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Antimicrobial activity of Dalchini (*Cinnamomum zeylanicum* bark) extracts on some dental caries pathogens

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ABSTRACT

The objective of the study was to assess the antimicrobial potential of the cinnamon bark with a view of searching a novel extract as a remedy for dental caries pathogens. Acetone, ethanol, methanol and aqueous (cold and hot) extracts of dalchini, the bark of *Cinnamomum zeylanicum*, were tested against three bacteria and two yeasts causing dental caries using agar well diffusion method. The ethanolic, methanolic and acetonic cinnamon bark extracts showed greater antimicrobial activities than the water extracts against the tested bacteria (*Streptococcus mutans* and *Staphylococcus aureus*) and the yeasts (*Candida albicans* and *Saccharomyces cerevisiae*) while *Lactobacillus acidophilus* was resistant to all the five extracts. Of the five extracts of cinnamon bark screened, the acetonic extract showed greater antimicrobial activity than the corresponding water and alcoholic extracts. Strongest antimicrobial activity was observed in the acetonic extract against *C.albicans* (zone of inhibition 29.30mm and 12.5mg/ml MIC) showing higher inhibition zone than the standard antifungal drug amphotericin B (zone of inhibition 13mm).

Keywords: Cinnamon bark, antibacterial activity, antifungal activity, minimum inhibitory concentration (MIC), Dental caries.

INTRODUCTION

Medicinal plants are part and parcel of humans since the dawn of civilization. In India they form the backbone of several indigenous traditional systems of medicine. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (1, 2, 3, 4, 5). The emergence of resistant bacterial and fungal strains due to overuse of antibiotics is a cause of worldwide concern (6). The use of plant extracts and phytochemicals with known antimicrobial properties may have great significance in therapeutic treatments (1, 7, 8, 9, 10). *Cinnamomum zeylanicum* Blume, a member of the family Lauraceae, is a tropical evergreen tree, native to Sri Lanka and the Malabar Coast of India, called differently in different languages such as *dalchini* in Hindi, *cannelle* in French, *kaneel* in German, *canela* in Spanish, *yook gway* in Chinese and *kurunda* in Sinhalese. The botanical name *Cinnamomum* is derived from the Hebraic and Arabic term *amomon*, meaning fragrant spice plant (11). In India, Southeast Asia, United States and in the European countries, cinnamon is used for flavouring foods, beverages, boiled beef, pickles, chutneys and ketchup. Medicinally, cinnamon is used in the treatment of diarrhoea, flatulent dyspesia, poor appetite, low vitality, kidney weakness and rheumatism, influenza, cough, bronchitis, fever, arthritic angina, palpitations, hypertension and nervous disorders, stimulating the circulatory system and capillary circulation, spasms, vomiting and controlling infections, reducing blood sugar levels in diabetics and as a skin antiseptic (8, 12, 13, 14, 15). It is sometimes used alternatively with damiana (*Turnera diffusa*) to promote conception (16). It is proven to be particularly effective against some species of toxicogenic fungi as well as respiratory tract pathogens, including species belonging to the genera *Aspergillus*, *Candida*, *Cryptococcus* and *Histoplasma* (17). Aqueous and alcoholic extracts of cinnamon have demonstrated antibacterial effects against *Helicobacter pylori* (18, 19). The antimicrobial properties of some spices and their components have been documented (20, 21, 22, 23) and the studies confirm that cinnamon inhibits the growth of both Gram positive and Gram negative food borne pathogens or spoilage bacteria, yeast and molds (24, 25).

The objective of this study was to assess 1) the *in vitro* antibacterial and antifungal properties of different extracts of cinnamon against common dental caries pathogens, 2) determination of minimum inhibitory concentration (MIC) of each extract against each pathogen with a view of searching a novel extract as a remedy for dental caries.

MATERIALS AND METHODS

Bark of *Cinnamomum zeylanicum* (dalchini) was collected from the local market of Delhi, India. It had a pale yellowish colour, sweet aroma and had a sweet finish in the after taste. Dr. B.D.Vashishta (Botany Department,Kurukshetra University, Kurukshetra) confirmed the identification of the specimen.

Extraction

The samples were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (30^{o}C) for two days and pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight bottles. Four different solvents namely ethanol, methanol, acetone and aqueous (hot and cold) were used for extraction. A 10g amount of pulverized bark was separately

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soaked in 100ml of acetone, ethanol, methanol, and cold sterile distilled water for 24h. Also the same amount (i.e. 10g) of pulverized bark was immersed in 100ml of hot sterile distilled water (100^{O}C) and allowed to stand for 30min on a waterbath with occasional shaking and kept undisturbed for 24h. Each preparation was filtered through a sterilized Whatman No.1 filter paper and the filtered extract was concentrated under vacuum below 40^{0}C using Heidolph, VE-11 rotaevaporator (7, 26). The dried extract thus obtained was exposed to UV rays for 24h and checked for sterility on nutrient agar plates and stored in labelled sterile bottles in a freezer at 4^{o}C until further use (27).

