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Antimicrobial Activity of Aqueous Extracts of Cinnamon and Ginger on Two Oral Pathogens Causing Dental Caries

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ABSTRACT

Streptococcus mutans is the most common cariogenic bacteria associated with dental caries. It is believed to be the chief etiologic agent in human dental caries, while, *Lactobacillus* characteristically cause existing carious lesions to progress, especially those in coronal caries. The purpose of this study was to evaluate the antimicrobial activity of different concentrations of aqueous extracts of cinnamon and garlic on these two oral pathogens. Stimulated saliva was collected from forty two healthy looking subjects aged (18-41 years) from which mutans streptococci and lactobacilli were isolated. The effect of different concentrations on the antimicrobial activity of cinnamon and ginger against Mutans streptococcus and lactobacilli was evaluated using the agar diffusion. The antimicrobial activity as determined by paper disc diffusion method demonstrated that the antimicrobial activity of aqueous extract of cinnamon is more against lactobacilli than its activity against mutant streptococci in especially with high concentrations.These results are the same regarding the antimicrobial activity of aqueous extract of ginger which was more against lactobacilli than its activity against mutant streptococci with all concentrations. Cinnamon and ginger have excellent antimicrobial activity on the growth of Mutans streptococci and lactobacilli, but their effects are more on lactobacilli than mutant streptococci. Cinnamon should be prescribed prior to ginger because it has more antibacterial activity on the growth on both bacteria types even in small concentrations (50mg/ml). Both plants may have potential for use in mouthwash for preventing dental caries.

Keywords: Antimicrobial activity; Aqueous cinnamon extract; Aqueous ginger extract; Mutans streptococci; Lactobacilli.

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INTRODUCTION

Oral diseases impact the quality of life and may lead to systemic and threatening diseases [1]. Dental caries is one of the most common infections of all oral diseases. It is proved that cariogenic microorganisms, especially *Streptococcus. mutans* plays an essential role in the pathogenesis of dental caries. It is involved in the initiation of almost all carious lesions in enamel [2]. The main cause of dental caries was attributed to oral biofilm, also known as dental plaque, a film of microorganisms sticking to the tooth surface [3].

Dental biofilms constitute an ecosystem of bacteria. They produce acids from carbohydrate metabolism, subsequent decrease in environmental pH causing demineralization of tooth surface and hence caries. The development of dental caries involves acidogenic and aciduric Gram-positive bacteria (Mutans streptococci, Lactobacilli and Actinomycetes). However, much research has identified Mutans streptococci (MS) as the major pathogens of dental caries. This is because, first, MS are frequently isolated from cavitated caries lesions; second, MS induce caries formation in animals which are fed with a sucrose-rich diet; third, MS are highly acidogenic and aciduric and fourth, MS are able to produce surface antigens I/II and water-insoluble glycan, which promote bacterial adhesion to the tooth surface and to other bacteria [4]. Gum disease involves bacterial growth & production of metabolic substances that gradually destroy the tissue surrounding and supporting the teeth. Oral cavity pathogens include *Streptococcus mutans*, lactobacilli, *Streptococcus salivarius, Halobacterium sp., Veilonella sp.* etc. These bacteria grow & attack the tissues causing gingivitis, characterized by inflamed gums that bleed easily. If left untreated the condition progresses to periodontal disease with severe inflammation, bone damage & tooth loss. The causative bacteria reside in plaque, the deposit that forms on the base of the teeth & hardens to form tartar. [5]

While *Streptococcus mutans* bacteria is the main cause of tooth decay, other varieties of microbes can cause dental caries, but to a lesser extent. For example, although considered beneficial, some *Lactobacillus* species have been associated with dental caries. The *Lactobacillus* count in saliva has been used as a "caries test" for many years. This is one of the arguments used in support of the use of fluoride in toothpaste. Lactobacilli characteristically cause existing carious lesions to progress, especially those in coronal caries. [6]

Many attempts have been made to eliminate Streptococcus mutans and other pathogenic bacteria from the oral cavity. Chlorhexidine is the antimicrobial agent most commonly used as oral rinse for prevention of dental caries. However its excessive use can result in side effects such as unpleasant taste, staining of restorations, discoloration of tongue, desquamation and soreness in the oral mucosa [7]. These problems necessitate further research for natural antimicrobial agents that are safe for humans and specific for oral pathogens [7-9].

