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The antibacterial efficacy of aqueous cinnamon extract on potential periodontal pathogens:Invitro study.

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Abstract

Aim: The role of microorganisms in causing periodontitis is well established. Many studies have evaluated the antimicrobial efficacy of Cinnamon on oral pathogens but very few literatures exist with respect to periodontal pathogens. In this study the in vitro antibacterial activity of aqueous cinnamon extract on some potential periodontal pathogens were evaluated using the minimum inhibitory concentration(MIC), zone of inhibition and time kill curve for predominant periodontal pathogens like Porpyromonas gingivalis (Pg), Prevotella intermedia(Pi) ,Tanerella forsythia(Tf) and Fusobacterium nucleatum(Fn).

Materials and Methods: MIC was determined by broth dilution. Disc diffusion method was used for zone of inhibition and serial dilution was used to determine time kill curve.

Results: The MIC of Prevotella intermedia, Tanerella forsythia and Fusobacterium nucleatum is 25mg/ml whereas of Porpyromonas gingivalis its 12.5 mg/ml. At 75µ/ml of cinnamon extract the zone of inhibition for Tf and Pi was 18mm, for Pg it was 15mm and 8mm for Fn. At 2µ/ml only Pi was found to have a zone of inhibition of 13 mm. At 2hours all microbes were sensitive to cinnamon extract except Pg.

Conclusion: The results of the study suggest that cinnamon has potential antimicrobial activity against periodontal pathogens and can be used as an alternative to synthetic substitutes for oral health care.

Keywords: cinnamon, minimum inhibitory concentration, periodontitis, Porpyromonas gingivalis ,time kill curve, herbal medicine,zone of inhibition,

Introduction

Periodontitis is a complex disease with multiple component causes, like genetics, epigenetic influences and environmental factors, and the host immune inflammatory reaction all of which conspire to establish and propagate the periodontitis lesion. But the role of periodontal pathogens is the prerequisite which is mainly composed of gram negative anaerobic bacteria.[1]

Many plants used in traditional medicine represent rich sources of natural bioactive substances with health-promoting effects and no side effects. Nowadays, over 65% of the world population depends on traditional

medicine for health care.[2] Cinnamon has been used as medicine in Indian and Chinese traditional medicine for decades. There are more than 300 evergreen aromatic trees and shrubs in the genus Cinnamomum (family Lauraceae).The four species of great economic importance for their multiple culinary uses as common spices worldwide are *Cinnamon zeylanicum Blume* (Cinnamon verum /Sri Lanka cinnamon)(CZ), *Cinnamon loureiroi Nees (*Vietnamese cinnamon), *Cinnamon burmanni Blume* (Indonesian cinnamon) and *Cinnamon aromaticum Nees* (Cinnamon cassia/Chinese cinnamon)(CC).Cinnamon bark, leaves, flowers and fruits are used to prepare essential oils, which are destined for use in cosmetics, food and in medicinal products.[2]

CZ, also known as Ceylon cinnamon or 'true cinnamon' indigenous to Sri Lanka and southern parts of India and CC also known as Chinese cinnamon differ in their content of coumarin which possess strong anticoagulant, carcinogenic and hepato-toxic properties. Because of very high coumarin content regular use of CC is not advocated where as CZ with hardly any coumarin has not shown to carry such risks.[3] Cinnamon and cinnamon extracts have been shown to have anti-inflammatory, anti-oxidant, antidiabetic, anticancer, cholesterol and lipid lowering function and used in neurologic and cardiovascular diseases.[2] The bark of the cinnamon tree contains an essential oil called cinnamonaldehyde, which give cinnamon its characteristic flavor and aroma.[4] Cinnamon have been used in for its antiplaque and anti-gingivitis properties [4] and against carious pathogens.

There are many studies evaluating the antibacterial activity of cinnamon extract against oral pathogens, but very few in relation to periodontal pathogens. So in this study we try to evaluate the minimum inhibitory concentration (MIC),zone of inhibition and time kill curve of cinnamon extract against the periodontal pathogens like *Porpyromonas gingivalis* , *Prevotella intermedia, Tanerella forsythia* and *Fusobacterium nucleatum* .

