









# Inhibitory Effects of Cardamom Seed Extract on Streptococcus mutans: A Study on Gum Infection Isolates from Al-Muthanna, Iraq

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## ABSTRACT

**Background:** *S. mutans* is one of the most bacterial pathogens causing periodontal disease worldwide. Aim: This study aimed to evaluate the effectiveness of cardamom seed extract concentrations in inhibiting the growth of clinically isolated bacteria from patients with gingivitis caused by Streptococcus mutans, given the traditional use of cardamom seeds in Iraqi folk medicine for oral and dental health.

**Methods:** *S. mutans* bacteria were clinically isolated from patients with periodontal disease in Al-Muthanna Governorate and identified using the VITEK system. The effect of cardamom seed extracts on the bacterial growth activity of Streptococcus mutans was then evaluated.

**Results:** The study showed that ethanolic extracts of cardamom seeds (*Elettaria cardamomum*) have strong antibacterial effects against Streptococcus mutans (*S. mutans*), and the larger inhibition zones (12-16 mm) observed in the ethanolic extract indicate better efficacy than the aqueous extract.

**Conclusion:** The properties of ethanolic cardamom extract have demonstrated its ability to inhibit bacterial cell membrane enzymes, thus controlling bacteria. Furthermore, it is necessary to determine safe concentrations for human use and assess their suitability for use without the appearance of side effects.

**Keywords:** Gum Infection, Cardamon, Bacteria, Periodontal Disease, Evaluation.

## 1. Introduction

The unjustified overuse and indiscriminate misuse of antibiotics in the treatment of bacterial infections, including gingivitis, has led to very serious problems, including the emergence of antibiotic-resistant

bacterial strains [1]. The clinical symptoms of gingivitis include redness, inflammation, and bleeding at the gum line, as well as ulceration [2]. Bacterial gingivitis may develop into acute periodontitis and periodontal disease due to bacterial attack, ultimately leading to a deterioration of both oral and dental health [3,4]. One of the main factors that contributes to an unhealthy gum environment is creating conditions conducive to the proliferation of acid-producing bacteria, the most important of which is the failure to clean teeth regularly, including streptococci mutans.

This leads to the replacement of the normal bacteria coexisting in the oral cavity with other harmful bacteria [5,6]. Rapid sugar metabolism and the production of various acids are among the most important virulence factors exhibited by *Streptococcus mutans* bacteria, as is their high resistance to low pH conditions in the mouth. [7].

Currently, plant-derived compounds are emerging as promising alternatives to antibiotics due to their biologically active properties and safe profile with no side effects [8]. The therapeutic effect of substances extracted from different parts of the plant, including roots, seeds, leaves, and bark, depends on different extraction techniques such as distillation, evaporation, or hydro-infusion [9, 10, 11]. Cardamom seeds contain important compounds such as myrcene, limonene and cineole, as well as some vitamins, calcium, niacin and riboflavin, making it an anti-inflammatory and antimicrobial substance in the form of a natural substance [12, 13].

This study evaluates the properties of cardamom seed extract against clinically isolated *Streptococcus mutans* strains from patients with periodontal disease in Al-Muthanna Governorate, Iraq, to demonstrate its efficacy in traditional herbal medicine and assess its suitability as an alternative to antibiotics and chemical treatments.

## 2. Materials and methods

### 2.1. Preparing the media

#### 2.1.1. Mitis Salivarius Bacitracin Agar

Medium was prepared by adding 90 grams of Mitis salivarius with 150 grams of sucrose to one liter of distilled water, mixing well, then sterilizing it well in an incubator, and after cooling, we add 1 ml of bacitracin solutions and mix with 9 grams of agar, then pour the medium into dishes [14].

#### 2.1.2. Hinton Muller agar

Medium was prepared according to the manufacturer's instructions, and after completing the sterilization process and cooling the medium to the appropriate temperature 40-45, this medium is used for the purpose of examining the sensitivity of bacteria to the alcoholic and aqueous extract of cardamom seeds to inhibit bacteria [15].

#### 2.1.3. Preparation of bacterial isolates

Samples taken from the mouths of people suffering from gingivitis, specifically from the gums and from both sexes, were collected from private clinics in Al-Muthanna Governorate. Then the diagnosis of the isolated bacteria was confirmed according to the use of the Vitech system to identify the bacterial isolates according to the manufacturer's instructions [16]. *Streptococcus mutans* bacteria were isolated using cotton swabs and plotted on the diagnostic medium mitis salivarius bacitracin agar and incubated at 37 degrees for 24 hours in a candle jar [17].