Hence in search of novel antimicrobial agents, two plants have been chosen in this study because of their antimicrobial activities [10-11]. First; Cinnamon (Cinnamomum zeylanicum) which is thought to have health benefits [12]m and has been used in traditional medicine for

colds, flatulence, nausea and diarrhea, besides also believed to improve energy, vitality, and circulation [13]. Studies have found that cinnamon may have antibacterial and antifungal properties [14]. Second; ginger (*Zinziber officinale*), which has been shown to have antimicrobial activity [15-16]. This study was done to evaluate and compare the antimicrobial activity of aqueous extracts of cinnamon and ginger on oral mutans streptococci and lactobacilli.

MATERIALS AND METHODS

Materials:

All chemicals used were of analytical-reagent grade and obtained. From Scientific Company (Baghdad, Iraq). Cinnamon (*Cinnamomum* zeylanicum), and ginger (*Zingiber officinale*) were purchased from local market of medical herbs (Baghdad, Iraq). The plants were brought to the laboratory and thoroughly washed in distilled water and dried in shade in room temperature then stored in a plastic zip bag in 4 ^{0}C until use.

Plant Extracts Preparation:

Plant materials were finely grinded to powder by using a blender. Fifty grams of each plant material in powder form was weighed in an Erlenmeyer of one hundred gram of finely powdered cinnamon and one hundred gram of ginger each mixed with one liter of sterile deionized water and kept in a water bath at 60 ^{0}C for five hours, then filtered through sterile filter paper (Whatman, UK). The filtrates were exposed to 40 ^{o}C in hot air oven for evaporation of water. The filtrates were kept at 4 ^{o}C until use [17].

Sample collection:

Forty two normal adults volunteers (15 females and 27 males), aged 18-41 years were recruited in this study which was conducted at dental clinics in the College of Dentistry, University of Baghdad. Stimulated saliva samples were collected under standard conditions from the participants. Each subject was instructed to chew a piece of Arabic chewing gum (0.4-0.5g) for five minutes to stimulate salivary flow as much as possible then saliva was collected in sterilized screw capped bottles. [18]

Inoculation:

The collected saliva was homogenized by vortex mixer for two minutes. Ten-fold serial dilutions were prepared using sterile normal saline. Two dilutions were selected for each microbial type and inoculated on the following culture media:

1. Mitis-Salivarius Bacitracin Agar (MSB Agar), the selective media for *Streptococcus mutans:* 0.1ml was withdrawn from dilutions 10'^{2} and 10" using adjustable micropipette with disposable tips and then spread in duplicate by using sterile microbiological glass spreader

on the plates of MSB agar. The plates were then incubated anaerobically by using a gas pack supplied in an anaerobic jar for 48 hrs at 37°C followed by aerobic incubation for 24hrs at 37°C.

2. Rogosa Selective Lactobacilli Agar (RSL Agar), this is selective for cultivation of oral LB: The inoculum was withdrawn from 10" and 10"""' dilutions; 0.1ml from each dilution was inoculated by using pour plate method. The plates were incubated aerobically for 48 hrs at 37°C.

Identification of bacteria:

a. Colony morphology: the colony on MSB agar and RSL agar were examined directly, under dissecting microscope (magnification xl5).

b. Morphology of the Microbial Cells: a colony was picked up from MSB agar, and RSL agar plates separately under sterilized conditions and subjected to gram's stain.

c. Biochemical Tests: Bacterial colonies of different morphology were picked up from MSB agar and RSL agar separately under sterilized conditions using inoculating loop and then inoculated in 10 ml of sterilized (BH1-B) and incubated aerobically at 37 C for 18 hrs. The following tests were conducted; (1) Catalase Production test: this test was conducted on both types of cells *(S. mutans* and LB) separately. Hydrogen peroxide 3% (H_20_2) had been used to detect the activity of catalase enzyme production. (2) Carbohydrate fermentation test for: *S. mutans* - CTA- mannitol media had been used to test the ability of *S. mutans* to ferment the mannitol which was added in a concentration of 1% to the CTA-mannitol media.

d. Identification system of API (analytical profile index) strep; API 20 strep was a standardized system used in the identification of *S. mutans.* It is combining of 20 biochemical tests that offer wide spread capabilities. The strip consists of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of sugars. The enzymatic tests were inoculated with a dense suspension of organisms made from a pure culture Microbial counts of *S. Mutans and Lactobacilli* were recorded by colony counter taking in consideration the dilution factor and expressed as colony forming unit multiplied by the dilution factor per milliliter saliva (CFU/ml) [19-20].

