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Comparison of Cytotoxic and Antibacterial Effects of Elettaria cardamomum Extract and Essential Oil

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Objectives This study aimed to investigate the cytotoxic and antibacterial properties of essential oils and hydroalcoholic extracts from Elettaria cardamomum fruit (E. cardamomum).
Methods Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) was used as the test subject for the agar diffusion test in this in-vitro investigation to determine the antibacterial effect of the extract and essential oil. The broth microdilution method was used to calculate their minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC). The methyl thiazolyl tetrazolium (MTT) assay was used to assess their cytotoxicity against human gingival fibroblasts. Tukey's test (alpha=0.05) and ANOVA were used to evaluate the data.
Results The E. cardamomum hydroalcoholic extracts and essential oil demonstrated strong antibacterial activity on A. actinomycetemcomitans. E. cardamomum essential oil (91.5±1.1 mm) and E. cardamomum extract (9.5±0.4 mm) had the highest and lowest growth inhibition zones, respectively. For E. cardamomum essential oil, the MIC and MBC were 1.45%, and for E. cardamomum extract, they were 11.5% (v/v). The essential oil exhibited appreciable cytotoxicity at low doses, while the extract did not.
Conclusion Because of its antibacterial properties and low cytotoxicity at low concentrations, the hydroalcoholic extract of E. cardamomum, one of the compounds examined, may have applications as an organic mouthwash.
Keywords Plant Extracts; Cytotoxicity; Cardamom; Essential oil; Anti-Bacterial Agents

Introduction

Periodontal disease is one of the most common health problems in human society. Because of the ensuing clinical attachment loss, alveolar bone resorption, and eventual tooth loss, as well as its association with numerous systemic risk factors such as obesity, diabetes mellitus, stress, and genetic factors, periodontal disease is clinically significant and requires prompt management. [1] The underlying mechanism is the interaction between the microbial biofilm and the host inflammatory response. The illness would worsen, and eventual tooth loss would occur without any intervention. [2] The primary pathogenic microorganisms that cause periodontal diseases are gram-negative bacteria, including Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), Prevotella intermedia, Treponema denticola, Bacteroides forsythus, and Porphyromonas gingivalis (P. gingivalis). One of the primary causes of periodontitis development is A. actinomycetemcomitans. [3]
The traditional first therapy for periodontitis is scaling and root planning, which removes subgingival and supragingival microbial plaque and calculus. [4] However, even after scaling and root planning, microbial recolonization is still possible because some inaccessible places have not been fully debrided. As a result, chemical antimicrobial adjunct therapies can reduce bacterial recolonization, and there is a need for pocket surgery. [5] Given the rise in antibiotic resistance and the unfavorable side effects of most conventional antibacterial agents, safe, affordable, and effective alternatives are clearly needed. As a result, scientists have been working to create organic, non-chemical mouthwashes with fewer adverse side effects and a high efficiency against periopathogenic germs. [6] It has already been established that certain herbal extracts and essential oils play a part in preventing and treating oral diseases, suppressing oral pathogen growth, and preventing dental plaque and biofilm formation. [7]
Essential oils can have potent antibacterial properties and are produced as secondary metabolites in many plant sections. [8] Essential oil is a viscous, hydrophobic liquid that contains volatile components. On the other hand, the extract is made from inactive plant parts using a suitable solvent to boost the active ingredients' stability, concentration, and purity. [9] Elettaria cardamomum (E. cardamomum) hydroalcoholic extract has demonstrated superior antibacterial activity against Gram-negative periopathogens. [10] To the authors' knowledge, no prior research has been done on the antibacterial properties of essential oil derived from E. cardamomum.
An ideal mouthwash should not cause localized or systemic allergic reactions or have any cytotoxic effects on oral cells. This study examined the antibacterial and cytotoxic properties of essential oil and E. cardamomum extract.

Methods and Materials

In this in vitro study, human gingival fibroblasts (HGF1 PI 1[NCBI: C165]) were obtained from the Pasteur Institute of Iran's Cell Bank, and A. actinomycetemcomitans (Aa

jp2nov799; ATCC 700685) was obtained from the Oral Microbiology Laboratory of Shahid Beheshti Dental School. The Traditional Medicine Department at Tehran University of Medical Sciences provided the E. cardamomum fruit hydroalcoholic extracts and essential oils.