Materials And Methods

preparation Of Cinnamon Extract

Cinnamon exract preparation was done at Bapuji Pharmacy College, Davangere. Fresh cinnamon bark purchased from local market in Davangere was ground to a fine powder in a mechanical grinder. To 100 ml of sterile deionized water ten grams of finely powdered cinnamon were mixed and kept in a water bath in a round-bottomed flask at 55 - 60° C. After five hours this mixture was then filtered through sterile filter paper (Whatman, UK). The aqueous extract was filtered through a muslin cloth. A dried extract was obtained by evaporation of the filtrate in a porcelain dish at 40° C.This dried extract was suspended in polyethylene glycol (PEG) 400 (20% v:v) and sterile distilled water to give a final concentration of 20% w:v. [4]

Microbial analysis

The antibacterial activity of cinnamon extract was carried out at department of microbiology and immunology in NGH Maratha Mandal Institute of Dental Sciences and Research Centre,Belgaum, Karnataka .The study was conducted on the following periodontal pathogens and these are their standard strain numbers : *Porpyromonas gingivalis* (ATCC no.33277) (American Type Culture), *Prevotella intermedia* (ATCC no.25611), *Tanerella forsythia* (ATCC no. 43037) and *Fusobacterium nucleatum* (ATCC no. 25586).

Determination of MIC

Brain heart infusion (BHI) broth contained the following ingredients: Calf brain, Beef heart, Proteose peptone, Dextrose, Sodium chloride, and Disodium phosphate .The final pH at 25ºC was around 7.4+/-0.2. 9 dilutions of cinnamon extract was done with BHI for MIC. In the initial tube 20microliter of cinnamon extract was added

into the 380microliter of BHI broth. For dilutions 200microliter of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200microliter was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI broth. In each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated in an anaerobic jar for 48-72 hrs and observed for turbidity.[5] (Fig. 1)

Determination of Time kill curve

Broth macrodilution method was used. Equal quantity of the broth with organism and cinnamon extract was mixed and immediately it was plated, this was noted as 0 hrs. Plates were kept in anaerobic jar and were cultured or plated at every 5mins, 10mins, 30mins and 2hrs. It was after 48-72 hrs of incubation the plates were removed and the colony count was noted. (Fig. 2)

Determination of zone of inhibition by disc diffusion

BHI agar was used as the medium. The inoculated cultured media was pressed with a heated tube of 5 mm diameter, and removed immediately in the plate.5 such wells were made on each plate. Cinnamon extract wss diluted to 5mg/ml, 10mg/ml, 25mg/ml, 50mg/ml and 75 mg/ml and 50 µl of each of these was added into the each of the wells in each plate. The plates were then incubated in an anaerobic jar for 18-24 hr and kept at 37 ºC. After incubation, the diameter of zone of inhibition for each extract was measured in millimeters as its diameter between the edges of the lawn.[6] (Fig. 3)

Results

The mean diameter of zone of inhibition for all groups for P.intermedia, P.gingivalis ,T. forsythia and F.nucleatum are shown in Table 1.At 75µ/ml of cinnamon extract the zone of inhibition was greatest for T. forsythia and P. intermedia (18 mm), followed by P.gingivalis (15 mm) and F.nucleatum (8 mm). At 50µ/ml T. forsythia and P. intermedia had the highest zone of inhibition of 15 mm followed by 12 mm for P. gingivalis. At this concentration F. nucleatum was found to be resistant to cinnamon extract. At 25 µ/ml only P.intermedia was found to have a zone of inhibition of 13 mm. All other microbe were resistant. F. nucleatum was sensitive only at 75µ/ml. P. gingivalis and T. forsythia are sensitive at 75 µ/ml and 50 µ/ml. P. intermedia is sensitive at 75 µ/ml,50 µ/ml and 25 µ/ml.

The results of MIC values of all groups by broth dilution method are shown in Table 2.For 25mg/ml of cinnamon extract all pathogens were sensitive.For 12.5mg/ml of cinnamon all cultured organisms were resistant except P. intermedia. The results of time kill curve (Table 3,Graph 1) showed that the number of colonies of all pathogens gradually decreased from 0 min, 5 min,10 min ,30 min and 2 hrs.At 2 hrs all microbes were showing colonies except *P. gingivalis.P.intermedia* had the highest colony count of 120, followed by 98 and 32 respectively for *F.nucleatum* and *T. forsythia* .