Concentrations of 50mg/ml, 100mg/ml, and 150mg/ml were used to study the antimicrobial activity of aqueous extracts of cinnamon and ginger against *S. mutans and lactobacilli* using agar diffusion method technique. Seven isolates of *S. mutans and lactobacilli* were used in this study.

Data were expressed as mean±standard deviation and a statistical significance was calculated by mean of ANOVA test.

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RESULTS

The antimicrobial activity as determined by paper disc diffusion method demonstrated that the antimicrobial activity of aqueous extract of cinnamon is more against lactobacilli than its activity against mutant streptococci in especially with high concentrations (Fig 1 & Fig 2). These results are the same regarding the antimicrobial activity of aqueous extract of ginger which was more against lactobacilli than its activity against mutant streptococci with all concentrations (Fig. 3 & Fig. 4).

However to compare between the two plants which one more effective on major pathogenic of dental caries the results showed that the priority will be given to cinnamon regarding its antimicrobial activity than ginger on both types of bacteria unless ginger is used in high concentrations (200 mg/ml and above), fig 5. With using very high concentration ginger will be more effective than cinnamon on lactobacilli only (fig.5)

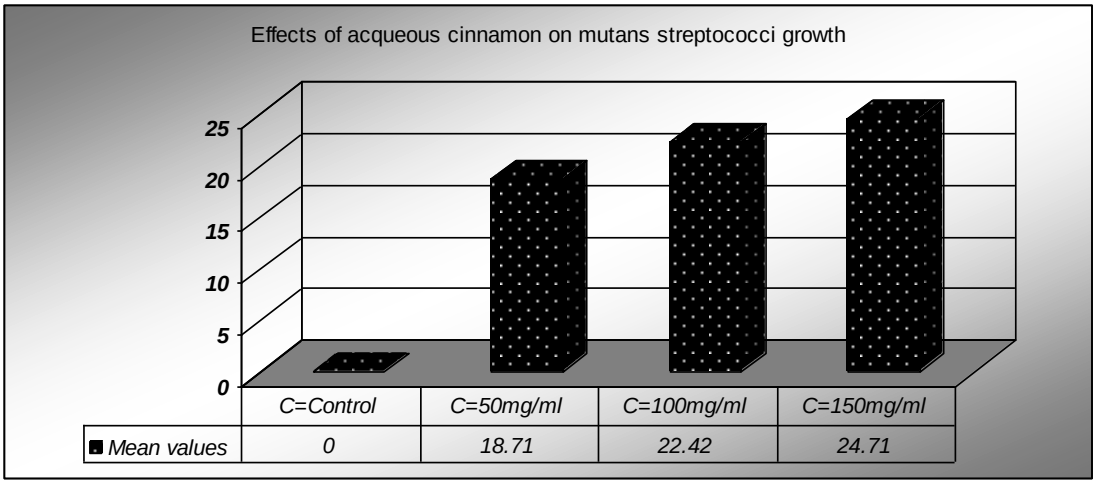

Figure1. Antimicrobial activity of aqueous extract of cinnamon on *matuns streptococci* growth.
C= concentration (mg/ml)

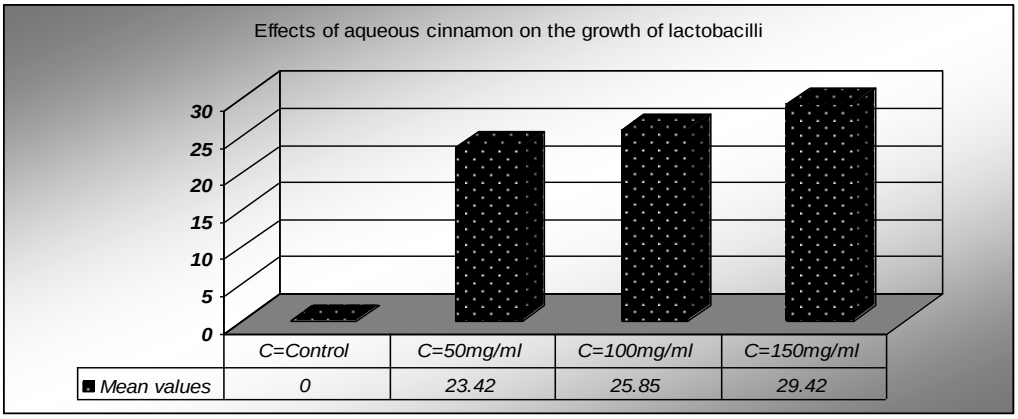

Figure2. Antimicrobial activity of aqueous extract of cinnamon on *lactobacilli* growth.
C= concentration (mg/ml)