Preparation of bacterial suspension:

A. actinomycetemcomitans was kept at -20°C for a whole day, then chilled for three hours at 4–8°C. Once it was cultivated on brain heart infusion (BHI) agar. After that, the bacterial culture was cultured for 48 hours at 37°C in an anaerobic jar (Gas-Pak A). Using a sterile loop, a glass test tube filled with BHI broth was used to hold the single colonies extracted from the solid BHI agar culture. The tube's contents were vortexed to create a uniform microbial solution. Using spectrophotometry, the optical density (OD) of one milliliter of the suspension was measured at a wavelength of 600 nm. 0.5 McFarland standard concentration was defined as a microbial suspension with 0.08 to 0.1 OD, with the control culture medium devoid of bacteria.

Assessment of antibacterial effects of extract and essential oil:

Agar well diffusion test: The first evaluation of microbiological susceptibility to the corresponding herbal extracts and essential oils (presence/absence of antibacterial properties) was conducted using the agar well diffusion or the well-plate technique. A sterile cotton swab was soaked in 0.5 McFarland standard bacterial suspension to lawn-culture the bacteria on solid BHI agar plates. Next, a sterile Pasteur pipette was used to produce a well in each of the four notional segments divided into each plate. Next, a sampler injected 50 µL of each sterilized extract or essential oil into each well. The positive control was mouthwash containing 0.2% chlorhexidine (CHX). The same test and control groups were used in three separate plates for the three repetitions of this experiment. Lastly, the plates were placed in an anaerobic jar (Gas-Pak A) and incubated for 24 hours at 37°C. The diameter of the growth inhibition zones quantitatively using a millimeter ruler and qualitatively assess their creation.

Determination of minimum inhibitory concentration (MIC):

Colorimetry (resazurin dye) was used in the broth microdilution procedure to measure the MIC of the extract and essential oil. To do this, 96-well plate wells were filled with 100 µL of sterile BHI broth. The first well of the test row was then filled with 100 µL of the appropriate essential oil or extract, which was then combined with the culture medium using a sampler. Subsequently, 100 microliters of the contents of this well were moved to the second well, which was repeated up to the 12th well. After that, all the wells received 10 µL of bacterial suspension containing 107 colony-forming units (CFUs)/mL, except for the negative control wells, which received 106 CFUs at the end. Next, 100 µL of the contents of this well were transferred to the second well, and this process was repeated until the 12th well. BHI broth and an essential oil or extract without bacterial suspension were found in the negative control wells. A single row served as the positive control, with BHI and bacterial suspension in its wells devoid of extract or essential oil. After that, the plates were incubated in an anaerobic environment for 48 hours at 37°C. Subsequently, each well received 20 µL of 0.01% resazurin blue dye, and the plates were incubated for three hours at 37°C. The MIC of the corresponding extract or essential oil was determined by measuring the lowest concentration at which the substance did not turn pink but instead remained blue.

Determination of minimum bactericidal concentration (MBC):

Samples were taken from all blue-colored wells before the MIC well, from the MIC well itself, and from all pink-colored wells (indicating bacterial growth) following the MIC well using a sterile loop. The samples were then cultured on BHI agar, with three repetitions for each specimen, to ascertain the MBC of the essential oils or extracts. The MBC of each extract or essential oil was determined by measuring the lowest concentration of that material that did not result in any bacterial growth after the plates were incubated for 72 hours at 37°C in an anaerobic jar.