After that, the isolates of *S. mutans* bacteria were added to the dishes containing the medium Muller hinton agar, and the dishes were left for a quarter of an hour to test the sensitivity of the bacteria to the aqueous and alcoholic extract of cardamom seeds. Then, the discs made from filter papers were immersed

in the previously prepared extracts and placed on the soil planted with it. The bacteria were placed at equal distances between the discs and incubated for 24 hours at a temperature of 37 degrees. The diameters of bacterial growth inhibition were measured using a ruler after the incubation process was completed.

#### 2.1.4. Preparation of cardamom extract

Indian green cardamom was used and ground by an electric grinder into a fine powder. Weigh (50 mg) of powder and place it in a Sechelt extractor with 250 ml of distilled water, absolute ethanol to obtain two different extracts according to the solvent used for 3 hours. the extract obtained from the Sechelt device was placed in the rotary evaporator to obtain the solid substance of the extract. This Solution is subject to damage when kept at room temperature after 10 days of extraction.

### 3. Results and discussion

#### 3.1 UV-Vis Absorbance Spectrum

The Shimadzu UV-1900 spectrophotometer (Serial No A12536003441) acquired the UV-Vis absorbance spectrum (File: 1%AA - RawData) on April 16, 2024 at 02:53:00 PM. The ASEEL sample received the following measurement settings:

Spectrum Type: Absorbance. Slit Width: 1.0 nm. Light Source Switch Wavelength: Standard (typically 340 nm for this instrument class). Measurement Range: 600.00 nm to 900.00 nm (as indicated by Sample ID). The raw data shows the measurement happened in the visible to near-infrared region (600-900 nm), which serves well for studying electronic transitions in conjugated systems, charge-transfer complexes, and semiconductor materials (Figure 1).

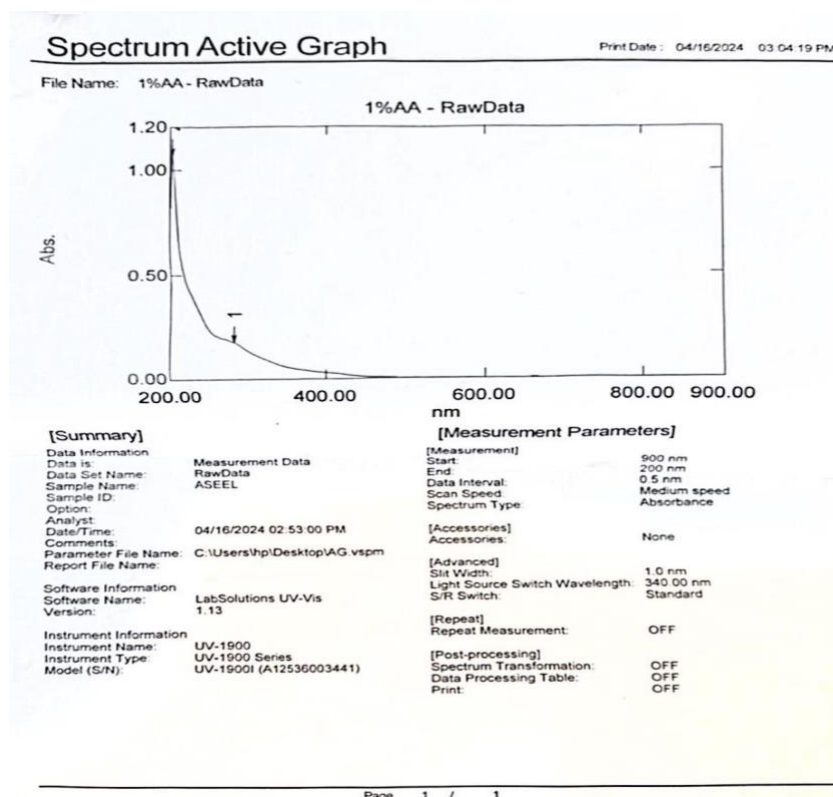


Figure 1. UV-Vis Absorbance Spectrum of Cardamom extracts

The selected wavelength ranges from 600-900 nm matches photon energies between 2.07 eV and 1.38 eV, which scientists use for various scientific purposes. Conjugated Organic Materials [18]:

- The Shimadzu UV-1900 series instruments provide a photometric accuracy of  $\pm 0.002$  Abs for measurements between 0-0.5 Abs and  $\pm 0.004$  Abs for measurements between 0.5-1.0 Abs and wavelength accuracy of  $\pm 0.1$  nm (Shimadzu specifications).
- The bacterium isolated from the gums of the human mouth showed good growth on MSB culture medium, where it appeared in the form of small, circular, irregularly shaped, semi-transparent colonies with a granular appearance, raised above the surface of the medium agar [19] (Figure 2).