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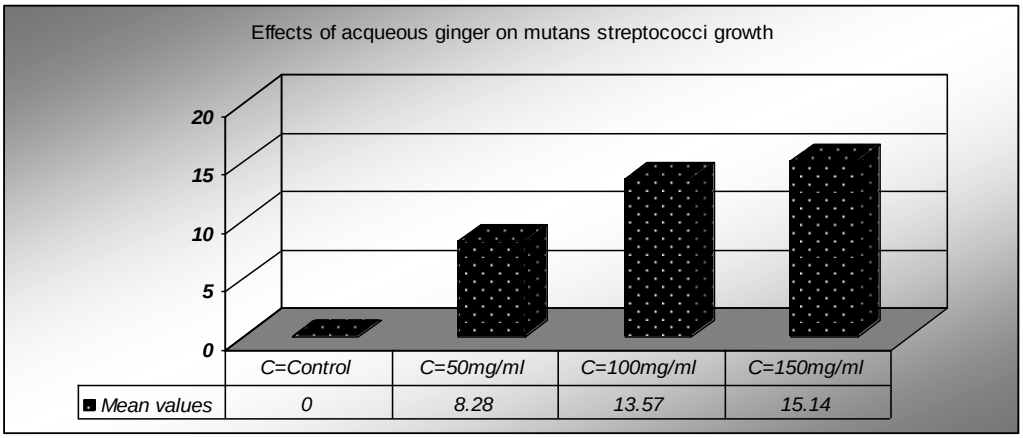

Figure3. Antimicrobial activity of aqueous extract of ginger on *matuns streptococci* growth.
C= concentration (mg/ml)

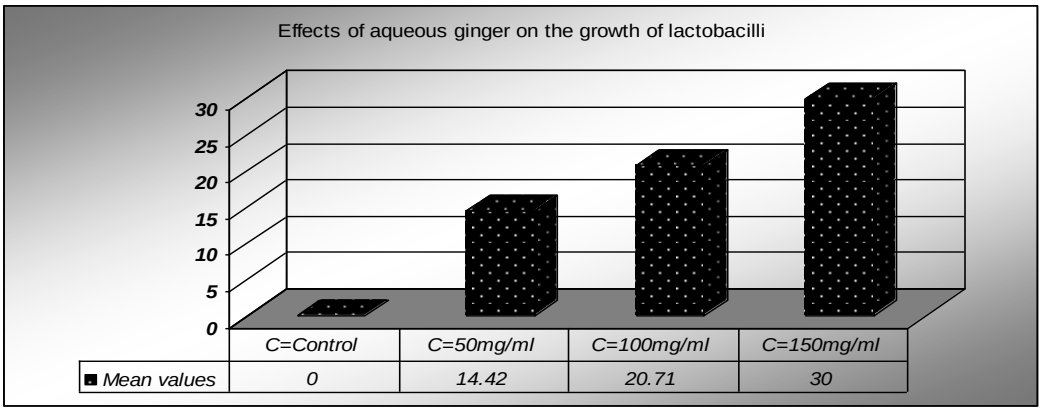

Figure4. Antimicrobial activity of aqueous extract of ginger on *lactobacilli* growth.
C= concentration (mg/ml)