Assessment of cytotoxicity by the methyl thiazolyl tetrazolium (MTT) assay:

The cytotoxic effects of essential oils and extracts were assessed at 1%, 5%, and 10% (v/v). Human gingival fibroblasts in the logarithmic growth phase were seeded with 100 µL of Dulbecco's modified Eagle's medium, an antibiotic-containing medium, and 5000 cells/well in each well of 96-well culture plates. The counts were performed under aseptic conditions using a hemocytometer and Trypan Blue dye. After that, the plates were incubated for 24 hours at 37°C, 95% humidity, and 5% CO2. On the second day, adding various extract/essential oil concentrations to the cells in isolation after checking the cells' condition, checking for contamination, and ensuring they were at least 50% confluent were performed. Two plates were considered to evaluate cell viability and proliferation as well as acute cytotoxicity after 24 hours of exposure and cell viability and proliferation as well as chronic cytotoxicity after 72 hours of exposure. Following the plates' incubation, the overlaying medium was carefully removed at each 24- or 72-hour mark, the contents of each well were rinsed with phosphate-buffered saline, and 100 µL of culture medium (devoid of serum and antibiotics) containing 10% MTT dye (Sigma-Aldrich) was added to each well. After three hours of incubation, the plates were checked under an inverted microscope to confirm the production of formazan crystals. The MTT dye (Sigma-Aldrich) was then withdrawn and replaced with an equal

volume of dimethyl sulfoxide (Sigma-Aldrich). The percentage of cell viability was then determined by dividing the mean OD of the treated cells in each group by the mean OD of the control wells (non-treated cells with 100% viability) and multiplying the result by 100. The OD of the wells was then read using an ELISA Reader (Anthus 2020). According to the ISO-10993-5, a substance was deemed cytotoxic if it resulted in a reduction of over 30% in the percentage of cell viability when compared to the control group, meaning that the viability was less than 70%.

Statistical analysis:

The mean amount of the three repeats was given for the MTT experiment. First, each test group was contrasted individually with the control group. Then, in GraphPad Prism version 9, one-way ANOVA and Tukey's test were used to examine the data at the 0.05 significance level.

Results

Antibacterial effects of the tested extract and essential oil on A. actinomycetemcomitans:

Well, plate results: Growth inhibition zones around the hydroalcoholic extract and essential oil of E. cardamomum and the positive control group (0.2% CHX) were identified after 24 hours, as shown in Figure 1, suggesting their antibacterial efficacy against A. actinomycetemcomitans. With a mean of 91.5±1.1 mm, E. cardamomum essential oil had the highest growth inhibition zone, followed by CHX with a mean of 42.3±2.6 mm. Extract growth inhibition zones were smaller than essential oil growth inhibition zones (Table 1), with E. cardamomum extract having the smallest growth inhibition zone (9.5±0.4 mm).

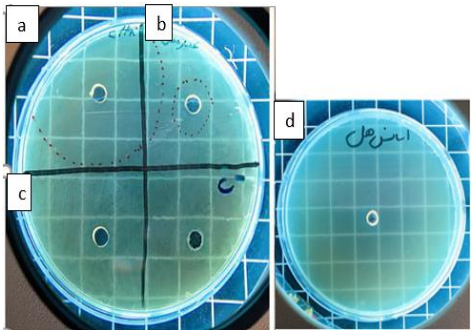

Figure 1. Assessment of antibacterial activity of the respective essential oil and extract against A. actinomycetemcomitans by the well plate technique. a:CHX. b: E. cardamomum extract. c: negative control (The negative control wells contained BHI broth and essential oil/extract without bacterial suspension.). d: E. cardamomum essential oil

MIC outcomes: CHX had the lowest MIC (=MBC), at 0.0001% (v/v), followed by 1.45% (v/v) for E. cardamomum essential oil and 11.5% (v/v) for E. cardamomum extract. The MIC and MBC of the extract and essential oil are shown in Table 2.

Table 1- Growth inhibition zones of extract and essential oil

Extract/essential oil	Zone of growth inhibition in mm (Mean±SD)
E. cardamomum	9.5 ± 0.4
E. cardamomum	91.5 ± 1.1
CHX	42.3 ± 2.6

Table 2- MIC and MBC of the extract and essential oil

Extract/essential oil	MIC=MBC (% v/v)
E. cardamomum extract	11.5
E. cardamomum essential oil	1.45
CHX	0.0001

Cytotoxicity results:

At 24 hours, E. cardamomum showed >70% cell viability at 1% and 5% doses, but a 10% concentration was cytotoxic (Figure 2A). Regarding the viability, there was no discernible difference between the E. cardamomum extracts and the control group. The quantities of all essential oils were cytotoxic.