Discussion

This study used the antimicrobial susceptibility test to determine the antimicrobial efficacy of cinnamon over oral pathogens and it clearly showed that cinnamon extract obtained from bark showed excellent antibacterial activity against periodontal pathogens like *P. gingivalis*, *P. intermedia*, *T. forsythia* and *F. nucleatum* which are among the secondary colonizers of the dental biofilm and have a potential role in development of periodontitis.[7] In

this study antimicrobial susceptibility test of cinnamon was done using MIC. Disc diffusion and broth dilusion have been used to determine the MIC which have shown consistently providing reproducible and repeatable results.

The results of our study suggest that the MIC value of aqueous cinnamon extract for *P. gingivalis*, *F. nucleatum*, *T. forsythia* is 25mg/ml and for *P. intermedia* its 12.5mg/ml. For *P.intermedia* 25µl/ml of cinnamon showed 13mm of zone of inhibition whereas all other pathogens were resistant at this concentration of cinnamon. For *P. intermedia* and *T. forsythia* the zone of inhibition was 18mm at 75µl/ml of cinnamon. In an in-vitro study Abidin et al in 2013, reported the anti-bacterial effect of cinnamon(*Cinnamomum zeylanicum* Blume) ,against major oral pathogens in caries and periodontal diseases *viz. Streptococcus mutans, Streptococcus mitis, Streptococcus salivarius, Aggregatibacter actinomycetemcomitans, P.gingivalis* and *F.nucleatum.* Following 2 hrs of exposure to the cinnamon bark oil (2.5 mg/mL) the bacterial cells of *Streptococcus mutans*, *P. gingivalis*, and *F.nucleatum* showed increased translucency.[8]

According to Gupta C et al [9] cinnamon oil produced maximum inhibition zone of diameter (IZD) of 24.0 mm against *Streptococcus mutans* as compared to clove oil (IZD = 13.0mm) whereas Kim et al [10] reported the minimum inhibition concentration of the cinnamon ethanol extract on the growth and development of *Streptococcus mutans*, *Streptococcus sanguinis*, and *Streptococcus sanguinis* as 4 mg/ml concentration.

A randomized control trial by Devanand et al., also showed the effectiveness of aqueous cinnamon extract to reduce plaque levels and gingivitis and they state that the antimicrobial activities may be due to the active substances like cinnamic aldehyde, an aromatic aldehyde and eugenol.[11]

Conclusion

The results of our study suggest that cinnamon extract showed high effects on zone of inhibition and MIC indicating that cinnamon bark extract and its constituents have the potential to be developed as a therapeutic agent in preventing bacteria related oral diseases. Further, invivo studies on the mechanism of antibacterial activity of cinnamon bark extract are warranted.

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Legends Tables,Graphs And Fjgure

Table 1: Mean diameter of zone of inhibition of periodontal pathogens by disc diffusion method

Cinnamon Extract(µl/ml)	75	50	25	10	05
Pg	15mm	12mm	R	R	R
Pi	18mm	15mm	13mm	R	R
Fn	08mm	R	R	R	R
Tf	18mm	15mm	R	R	R

S- sensitive,R- resistant,Pg - Porpyromonas gingivalis, Pi - Prevotella intermedia, Tf - Tanerella forsythia ,Fn - Fusobacterium nucleatum

Table 2: Determination of MIC by broth dilusion method.

Cinnamon Extract (mg/ml)	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Pg	S	S	S	R	R	R	R	R	R	R
Pi	S	S	S	S	R	R	R	R	R	R
Fn	S	S	S	R	R	R	R	R	R	R
Tf	S	S	S	R	R	R	R	R	R	R

S- sensitive,R- resistant,Pg - Porpyromonas gingivalis,Pi - Prevotella intermedia,Tf - Tanerella forsythia ,Fn - Fusobacterium nucleatum