Test Microorganisms

Three dental caries causing bacteria *Streptococcus mutans* (MTCC*497), *Staphylococcus aureus* (MTCC 740), *Lactobacillus acidophilus* (MTCC *447) and two yeasts *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170) were procured from Microbial Type Culture Collection, IMTECH, Chandigarh. The microorganisms were subcultured on the specific media recommended for different microorganisms such as Brain heart infusion agar (*S.mutans*), Nutrient agar (*S.aureus*), Lactobacillus MRS agar (*L.acidophilus*), Malt yeast agar (*C.albicans* and *S.cerevisiae*) and incubated aerobically at 37^{O}C. The media were procured from HiMedia Laboratory Pvt. Ltd., Bombay, India. Identification of all the strains was confirmed by standard biochemical and staining methods (28, 29, 30).

Screening for Antimicrobial Activity

The acetone, methanol, ethanol, cold and hot water cinnamon bark extracts were used for the screening. Antimicrobial activity of various extracts was determined by the agar well diffusion method (31). In this method, pure isolate of each microbe was subcultured on the recommended specific media for each microorganism at 37^{O}C for 24h. A plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10^{6} cfu/ml (standardized by 0.5McFarland standard) and used as the inoculum for performing agar well diffusion assay. One hundred microlitre (100µl) of inoculum of each test organism was spread onto the specific media plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells or cups of 8mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. The extracts were reconstituted in 20% DMSO for the bioassay analysis (32). A 100µl volume of each extract was propelled directly into the wells (in triplicates) of the inoculated specific media agar plates for each test organism. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37^{O}C for 24h (33, 34). Sterile DMSO served as the negative control and ciprofloxacin (for bacteria) and amphotericin-B (for fungi) served as the positive control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8mm (35). The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.

Determination of Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24h (36). The MIC for the acetonic, methanolic and ethanolic extract was determined by following the modified agar well diffusion method (31). A twofold serial dilution of each extract was prepared by first reconstituting the powder in 20% dimethylsulphoxide (DMSO) followed by dilution in sterile distilled water to achieve a decreasing concentration range of 50mg/ml to 0.39mg/ml. A 100 µl volume of each dilution was introduced into wells (triplicate) in the specific media agar plates already seeded with 100µl of standardized inoculum (10^{6} cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37^{o}C for 24 hrs and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition, considered as the MIC, was recorded for each test organism (27).

RESULTS AND DISCUSSION

We chose *Streptococcus mutans, Lactobacillus acidophilus* and *Candida albicans* as test microorganisms for our study because they have been implicated in dental caries (37, 38). *C.albicans* is also the

Table 1: Antimicrobial activity of Cinnamon bark extracts on dental caries pathogens determined by agar well diffusion method on specific media for each test microorganism.

Cinnamon bark extracts (mg/ml)	Diameter of growth of inhibition zones (mm)				
	Streptococcus mutans	*Staphylococcus aureus*	*Lactobacillus acidophilus*	*Candida albicans*	*Saccharomyces cerevisiae*
Acetone	12.31[a]±0.57[b]	16±0	-	29.30±1.15	16.65±0.57
Methanol	12.94±1	14.31±0.57	-	18.96±1	17.65±0.57
Ethanol	14.95±1	14.65±0.57	-	11.64±0.57	18.32±0.57
Hot water	10.64±0.57	14±0	-	-	-
Cold water	10±0	10.31±0.57	-	-	-
Ciprofloxacin (5 µg/ml)	27.32±0.57	34.66±0.57	25.65±0.57	Nt	Nt
Amphotericin B (100 units/ ml)	Nt	Nt	Nt	13±0	11.94±1
DMSO	0	0	0	0	0

(-) = no activity, Nt = not tested[a] ***Values, including diameter of the well (8 mm), are means of three replicates*** [b] ***± Standard deviation***

Table 2: MIC of Cinnamon bark extracts against dental caries pathogens on specific media for each microorganism determined by modified agar well diffusion method.

Cinnamon bark extracts	MIC (mg/ml)				
	Streptococcus mutans	*Staphylococcus aureus*	*Lactobacillus acidophilus*	*Candida albicans*	*Saccharomyces cerevisiae*
Acetone	-	25	Nt	12.5	50
Methanol	-	25	Nt	25	50
Ethanol	-	25	Nt	50	50

(-) = no activity, Nt = not tested

most common yeast isolated from the oral cavity, and is associated with fungal oral infections, endocarditis and septicemia (39). *Staphylococcus aureus*, a major human pathogen, is responsible for a number of hospital – acquired infections and propagates mainly in mouth and hands in the hospital environment (40, 41, 42). *Saccharomyces cerevisiae* considered to be an opportunistic pathogen in the oral cavity, may induce significant oral risks by acting as a tertiary colonizer in the progress of dental caries thus causing both superficial and invasive infections (43).

