs.ufl.edu/tests/test-directory-a/anaerobic-and-aerobic-culture/

Barta, T., Demchik, M., Moll, S., Gontkovic, A., Hall, J., & Fischbach, J. (n.d.). Antimicrobial Properties of American Hazelnut Oil and Extracts. *Essence Journal.* https://www.essencejournal.com/pdf/2020/vol8issue1/PartA/5-5-50-880.pdf

Cinnamon. (n.d.). *NCCIH*. Retrieved August 18, 2024, from https://www.nccih.nih.gov/health/cinnamon

Cinnamon | Plant, Spice, History, & Uses. *Britannica*. (n.d.). https://www.britannica.com/plant/cinnamon

Ginting, E. V., Retnaningrum, E., & Widiasih, D. A. (2021). Antibacterial activity of clove (Syzygium aromaticum) and cinnamon (Cinnamomum burmannii) essential oil against extended-spectrum β-lactamase-producing bacteria. *Veterinary World*, *14*(8), 2206–2211. https://doi.org/10.14202/vetworld.2021.2206-2211

Institute, M. S. (n.d.). *History of Spices*. McCormick Science Institute. https://www.mccormickscienceinstitute.com/resources/history-of-spices

Marya, C. M., Satija, G., J., A., Nagpal, R., Kapoor, R., & Ahmad, A. (2012). In Vitro Inhibitory Effect of Clove Essential Oil and Its Two Active Principles on Tooth Decalcification by Apple Juice. *International Journal of Dentistry*, *2012*, 759618. https://doi.org/10.1155/2012/759618

Nabavi, S. F., Di Lorenzo, A., Izadi, M., Sobarzo-Sánchez, E., Daglia, M., & Nabavi, S. M. (2015). Antibacterial Effects of Cinnamon: From Farm to Food, Cosmetic and Pharmaceutical Industries. *Nutrients*, *7*(9), 7729–7748. https://doi.org/10.3390/nu7095359

Nirmala, M. J., Durai, L., Gopakumar, V., & Nagarajan, R. (2019). Anticancer and antibacterial effects of a clove bud essential oil-based nanoscale emulsion system. *International Journal of Nanomedicine*, *14*, 6439–6450. https://doi.org/10.2147/IJN.S211047

Nuñez, L., & Aquino, M. D. (2012). Microbicide activity of clove essential oil (Eugenia caryophyllata). *Brazilian Journal of Microbiology*, *43*(4), 1255–1260. https://doi.org/10.1590/S1517-83822012000400003

Rao, P. V., & Gan, S. H. (2014). Cinnamon: A Multifaceted Medicinal Plant. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2014*, 642942. https://doi.org/10.1155/2014/642942

Usai, F., & Di Sotto, A. (2023). trans-Cinnamaldehyde as a Novel Candidate to Overcome Bacterial Resistance: An

Overview of In Vitro Studies. *Antibiotics*, *12*(2), 254. https://doi.org/10.3390/antibiotics12020254

Worreth, S., Bieger, V., Rohr, N., Astasov-Frauenhoffer, M., Töpper, T., Osmani, B., & Braissant, O. (2022). Cinnamaldehyde as antimicrobial in cellulose-based dental appliances. *Journal of Applied Microbiology*, *132*(2), 1018–1024. https://doi.org/10.1111/jam.15283

Yanakiev, S. (2020). Effects of Cinnamon (Cinnamomum spp.) in Dentistry: A Review. *Molecules*, *25*(18), 4184. https://doi.org/10.3390/molecules25184184

Zhang, J., Onakpoya, I. J., Posadzki, P., & Eddouks, M. (2015). The Safety of Herbal Medicine: From Prejudice to Evidence. *Evidence-Based Complementary and Alternative Medicine*, *2015*(1), 316706. https://doi.org/10.1155/2015/316706