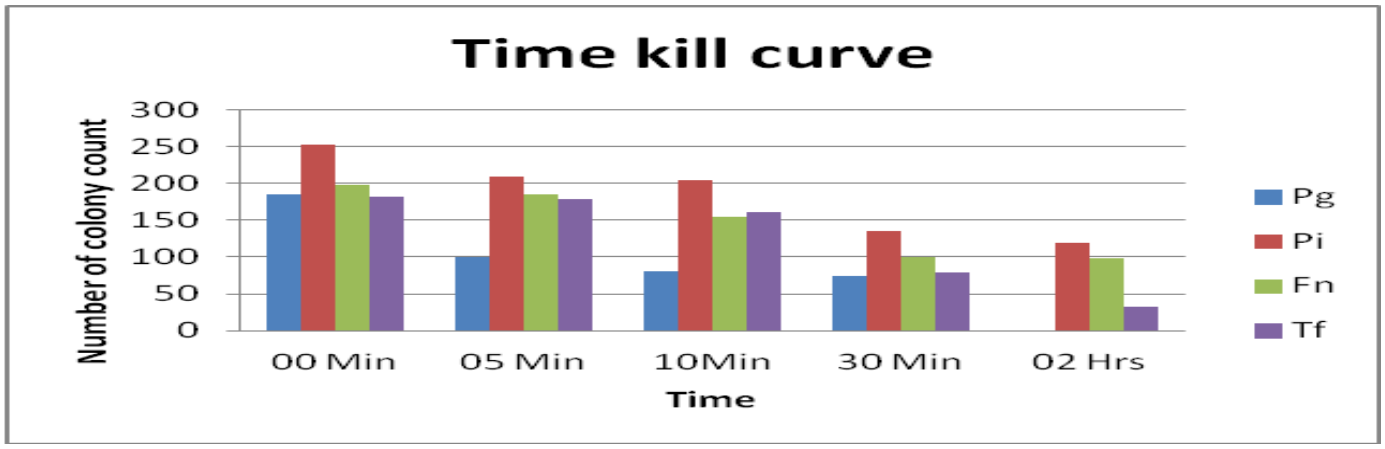
Table 3: Results of time kill curve.

Cinnamon Extract	00 Min	05 Min	10Min	30 Min	02 Hrs
Pg	186	100	81	74	NG
Pi	252	210	205	136	120
Fn	198	185	154	100	98
Tf	182	178	161	79	32

NG-no growth

Pg - Porpyromonas gingivalis,Pi - Prevotella intermedia,Tf - Tanerella forsythia ,Fn - Fusobacterium nucleatu

Graph 1:Time kill curve for tested periodontal pathogens against aqueous cinnamon extract.



Pg - Porpyromonas gingivalis, Pi - Prevotella intermedia, Tf - Tanerella forsythia ,Fn - Fusobacterium nucleatum

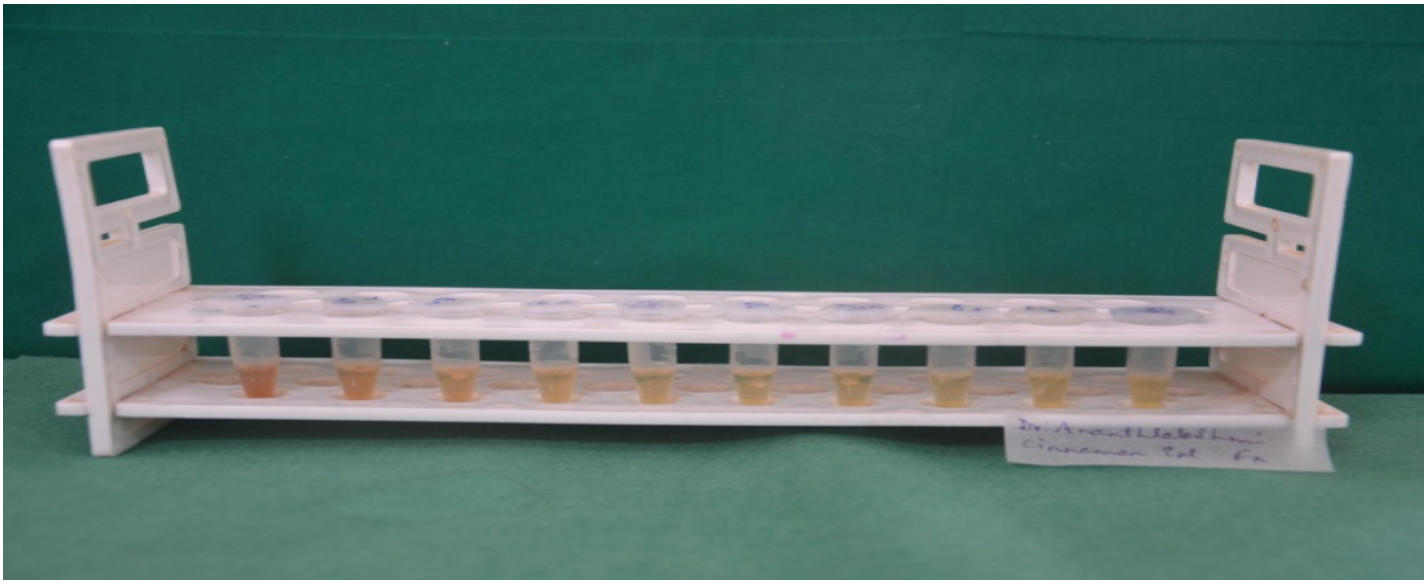


Figure 1:Serial dilution of cinnamon extract for detection of MIC.

