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Research Article

Antibacterial Activity of Crude *Cinnamomum zeylanicum* Ethanol Extract on Bacterial Isolates from Orofacial Infections

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Abstract

Local application of natural antimicrobial products on oral infection site can be considered useful alternative to common antibiotics. Cinnamomum zeylanicum (C.Z) has been found to be effective against both types of bacterial (Gram-negative and Gram- positive bacterial), which play roles in human infections responsible for human infectious diseases. This study aimed to assess the efficacy of (C.Z) antibacterial activity on isolates from orofacial infections. The antibacterial activity of crude (C.Z) ethanol extracts on four types of oro-facial infection microorganisms was studied; ciprofloxacin an antibiotic was used as positive control. The Results showed All four bacterial isolates (*Staphylococcus aureus, Escherichia coli, Peptococcus* and *S. Mutans*) were sensitive towards the ethanol extract of Cinnamomum. However, the maximum antimicrobial activity has been shown against *Staphylococcus aureus*. This study is the first of a series of studies to produce Cinnamon extract for local control of oral facial infections. Conclusion and clinical significance is Crude (C.Z) ethanol extract effective against aerobic, anaerobic, gram positive and gram negative oral pathogens. The extract might be employed to make an effective spray or ointment for orofacial infection.

Keywords: *Cinnamomum zeylanicum*; Antibacterial Activity; Oral Infection; Orofacial

Introduction

Oral infections are usually caused by poly bacterial. Approximately 60% of oral infections are caused by joint aerobic and anaerobic bacteria; 35% caused by anaerobic bacteria and 5% due to aerobic bacteria. When aerobic bacteria are involved, Gram positive aerobic cocci are the most common aerobic species involved in oral cavity infection (e.g., Streptococci (e.g., Streptococci "species" (spp), Staphylococci spp). In cases of anaerobic and/or mixed infection Streptococci spp, Peptostreptococci spp and gram-negative anaerobic bacilli (*Prevotella spp, Porphyromonas spp, Bacteroides spp, and Fusobacterium spp*) are involved. [1,2]. It has been hypothesized that synergism between more than one type of bacteria in a particular infection plays a key role in their pathogenicity. Accordingly the target of the antimicrobial control for such infection will be the synergism between key offending organisms and there is no need for specific medication for every involved bacteria in the infection process [1,3].

Recently there has been more focus natural materials, more specifically aromatic plants with wide biological medicinal applications [4-6]. Among them is Cinnamon, which is a spice, commonly used in food. The aroma its oil has makes it useful in food industry [7]. One of Cinnamon species is C. zeylanicum. C. zeylanicum contains eugenol [8-10], in addition to it Antimicrobial property of C. zeylaincum is attributed to the presence of cinnamyl acetate, methoxy cinnamaldehyde (MOCA) and other volatile compounds [11, 12]. Among cinnamon extracts, essential oils and components have been identified for their activity against

infectious Gram positive and Gram negative bacteria responsible for infectious diseases and food or cosmetics' degradation [13].

In addition, there are studies have shown that cinnamon extracts and essential oils might be effective against oral infections. Chaudhari., et al. in their study in 2012 demonstrated the effectiveness of cinnamon essential oil against *Streptococcus Mutans* and concluded that the use of cinnamon essential oils can be a good alternative to other antibacterial compounds against the bacteria responsible for oral infections [14]. More recently, Miller., *et al.* [2015] found that the essential oils extracted from C. zeylanicum leaves is effective against some of dental plaque forming bacterial, such as S. Mutans and Lactobaicllus Acidophilus. These bacterial spp. considered to play a significant role in dental caries development [15]. This is why Cinnamon oils have been suggested to be considered as useful alternative to synthetic antibiotics, especially for infection caused by antibiotic-resistant bacterial [16].

However, local application of Cinnamon extracts as useful measures in preventing oral wound infection has not been given enough attention. The aim of this study is to assess the efficacy of the antibacterial activity of *Cinnamomum zeylanicum* on isolates from orofacial acute infections.

Materials and Methods

Plant material

The spice cinnamon bark *Cinnamomum zeylanicum* (C.Z) were purchased from local market of Baghdad city. The spice were botanically identified. Different concentrations of the species of (C.Z) were prepared in Dimethyl sulfoxide (DMSO) (Table 1).

S. No.	Concentration of extract(w/v) mg/ml
1	1
2	1.875
3	3.75
4	15
5	30

Table 1: Preparation of Different Concentrations of extract.

Extract preparation

After cleaning of the spices with deionized water they were dried in sunlight for two days. This was supplemented with further dryness in an oven at 40^0C for about 24 hours. The dried material was pulverized into fine powdered substance by a grinder. Twenty grams of Cinnamon powder (weighted by electric balance) was transferred into 100 ml conical flask were 40ml of ethanol was added.