When compared to other evaluated materials, the 1% E. cardamomum extract did not exhibit cytotoxicity after 72 hours (>70% viability) (Figure 2B). This difference was statistically significant (P<0.05). The increase in cytotoxicity after 72 hours, as opposed to 24 hours, suggests that cytotoxicity was time-dependent. Furthermore, it was dose-dependent (P<0.05).

Antibacterial effects of the tested extract and essential oil on A. actinomycetemcomitans:

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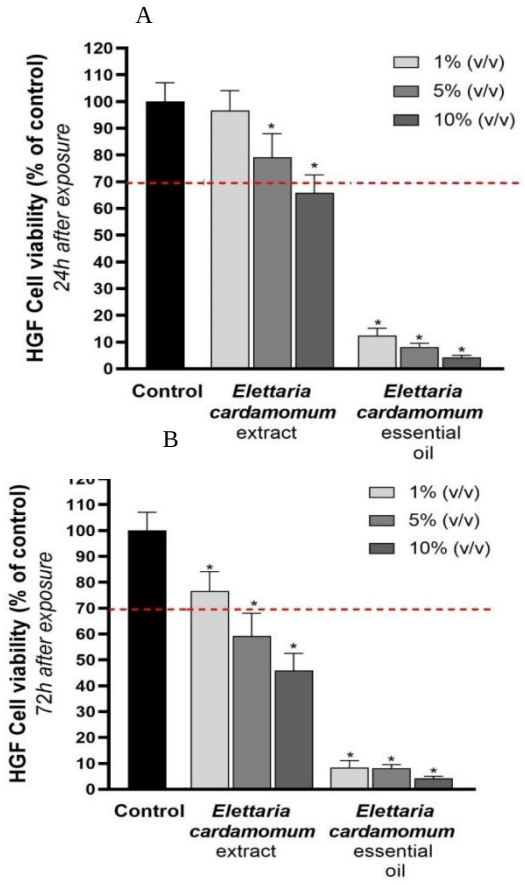
Cytotoxicity results:

At 24 hours, E. cardamomum showed over 70% cell viability at 1% and 5% doses, but a 10% concentration was cytotoxic (Figure 2A). Regarding the viability percent, there was no discernible difference between the E. cardamomum extracts and the control group. The quantities of all essential oils were cytotoxic.

Figure 2: Quantitative assessment of the cytotoxic effects of



extracts and essential oils on human gingival fibroblasts at 24 (A) and 72 (B) hours. stars over the columns indicate presence of a significant difference with control group (P<0.05). Stars between two columns indicate presence of a significant difference between the two groups (P<0.05).

When compared to other evaluated materials, the 1% E. cardamomum extract did not exhibit cytotoxicity after 72 hours (>70% viability) (Figure 2B). This difference was statistically significant (P<0.05). The increase in cytotoxicity after 72 hours, as opposed to 24 hours, suggested that cytotoxicity was time dependent. Furthermore, it was dose-dependent (P<0.05).