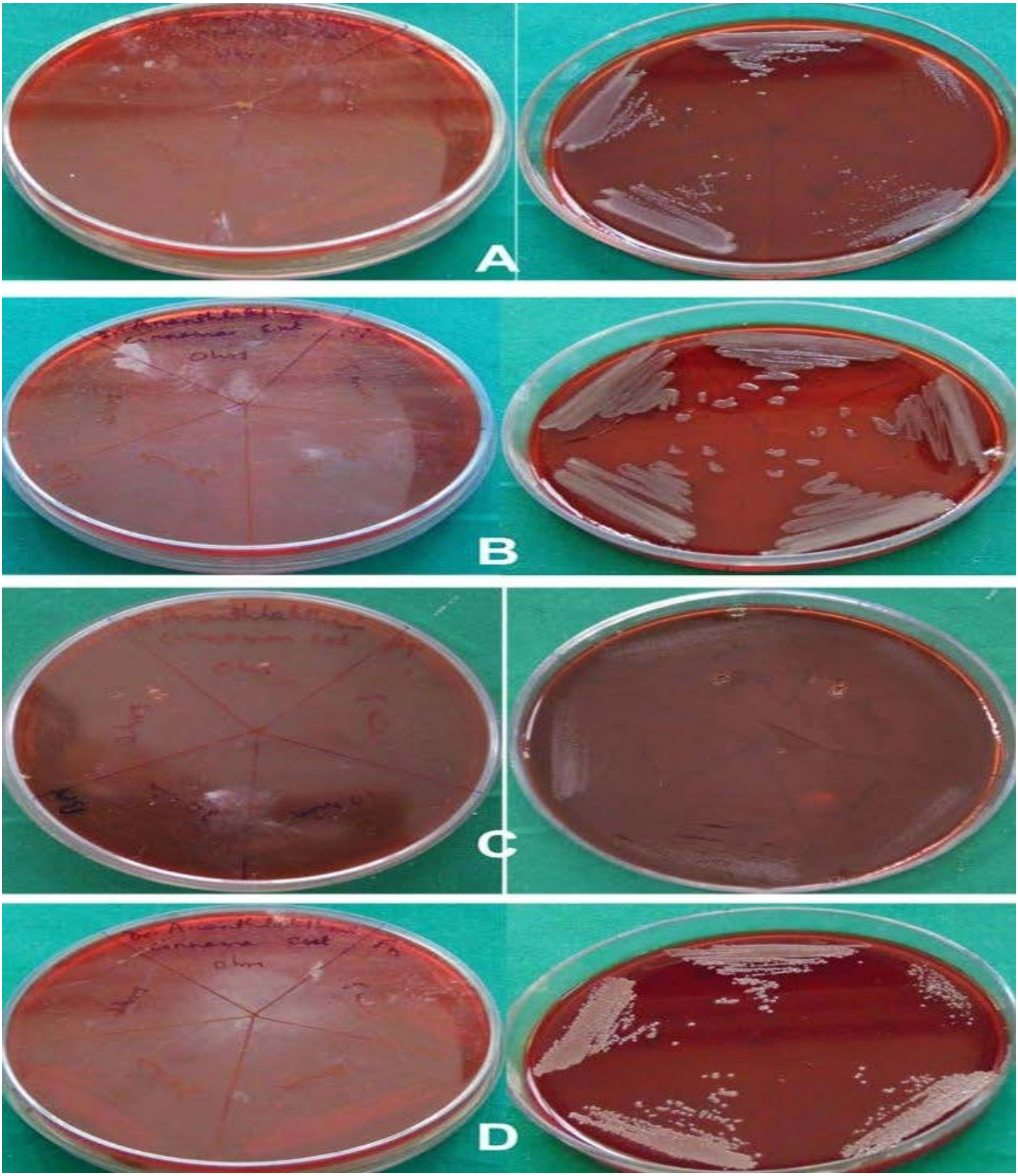


Figure 2: Plate with cinnamon extract showing the colony formation of microorganism, Tanerella forsythia (2.A), Prevotella intermedia (2.B), Porpyromonas gingivalis (2.C), and Fusobacterium nucleatum (2.D) at 0,10,30 minutes and 2 hours.