A foil paper was used to close the conical flask, which was stored in dark place for maximum seven days. The extracted ethanol crude was filtered by Whatman No. 1 filter paper. Then it was concentrated under 40^0C vacuum by using a rotary evaporator. The powder of (C.Z) was then dissolved with DMSO. Different concentrations of the extract were prepared. The residual extracts were stored in refrigerator at 4^0C.

Tested Bacteria

Escherichia coli (gram negative bacteria), *Streptococcus mutans, Staphylococcus Aureus* and *Peptococcus* (gram positive bacteria) were the types of bacteria on which the antibacterial activity of the extract was tested. The bacterial samples were obtained by swabs taken from odontogenic infection sites in the oral cavity. Nutrient Agar at 37 for 24 hors was used as culture media for tested bacteria. These cultures were sub-cultured on regular basis (every 30 days) then stored at 4C, in the Microbiology Lab, Department of Basic Sciences, College of Dentistry, Ibn Sina University of Medical and Pharmacological Sciences. Peptococcus is an aerobic was cultured at 37^0C for 6-7 days in an anaerobic chamber (Electrotek, United Kingdom) in the atmosphere consisting of 90% N2, 5% CO_2, and 5% H_2.

Sample preparation

Saline solution of concentration 0.9% w/v was prepared using sodium chloride (NaCl) and distilled water. Pure cultures of Staphylococcus aureus, Escherichia coli, S. Mutans and Peptococcus were taken and were serially diluted in 0.9% saline solution up to 10-8 dilution. MHA-GMB (Mueller Hinton Agar - Glucose Methylene Blue) media was used for the inoculation of each type of microbe. 250 mL of MHA-GMB media was prepared using, 5.5 g of Mueller Hinton Broth, 2% dextrose, 2-3drops of methylene blue and 1% of agar.

The antimicrobial disc of 6mm diameter was prepared using Whatman filter paper with different concentrations of cinnamon extract as mentioned in [table 1]. Plates of MHA media were prepared which were then inoculated with 2 loops of inoculum of 10-8 dilution. Discs were prepared with different concentrations of Cinnamon extract. These were then placed in the middle of plates already spread with inoculum. Sterile blank paper discs impregnated with only sterile DMSO served as negative control each time zone of inhibition of stocks at different concentration of extract was measured and similarly zone of inhibition of antibiotic ciprofloxacin was measured ciprofloxacin(5µg/disc) were used as positive control for comparison of the antibacterial activity.

Statistical Evaluation

The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the discs by millimeter scale The diameter of zone of inhibition mean of two replicates ± SD as indicated by clear area which was devoid growth of microbes. The experiment was replicated two times to confirm the reproducible results [14,17]. Measurement of Activity Index: Formula used Activity Index = Zone of inhibition of extract / Zone of inhibition of antibiotic.

Results and Discussion

The antimicrobial activity of C. Z was tested on four types of bacteria involved in orofacial infection (gram positive *Peptococcus, Staphylococcus* and gram negative *E. coli* bacteria). Analysis of the effect of the crude extract of C.Z was performed after an incubation period of 24 hour at 40^0C The zone of inhibition for positive control (Cip) and the negative control (DMSO) was measured (Table2) and Activity Index of Cinnamon extract for the gram positive *Streptococcus Peptococcus, Staphylococcus* and gram negative *E.coli* bacteria, was calculated at different concentrations. The higher activity index was for E.coli because when we applied the equation of the activity index, the value of zone of inhibition for cip was zero (no antibacterial effect to CIP on E.coli) [Table 3, 4, 5 and 6].

Among the four tested bacteria *Staph spp*. and *E. coli* Cinnamon crude extract has been found to be most effective against *Staph* spp. and *E. coli* with zone of inhibition for Staphylococcus aureus, 29mm(table 3), Escherichia coli 25mm [table 4], respectively at the higher concentration 30 mg/ml, whereas for *S. Mutans* and *Peptococcus* ZID, about 21mm [table 6], forand Peptococcus was 20mm [table 5]. The sensitivity increased for –all tested bacteria by increasing of the concentration, showing maximum

Bacteria	Negative control	Positive control; mm
	DMSO	CIP(5µg/ml)
Escherichia coli	0	0
Staphylococcus aureus	0	33
Strepto coccus mutans	0	26
Peptococcus	0	19

Table 2: Effect of the Negative control and Positive control on teasted bacteria.

S.No.	Conentration of Extract(mg/ml)w/v	Zone Inhibition (mm)	Activity index
1	0.00	0	0:0
2	1.875	3	0.09
3	3.75	5	0.15
4	7.5	18	0.54
5	15	19	0.57
6	30	29	0.87

Table 3: Effect of the of crude ethanol extract of *C. zeylanicum* staph.