### 3.2 Morphological and Cultural Characteristics

The fine, granular appearance of bacterial colonies is due to the production of extracellular polymers (EPS), often resulting from biofilm formation, a key factor in the virulence of oral bacteria [20]. A relatively high concentration of bacterial colonies growing in the medium indicates either active movement or the secretion of certain active substances onto the surface. [21]. As for partial transparency, it indicates thin or un-pigmented cell walls, a characteristic feature of anaerobic or facultative anaerobic bacteria found in the gingival fissure [22].

### 3.3 Implications for Oral Microbiology

MSB medium, which contains hemin and vitamin K, selectively helps the growth of anaerobic bacteria [19]. The observed colony morphology aligns with *Prevotella* or *Fusobacterium* spp., which are known to colonize subgingival plaques and contribute to periodontal disease [23].



**Figure 2.** Appearance of *Streptococcus mutnas* on MSBA differentiation medium

The sensitivity of *S. mutnas* against the aqueous and alcoholic (ethanol) extract of cardamom seeds was tested according to the etch diffusion technique. The bacteria showed clear inhibition against the ethanolic extract of cardamom seeds, as the diameter of inhibition of the alcoholic extract reached (12-16) mm, and this indicates a decrease in the number of bacteria, which indicates the sensitivity of the bacteria *S. mutnas* to the ethanol extract of cardamom seeds, while the aqueous extract gave an inhibition of (1- 2) mm, which is not considered a noticeable inhibition (Figure 3).

### 3.4 Ethanol Extract Superiority

The ethanolic extract produced a larger inhibition zone because cardamom bioactive compounds, such

as terpenes and flavonoids, and phenolic acids extracted better in ethanol than in water [24]. The extraction of non-polar antimicrobial agents, including 1,8-cineole and  $\alpha$ -terpinyl acetate, becomes more effective through ethanol because it disrupts bacterial cell membranes and blocks essential enzymes [25].

### 3.5 Aqueous Extract Limitations

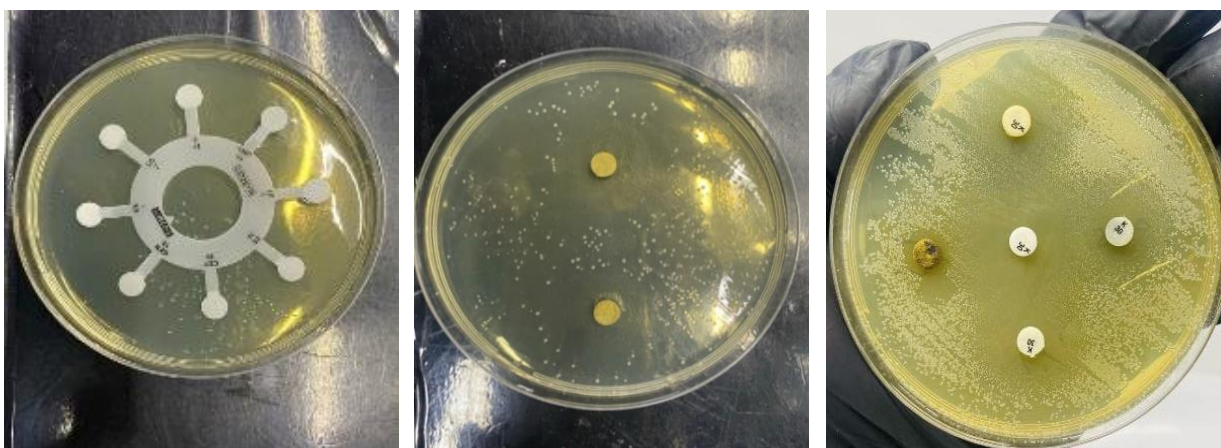
The aqueous extract showed no inhibition because water does not dissolve cardamom antimicrobial compounds according to [26]. Polar solvents lack the ability to extract lipid-soluble compounds, which are essential for antibacterial action.