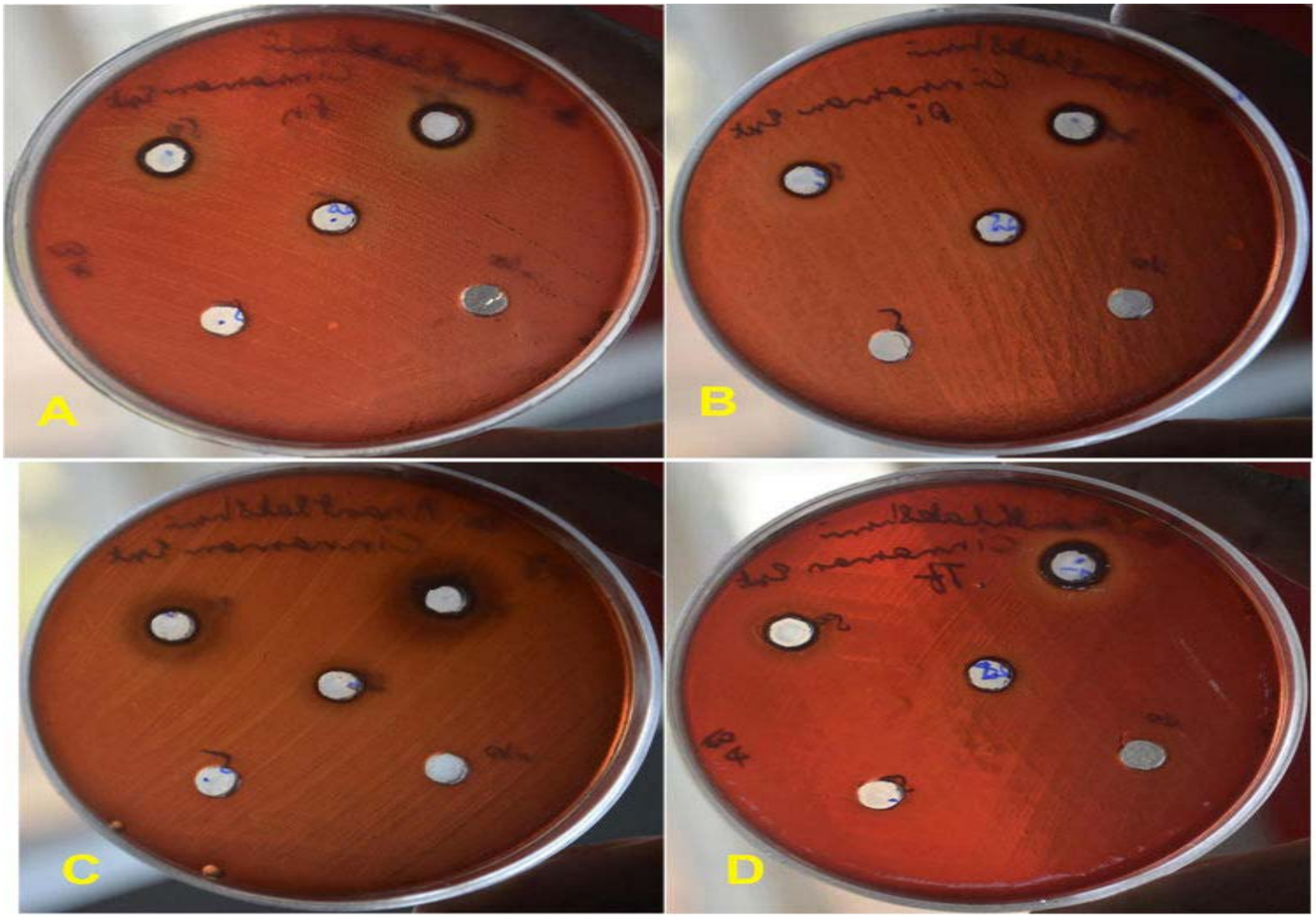


Figure 3:Disc diffusion assay showing the zone of inhibition for Fusobacterium nucleatum (3.A), Prevotella intermedia (3.B), Porpyromonas gingivalis (3.C), and Tanerella forsythia (3.D) at various concentrations of cinnamon extract namely 5 µl/ml, 10 µl/ml, 25 µl/ml, 50 µl/ml and 75µl/ml.