S. No.	Conentration of Extract (mg/ml) w/v	Zone Inhibition (mm)	Activity index
1	0.00	0	0:0
2	1.875	3	3:0
3	3.75	5	5:0
4	7.5	10	10:0
5	15	17	17:0
6	30	25	25:0

Table 4: Effect of the of crude ethanol extract of *C. zeylanicum* on *E.coli.*

S. No.	Conentration of Extract (mg/ml) w/v	Zone Inhibition (mm)	Activity index
1	0.00	0	0:0
2	1.875	2	0.01
3	3.75	3	0.15
4	7.5	12	0.63
5	15	15	0.78
6	30	20	1.05

Table5: Effect of the of crude ethanol extract of *C. zeylanicum* on *Peptococcus*.

sensitivity at the concentration 30 mg /ml(w/v). However, at lower concentration of 1.875mg/ml the four types of tested bacteria exhibit the same sensitivity and same DIZ, which was 3mm. There was no zone against any bacteria in the negative control group (DMSO containing disc), whereas CIP (positive control group) showed antibacterial activity against all tested bacteria except *E. coli.*

This study is the first of a series of studies to produce Cinnamon extract for local control of oral wound infections. The antibacterial activity was expressed as diameters of inhibition by using the disc diffusion test. The cinnamon extract, showed a diameter of inhibition zone ranging from 20 to 29 mm, when used a higher concentration of the ethanol extract(30mg/ml), for the tested bacteria after 24 h of incubation.

S. Aureus and *E. coli* were found to be the most sensitive towards the antimicrobial activity of Cinnamon crude extract with zone of inhibition 29mm,25mm respectively at this concentration, whereas for *S. Mutans* and *Peptococcus* ZID about 21mm, 20mm table 3,4 respectively, suggesting a high antibacterial activity against both gram positive and gram negative bacteria. The concentration chosen was based on literature review. The major chemical constituents in the (C.Z), crude extract was cinnamaldehyde which dissolved in solvents like 95% ethanol). The results showed tendency of C.Z. molecule for growth inhibition of wide range of bacteria. However, the molecular mechanism through which this has been achieved is still unknown.

This study finding concurs with other studies. Mandal., *et al*. from Kolkata, India, found that clinical isolates of Methicillin resistant *S. Aureus* (MRSA)are sensitive to ethanolic extract of stem bark *C. zeylanicum,* with a diameter of inhibition zone ranging from 22 to 27 mm [18]. The result of this study were also found to be consistent with works done by Prabuseenivasan., *et al*. in Chennai in 20l6 Kalemba., *et al.* in chenni in 2006 in Poland and kamal Rai Anej., *et al.* in Haryana , India in 2009, several bark extracts, which were prepared using different organic solvents were tested in vitro against *Klebsiella Pneumonae* 13883, *Bacillus Megatrium* NRS, *Pseudomonas Aerguinosa* ATTC 2789, *Escherichia coli* ATCC 8739, *Enterobacter cloacae* ATCC 13047, *Staphylococcus aureus* 6538 P, *Corynebacterium xerosis* UC 9165, *Streptococcus Faecalis* DC 74, by the disk-diffusion method. [19,20].

Furthermore, the effectiveness of both essential oils and other cinnmon extract could have an influence against oral infectious bacteria,has been investigated by other studies.

Chaudhari., *et al.* in 2012 [14], demonstrated the activity of cinnamon essential oil against Streptococcus Mutans and suggested cinnamons essential oils can be useful alternative to the usual antimicrobial compound, which are commonly used in treatment of oral infection.

The antibacterial activity of *C. Zeylanicum* fresh leaf extract on Enterococcus Faecalis was investigated. *E. Faecalis* is one of the main offending micro-organisms in pulp and periapical diseases of the oral cavity. The antibacterial activity of *E. Faecalis* sample, which was grown both on cellulose nitrate membrane and on a tooth model system, was determined by the agar diffusion test and microdilution method. The obtained inhibition zones have been found to vary according to the cinnamon fresh leaf extract concentration (5% to 20%). This suggests that cinnamon extract is active against both planktonic and biofilm forms. This, also, has been observed *in vivo* [21].

Moreover, Miller., *et al.* [2015] demonstrated the effectiveness of essential oil extracted from C. zelanicum fresh leaves against both S. Mutans and Lacto bacillus Acidophilus involved in in the formation of dental plaque and subsequent development of dental caries [22]. The differences between different studies' may be explained by the diversity of experimental methods with different inoculum size, culture media and bacterial strains with varying susceptibilities [23,24].

Conclusion and Clinical Significance

Cinnamomum Zeylanicum ethanol extract can provide valuable support for antibacterial therapy and contribute potential development antimicrobial agents against aerobic, anaerobic, gram positive and gram negative oral pathogens.

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Conflict of Interests

No conflict of interests is related to this study.

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