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Antibacterial Effect of Aqueous and Alcoholic ginger extracts against periodontal pathogens (*Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*) in Comparison to Chlorohexidine Gluconate (*in vitro* study)

A Thesis

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Abstract

Background: Ginger *(Zingiber officinale*) has been grown in China and India for centuries and it was used for cooking and for herbal medicine. It possess antimicrobial, antifungal and antioxidant properties due to the phenols – related constituents (gingerols) that inhibit the growth of many Gram positive and Gram negative bacteria including some periodontal bacteria. The main pathogens responsible for periodontal disease initiation and progression are *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

Aims of the study: This study was conducted to isolate and identify the periodontal pathogens (*Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis*), test the antibacterial effect of aqueous and alcoholic ginger extracts against both bacteria in comparison to 0.2% chlorohexidine gluconate and distilled water *in vitro*, determination of ginger extracts minimum inhibitory concentration and minimum bactericidal concentration , detection of active ingredients of ginger extracts using high-performance liquid chromatography and determination of chemical elements .

Materials and methods : *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* strains were isolated by careful collection of sub gingival plaque samples from 50 patients suffering from chronic periodontitis of pocket depth at least 8 mm to be cultured under anaerobic conditions for 48 hours in suitable culture media using anaerobic jar in the incubator. Presence of the target microorganisms is confirmed using morphological characteristics, Gram stain, biochemical tests (Indole test, oxidase test, catalase test, coagulase test, urease test and analytical profile index test), hemolytic ability and antibiotic sensitivity. Ginger extracts (aqueous and alcoholic) was extracted by using water and alcohol respectively.

For the first experiment, agar well diffusion technique was used to study the sensitivity of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* to different concentrations of ginger extracts (20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) and other control agents 0.2% chlorhexidine gluconate and distilled water. The second experiment involved determination of the minimum inhibitory concentration of the extracts that inhibits the bacterial growth and then determination of the minimum bactericidal concentration of the extracts that was required for killing the bacteria. Third experiment involved using high-performance liquid chromatography to detect the active ingredients of ginger extracts and determination of chemical elements.

Results: *Aggregatibacter actinomycetemcomitans,* colonies were about 1 mm in diameter and they adhered well to the agar. Microscopic examination showed that *Aggregatibacter actinomycetem comitans* were Gram negative rods.

Biochemical tests revealed that *Aggregatibacter actinomycetemcomitans* was oxidase and catalase positive, while, indole, coagulase and urease negative hence, according to analytical profile index test, *Aggregatibacter actinomycetemcomitans* was listed as (*Haemophilus actinomycetemcomitans*), also it showed positive hemolytic ability and it was resistant to both Kanamycin and Vancomycin antibiotics.

Regarding *Aggregatibacter actinomycetemcomitans* sensitivity to ginger extracts, all alcoholic ginger extract concentrations showed mean values of inhibition zones less than 0.2% chlorohexidine except, 90% and 100% showed higher mean values of inhibition zones than 0.2% chlorohexidine, while all aqueous ginger extract concentrations revealed mean values of inhibition zones less than 0.2% chlorohexidine. For *Porphyromonas gingivalis* colonies appeared as round spherical on agar plates with raised or convex surface, in microscopic examination they were Gram negative. Biochemical tests revealed that *P. gingivalis* was indole positive, while oxidase, catalase, coagulase and

urease negative hence, according to analytical profile index test of *P. gingivalis*, it was listed as *Porphyromonas asaccharolytica*, also it showed positive hemolytic ability and it was sensitive to both Kanamycin and Vancomycin.

Relating to *Porphyromonas gingivalis* sensitivity to ginger extracts, all alcoholic ginger extract concentrations showed mean values of inhibition zones less than 0.2% chlorohexidine, except the concentrations 80%,90% and 100% that showed higher mean values of inhibition zones than 0.2% chlorohexidine, also all aqueous ginger extract concentrations demonstrated mean values of inhibition zones less than 0.2% chlorohexidine, except, concentration 100% illustrated mean values of inhibition zones almost equal to 0.2% chlorohexidine. *Porphyromonas gingivalis* showed higher mean values of inhibition zones than *Aggregatibacter actinomycetemcomitans* to all concentrations of both extracts and chlorohexidine.

The minimum inhibitory concentration of alcoholic ginger extract against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were 50% (0.5 g/ml) and 30% (0.3 g/ml) respectively, while minimum inhibitory concentration of aqueous ginger extract against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were 80% (0.8 g/ml) and 50% (0.5 g/ml) respectively.

The minimum bactericidal concentration of alcoholic ginger extract against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were 80% (0.8 g/ml) and 60%(0.6 g/ml) respectively. While the minimum bactericidal concentration of aqueous ginger extract against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were 100% (1 g/ml) and 80%(0.8 g/ml) respectively.

According to the results of high-performance liquid chromatography analysis for both extracts, alcoholic extract had higher content of 10-gingerol and 6-shogoal than the aqueous extract, but aqueous extract had higher content of 6-gingerol and 8-gingerol. Hence, the results of chemical elements analysis

of ginger extracts revealed the presence of higher concentrations of both Potassium and Magnesium elements, also there were Phosphorous, Iron, Manganese, Zinc and Cupper elements.

Conclusion: Both ginger extracts were effective against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* with bacteriostatic action and bactericidal action, nonetheless, alcoholic extract was more effective than aqueous extract. Both anaerobic periodontal pathogens were sensitive to ginger extracts, but *Porphyromonas gingivalis* was more sensitive than *Aggregatibacter actinomycetemcomitans* to both extracts. Chlorohexidine revealed higher mean values of inhibition zones for all concentrations of both extracts against both bacteria , except for 90% and 100% concentrations of alcoholic extract against *Aggregatibacter actinomycetemcomitans* and 80%,90% and 100% against *Porphyromonas gingivalis* that showed higher mean values of inhibition zones than0.2% chlorohexidine , as well as 100% concentration of aqueous extract that showed mean values of inhibition zones almost equal to 0.2% chlorohexidine against *Aggregatibacter actinomycetemcomitans*.