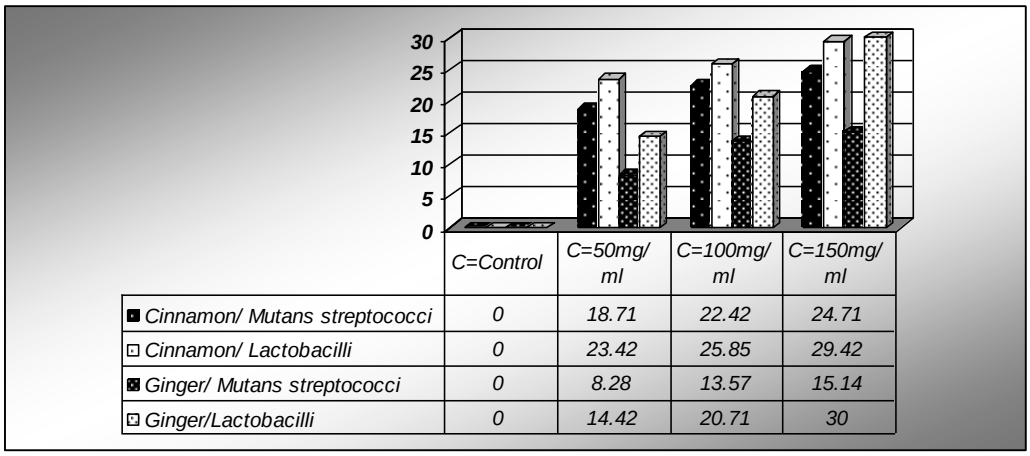

Figure5. Antimicrobial activity of aqueous extracts of Cinnamon & Ginger on Mutans streptococci & *lactobacilli* growth.
C= concentration (mg/ml)

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DISCUSSION

This study demonstrates the antimicrobial activity of aqueous extracts of cinnamon and ginger on the most causative agents of dental caries; *Streptococci mutans* and *lactobacilli* growth on saliva samples from normal adults population. Our principal findings are: (1) both plant candidates have excellent antimicrobial activity on the growth of both bacteria types but their effects are more on lactobacilli than mutant streptococci; (2) if one wants to prescribe one of these two plants to treat or prevent dental caries, cinnamon should be prescribed prior to ginger because cinnamon has more antibacterial activity on the growth on both bacteria types even in small concentration (50mg/ml), Fig 1&2.

It is well-known, that chemical agents such as fluoride and chlorhexidine, which have been used to prevent dental caries for several decades, were associated with some side effects such as staining of teeth and fluorosis. Thus, there is no perfect antimicrobial agent to prevent dental caries until now [7]. The use of natural products has been one of the most successful strategies for the discovery of new drugs [7]. Natural products have been used for thousands of years in folk medicine and they are believed to be the new source of antimicrobial agents [21].

The relationship between the high incidence of oral diseases and microorganisms is well known. Because of the increased bacterial resistance to antibiotics, toxic and harmful effects of few common antibacterial agents, there is a continuous need for alternative therapies which are affordable, not toxic and effective, such as plants [22-23].

Cinnamon and ginger are mainly used in foods because they give desirable flavors and aromas, in addition they showed medicinal and antimicrobial activities [12, 24-25]. The antimicrobial activity of cinnamon has been studied since the end of the last century and the active components were determined [26-28]. Results of current study regarding antimicrobial activity against mutans streptococci and lactobacilli are consistent with that reported by other studies. In an in-vitro study [29], demonstrated that cinnamon oil has excellent antibacterial activity, either alone or in combination with chlorhexidine, triclosan and gentamicin, against clinical streptococci isolates [29]. However, other study [30] determined the Minimum Inhibitory Concentrations (MIC) of the essential oils of cinnamon, thyme, and clove oil against Gram-positive bacteria and Gram negative bacteria. The results showed that cinnamon was a promising antibacterial substance with MIC ranged from 0.1 to 0.4µl/ml for the bacterial species [30].

Current study demonstrated the effects of ginger (*Z. officinale*)on antimicrobial activity of mutans streptococci and lactobacilli. Same results were reported by others [31-32]. Ethanolic extract of the rhizomes of *Z. officinale* showed significant inhibition of growth of both certain gram-positive and gram-negative bacteria. The essential oils of *Z. officinale* showed antimicrobial activity against gram-positive and gramnegative bacteria using the agar diffusion method [31]. Toxicity studies conducted on *Z. officinale*, used as aphrodisiacs in Arab Medicine showed no toxicity during acute toxicity test. The percent lethality was insignificant as compared to the control [32].

Current study demonstrated the comparison between two plants on the major causative pathogens of dental caries. This is an additional advantage in using these medical plants in the preventive dentistry, because, in the search for these novel antimicrobial compounds, traditional plants have been proved to be a better source. The advantage of traditional medicine is that it is less likely to form allergies and side effects. Now a days because of their high antimicrobial, anti-inflammatory, anti-oxidant and biocompatible properties, their use in dentistry is becoming more popular [33].

CONCLUSION

Cinnamon and ginger have excellent antimicrobial activity on the growth of Mutans streptococci and lactobacilli, but their effects are more on lactobacilli than mutant streptococci. Cinnamon should be prescribed prior to ginger because it has more antibacterial activity on the growth on both bacteria types even in small concentration (50mg/ml).

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