### 3.6 Clinical Relevance

*S. mutans* functions as a major cariogenic bacterium through its ability to create biofilms and generate acids [27]. Further research must be conducted to establish the in vivo safety of using the ethanolic extract in natural oral care products that target dental caries despite its demonstrated effectiveness against *S. mutans*.

### 3.7 Comparative Analysis with Literature

The measured inhibition zone (12–16 mm) of the ethanolic extract matches previous research on cardamom's effectiveness against Gram-positive bacteria [28]. The minimal aqueous inhibition matches the observation of [29] that water extracts of spices typically produce weak antimicrobial effects.



**Figure 3.** left panel: Control group with extracts on the medium of allergy test Muller Hinton agar. Middle panel: Inhibition of alcoholic extract of cardamom seeds against *Streptococcus mutans* bacteria. Right panel: Inhibition of aqueous extract of cardamom seeds against *Streptococcus mutans* bacteria

## 4. Conclusion

The current research shows that ethanolic extracts of *Elettaria cardamomum* seeds have strong antibacterial effects against *S. mutans* which is a main dental pathogen responsible for periodontal diseases and dental caries. The larger inhibition zones (12–16 mm) observed in ethanolic extraction demonstrate better effectiveness than aqueous extraction because organic solvents better extract terpenes, flavonoids and phenolic acids.

This study highlights the importance of the traditional use of cardamom seeds in folk medicine for oral and dental health. It demonstrates the value and potential of cardamom seeds as an antibacterial agent that inhibits bacterial growth, specifically targeting gingivitis and tooth decay. Cardamom seeds

achieve this by disrupting bacterial cell wall formation and inhibiting enzymatic activity.

Further research is needed due to the significant health implications of this study. This research should explore the potential for widespread use of oral care products based on cardamom seed extract, establish safe and appropriate concentrations for human use without side effects, and determine the possibility of combining it with other plant extracts to ensure long-term effectiveness.

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## REFERENCES

1. Yang J, Wang S, Luo X, Yu Z, Zhou Y. Fenton-like process in antibiotic-containing wastewater treatment: applications and toxicity evaluation. *Chin Chem Lett.* 2025;110996. <https://doi.org/10.1016/j.ccllet.2025.110996>
2. Susin C, Stadler AF, Haas A, Albandar JM. Periodontal manifestations of systemic diseases. *J Periodontol Res.* 2025;1–26. <https://doi.org/10.1111/jre.70034>
3. Dye BA. Global periodontal disease epidemiology. *Periodontol* 2000. 2012;58(1):10–25. <https://doi.org/10.1902/jop.2011.110320>
4. Cafiero C, Matarasso S. Predictive, preventive, personalised and participatory periodontology: the 5Ps age has already started. *EPMA J.* 2013;4:16. <https://doi.org/10.1186/1878-5085-4-16>
5. Lamont RJ, Jenkinson HF. *Oral Microbiology at a Glance.* Singapore: Wiley Blackwell; 2010. <https://doi.org/10.1002/9781444325556>
6. Rogers AH. *Molecular Oral Microbiology.* Norfolk: Caister Academic Press; 2008.
7. Liang HJ, Tan XY, Li D, Lin C, Huang SY, Nie GC, et al. New advances in oral microbiology and tumor research. *World J Clin Oncol.* 2025;16(7):106981. <https://doi.org/10.5306/wjco.v16.i7.106981>
8. Bjarnsholt T, Buhlin K, Dufrêne YF, Gomelsky M, Moroni A, Ramstedt M, et al. Biofilm formation – what we can learn from recent developments. *J Intern Med.* 2018;284(4):332–345. <https://doi.org/10.1111/joim.12782>
9. de Sousa Araújo TA, Gomes de Melo J, Soares Ferreira Júnior W, Paulino Albuquerque U. Medicinal plants. In: *Introduction to Ethnobiology.* Cham: Springer; 2016. p. 143–149. [https://doi.org/10.1007/978-3-319-28155-1\\_23](https://doi.org/10.1007/978-3-319-28155-1_23)

10. Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, et al. Techniques for extraction of bioactive compounds from plant materials: a review. *J Food Eng.* 2013;117(4):426–436. <https://doi.org/10.1016/j.jfoodeng.2013.01.014>
11. Nambiar R, Raveendran K. Indigenous medicinal plants scripted in Amarakosam. *Am J Bot.* 2008;1(3):68–72.
12. Grieve M. Cardamoms botanical: *Elettaria cardamomum* Maton – a modern herbal. *Ind J.* 2006;1–4.
13. Christman S. Flavidata: *Elettaria cardamomum* [Internet]. Tallahassee: Flavide; 2006 [cited 2025]. Available from: [www.flavidata.com](http://www.flavidata.com)
14. Bansal K, Shamoo A, Atif M, Batra P, Chaudhry R. Isolation and detection of bacterial species on mitis salivarius bacitracin agar from the plaque samples of caries active children. *J Indian Soc Pedod Prev Dent.* 2024;42(1):15–21. [https://doi.org/10.4103/jisppd.jisppd\\_17\\_24](https://doi.org/10.4103/jisppd.jisppd_17_24)
15. McFadden LRM. Antimicrobial assay of sagebrush roots [thesis]. 2022. <https://doi.org/10.26076/ae7e-09c8>
16. Karagöz A, Acar S, Körkoca H. Characterization of *Klebsiella* isolates by MALDI-TOF MS and determination of antimicrobial resistance with VITEK 2 advanced expert system. *Turk J Med Sci.* 2015;45(6):1335–1344. <https://doi.org/10.3906/sag-1407-31>
17. Hardie JM, Whiley RA. The genus *Streptococcus* – oral. In: *The Prokaryotes*. Vol. 4. New York: Springer; 2006. p. 76–107. [https://doi.org/10.1007/0-387-30744-3\\_4](https://doi.org/10.1007/0-387-30744-3_4)
18. Sekar SKV, Dalla Mora A, Bargigia I, Martinenghi E, Lindner C, Farzam P, et al. Broadband (600–1350 nm) time-resolved diffuse optical spectrometer for clinical use. *IEEE J Sel Top Quantum Electron.* 2015;22(3):406–414. <https://doi.org/10.1109/JSTQE.2015.2506613>
19. Valm AM. The structure of dental plaque microbial communities in the transition from health to dental caries and periodontal disease. *J Mol Biol.* 2019;431(16):2957–2969. <https://doi.org/10.1016/j.jmb.2019.05.016>
20. Marsh PD, Zaura E. Dental biofilm: ecological interactions in health and disease. *J Clin Periodontol.* 2017;44(S18):S12–S22. <https://doi.org/10.1111/jcpe.12679>
21. Almatroudi A. Biofilm resilience: molecular mechanisms driving antibiotic resistance in clinical contexts. *Biology.* 2025;14(2):165. <https://doi.org/10.3390/biology14020165>
22. Belibasakis GN, Belstrøm D, Eick S, Gursoy UK, Johansson A, Könönen E. Periodontal microbiology and microbial etiology of periodontal diseases: historical concepts and contemporary perspectives. *Periodontol 2000.* 2023;00:1–17. <https://doi.org/10.1111/prd.12473>
23. Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. *Nat Rev Microbiol.* 2010;8(7):481–490. <https://doi.org/10.1038/nrmicro2337>
24. Bhatt P, et al. Phytochemistry and antimicrobial properties of cardamom. *J Essent Oil Res.* 2014;26(1):1–12. <https://doi.org/10.1080/10412905.2013.860409>
25. Kaushik P, et al. Antimicrobial activity of cardamom essential oil. *Food Chem.* 2010;119(1):196–201. <https://doi.org/10.1016/j.foodchem.2009.06.025>
26. Abdel-Rasoul AA, Saleh NA, Hosny EN, El-Gizawy MM, Ibrahim EA. Cardamom oil ameliorates behavioral and neuropathological disorders in a rat model of depression induced by reserpine. *J Ethnopharmacol.* 2023;308:116254. <https://doi.org/10.1016/j.jep.2023.116254>
27. Lemos JA, et al. The biology of *Streptococcus mutans*. *Microbiol Spectr.* 2019;7(1). <https://doi.org/10.1128/microbiolspec.GPP3-0051-2018>

28. Aghamiri V, et al. Antimicrobial activity of *Elettaria cardamomum* against oral pathogens. *J Herbal Med.* 2016;6(2):78–84. <https://doi.org/10.1016/j.hermed.2016.02.001>
29. Huang W, Wang Y, Tian W, Cui X, Tu P, Li J, et al. Biosynthesis investigations of terpenoid, alkaloid, and flavonoid antimicrobial agents derived from medicinal plants. *Antibiotics.* 2022;11(10):1380. <https://doi.org/10.3390/antibiotics11101380>

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