The results of antimicrobial properties of ethanol, methanol, acetone and aqueous (hot and cold) extracts of cinnamon bark as well as the positive control ciprofloxacin (for bacteria) and amphotericin-B (for fungi) are presented in Table 1 and the MIC of the three extracts (ethanol, methanol and acetone) against the test pathogens are presented in Table 2 . The antimicrobial activity of cinnamon bark extracts on the agar plates varied greatly in different solvents. Both the positive controls produced significantly sized inhibition zones against the test bacteria (ciprofloxacin) and yeasts (amphotericin-B). However, the negative control produced no observable inhibitory effect. Of the five extracts screened for antifungal activity, acetone, methanol and ethanol showed antifungal activity against both the yeasts *Candida albicans* and *Saccharomyces cerevisiae*. However, water extracts, both hot and cold, showed no activity against the test strains. The acetonic extract was most effective against *C.albicans* showing the highest zone of inhibition (29.30mm) followed by the methanolic (18.96mm) and ethanolic extract (11.64mm). *C.albicans* was found to be most sensitive pathogen which survived upto 6.25mg/ml in the acetonic extract, thus having a MIC of 12.5mg/ml followed by the methanolic extract (25mg/ml) and the ethanolic extract (50mg/ml). The inhibition zones produced by the three solvents against *S.cerevisiae* ranged between 16.65mm and 18.32mm. *S.cerevisiae* was found to be comparatively more resistant than *C.albicans* as it survived upto 25mg/ml, thus having a MIC of 50mg/ml in all the three extracts tested. Interestingly the acetonic extract showed much more potent activity against *C.albicans* (29.30mm) than that of the standard drug amphotericin-B (13mm) thus having a great potential to control candidiasis. Methanolic extract showed almost equal antibacterial activity against both *Streptococcus mutans* (14.95mm) and *Staphylococcus aureus* (14.65mm). *S.aureus* was found to be more sensitive to all three extracts and survived upto 12.5mg/ml of the extract, thus having a MIC of 25mg/ml. *S.mutans* was found to be comparatively resistant bacterium as it survived upto 50mg/ml of each extract, thus having a MIC of 100mg/ml (Table 2). The ethanolic, acetonic and methanolic bark extracts showed greater antimicrobial activities than the water extracts against the tested bacteria and yeasts while *Lactobacillus acidophilus* was resistant to all the five extracts. Lactic acid bacteria are known to produce acetaldehyde, hydrogen peroxide, diacetyl, carbon dioxide, polysaccharides and bacteriocins (44, 45) some of which may act antagonistically as antimicrobials. The absence of antifungal activity and limited spectrum of antibacterial activity in the aqueous extracts of cinnamon might either be due to the more solubility of the active principles in analytical solvents than the aqueous solvents (3, 27) or presence of active components in insufficient quantities in the crude extracts to show the activity with the dose levels employed (46).

The antimicrobial activity shown by the cinnamon extracts may be due to the presence of cinnamaldehyde, an aromatic aldehyde. Cinnamon bark is rich in cinnamaldehyde (50.5%) which is highly electronegative and interferes in biological processes involving electron transfer and react with nitrogen containing components, e.g. proteins and nucleic acids and therefore inhibit the growth of the microorganisms (47). Cinnamaldehyde has been proven to be active against many pathogenic bacteria (48, 49) including *S.aureus, E.coli* 0157:H7 (50) and *Salmonella typhimurium* (51). It is among the most active component against Gram positive and Gram negative bacteria (52). The cinnamon bark also contains tannins consisting of polymeric 5,7,3',4'-tetrahydroxy flavan-3,4–diol units (10, 53). Flavanols present in the bark might be responsible for the highly significant antifungal activity exhibited by the acetonic extract of cinnamon against *C.albicans*, as the active components extracted in acetonic extracts are flavanols (1). Of the five extracts of cinnamon bark screened, the acetonic extract showed greater antimicrobial activity than the corresponding water and alcoholic extracts. Thus from the overall result it is evident that the acetonic extract has been found to have a better antimicrobial activity, substituting the findings of earlier workers (1, 2, 54), who rated acetone as the best solvent followed by methanol, ethanol and water.

CONCLUSION

On comparison of the antimicrobial activities of all the five extracts tested against the bacterial and fungal strains, it was finally concluded that acetone cinnamon extract emerged as the potent agent exhibiting even much higher antifungal activity than the standard antifungal drug amphotericin-B. The need of the hour is to perform more and more screening of the natural products or plant parts as such screening experiments form a primary platform for further phytochemical and pharmacological studies that may open the possibilities of finding new clinically effective antifungal and antibacterial compounds against the dental caries pathogens and the resistant bacterial and fungal pathogens.

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