Discussion

The cytotoxic and antibacterial properties of essential oil and E. cardamomum extract were examined in this study. The findings demonstrated that A. actinomycetemcomitans was susceptible to the antibacterial properties of the hydroalcoholic extract and the essential oil of eucalyptus cardamomum. The essential oil of E. cardamomum had the most significant growth inhibition zone, followed by CHX. Extracts had smaller growth inhibition zones than essential oils, with E. cardamomum extract having the smallest growth inhibition zone. CHX had the lowest MIC and MBC, at 0.0001% (v/v), followed by 1.45% (v/v) E. cardamomum essential oil, and 11.5% (v/v) E. cardamomum extract. Gram-positive bacteria were shown to be very sensitive to the antibacterial actions of E. cardamomum essential oil by Noshad and Behbahani. [11] Since gram-negative bacteria have distinct cell wall compositions, gram-positive bacteria are generally more sensitive to herbal extracts and essential oils than gram-negative bacteria. Gram-negative bacteria are more resistant because their outer cell membranes contain complex hydrophobic lipopolysaccharide compositions impervious to hydrophilic substances. [11] The ethanolic extract of E. cardamomum had the most potent antibacterial action against Streptococcus pyogenes, as reported by Utami et al. [12], with a growth inhibition zone measuring 1425.96 mm^2. Similar antibacterial benefits of an herbal mouthwash containing E. cardamomum and CHX on periopathogenic microorganisms were described by Mufti et al. [13]. Some of their findings about the best antibacterial effects of E. cardamomum agreed with the current findings; however, the herbal mouthwash and CHX in their study had different comparable antibacterial activity from the current study. This could be because the herbal mouthwash in their study contained several ingredients, one of them being E. cardamomum. The MIC and MBC of E. cardamomum extract in this investigation were both 11.5% (v/v). A. actinomycetemcomitans, Prevotella intermedia, P. gingivalis, and Fusobacterium nucleatum were the periopathogens on which Souissi et al.'s [14] evaluation of the antibacterial activities of E. cardamomum fruit and seed extract revealed that both of them had the best antibacterial effects. Concerning A. actinomycetemcomitans, the fruit and seed extracts had MICs of 5% and 0.25%, respectively, and MBCs of 1% and 0.5%, respectively. They concluded that while both extracts might be used to treat periodontal infections, the seed extract worked better. Variations in the extract types between the two studies (the current study solely used fruit extract), E. cardamomum species, and extraction methods can be responsible for the discrepancies between the results.

The MIC and MBC of E. cardamomum essential oil against A. actinomycetemcomitans were found to be 1.45% (v/v) in the current investigation. Tabibzadeh et al. [15] evaluated E. cardamomum hydroalcoholic extract and found antibacterial properties on A.a. Compared to 0.00065 mg/ml and 0.00098 mg/ml for 0.2% of chlorhexidine, the MIC and MBC values for this bacterium were 0.0039 mg/ml and 0.0039 mg/ml, respectively. To the authors' knowledge, no prior investigation has assessed the MIC and MBC of E. cardamomum essential oil against A. actinomycetemcomitans in order to compare the findings with the present study.

After checking the antibacterial property, the cytotoxic effect should also be checked on the oral cells. The essential oil at all concentrations was cytotoxic at 24 and 72 hours. According to cytotoxicity tests of the extract and essential oil in this study, there was a significant and possibly dose-

dependent reduction in cell viability of about 90%. At 24 hours, neither the 1% nor the 5% extracts of E. cardamomum nor their 1% concentration in 72 hours were cytotoxic. E.cardamomom essential oils were cytotoxic in all concentrations.

Sreedharan et al. [16] demonstrated that E. cardamomum extract was not cytotoxic to colon cells or macrophages at doses of 200–800 µg/mL, and they suggested that the extract's anti-inflammatory properties were due to the phenol in its composition. In 2021, Gustavo R. et al. [17] investigated the cytotoxic effect of cardamom extract on peritoneal cells and showed that natural cardamom extract has no cytotoxic effect on peritoneal cell lines. Manjunath et al. [18] demonstrated that limonene, an active ingredient of E. cardamomum, has chemical cancer-preventive properties and inhibits cancer cell growth with no significant toxicity. The present results also showed that E. cardamomum essential oil in high concentrations caused a substantial reduction in murine fibroblasts and was cytotoxic. A dose–response relationship was noted regarding the cytotoxic action, meaning that the cytotoxic activity increased with concentration. [18]

One of the study's shortcomings was its in vitro design. Future research is needed to evaluate the antibacterial properties of essential oil and extract from E. cardamomum on other periopathogenic microorganisms. Furthermore, more in vitro research on complex bacterial plaque media and in clinical settings is necessary to validate the current findings.

Conclusion

E. cardamomum extract demonstrated the potential to be used as an organic mouthwash due to its antibacterial effects and absence of cytotoxicity in low concentrations.

Conflict of Interest

The authors declare that they have no conflict of interest.

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