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Assessment of Antibacterial, Anti-Inflammatory and Antioxidant Effects of Cinnamommum Zeylanicum (Cinnamon) – An In-Vitro Study

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Abstract

Background and Objectives: Various phytochemicals-based plant extracts have been utilized in the management of periodontal diseases. Cinnamommum zeylanicum (cinnamon) is one such plant with medicinal property. The aim of this study was to assess the antibacterial, anti-inflammatory and antioxidant activity of cinnamon water extract (CWE) under laboratory condition.

Methods: In the present study, the water extract of Cinnamommum zeylanicum bark was assessed for its antibacterial effects against Streptococcus mutans, Streptococcus sanguis, Staphylococcus aureus and porphyromonas gingivalis using agar disc diffusion method and minimum inhibition concentration (MIC). The anti-inflammatory effect was assessed by lipoxygenase inhibition assay and hyaluronidase inhibition assay. The antioxidant effects were assessed by ABTS radical scavenging assay and DPPH antioxidant assay. The statistical analysis was done by Kruskal wallis test for disc diffusion, Independent Student t-test for MIC, Lipoxygenase, Hyaluronidase, ABTS and DPPH assays.

Results: The MIC of chlorhexidine showed higher percentage of inhibition of Streptococcus sanguis, Streptococcus mutans, Staphylococcus aureus and Porphyromonas gingivalis bacteria than the water extract of cinnamon bark and the difference found was statistically significant. DPPH assay results revealed that CWE showed lower radical scavenging property as compared to quercetin group with statistically insignificant difference, (P Value -0.20). Lipoxygenase inhibition assay and Hyaluronidase inhibition assay results revealed that the Indomethacin group and Cromolyn group showed higher percentage of inhibition as compared to the CWE and the differences obtained were statistically insignificant, (P Value -0.27,0.09). ABTS assay results revealed that the quercetin group had higher radical scavenging property as compared to CWE with statistically insignificant difference, (P Value -0.75).

Conclusion: Cinnamommum zeylanicum (cinnamon) possesess antibacterial, anti-inflammatory and antioxidant property, but less when compared with standards. Further studies with large sample size and under in-vivo condition have to be conducted.

Keywords: Periodontitis, Cinnamommum zeylanicum (cinnamon), lipoxygenase, hyaluronidase, antioxidant, anti-inflammatory, DPPH, ABTS.

Introduction

Dental caries and periodontal disease are the most common oral diseases worldwide. One of the most important contributing factors for the progression of gingivitis or periodontitis is the biodiversity and complex nature of the dental plaque. A shift is seen in microflora from primary to secondary colonizers that results in colonization of subgingival plaque.1

Secondary colonizers like Porphyromonas gingivalis in subgingival plaque are known to express a variety of virulence factors like capsule, fimbriae, proteases that modulates the immune responses of the host cells resulting in progression of periodontitis and bone resorption.2

The goal of periodontal treatment is to downregulate the inflammation of the tissues, reduce the bacterial load and alter the host response. This can be achieved through mechanical debridement or the use of chemotherapeutic agents. Although mechanical plaque control methods are efficient in maintaining adequate levels of oral hygiene, studies have shown that patient compliance in following these methods are not adequate in a large population. In order to overcome the shortcomings of mechanical plaque control methods, various chemotherapeutic agents have been employed to improve the efficacy of daily oral hygiene. Chemotherapeutic agents including systemic antibiotics, antiseptic mouthwashes, local drug delivery of antiseptics and antibiotics, various host modulating agents, and advanced periodontal therapeutic modalities such as lasers have been used as an adjunct to the conventional periodontal therapy. The major disadvantage of these chemotherapeutic agents is the toxicity and development of bacterial resistance.3

The rapid emergence of multiple drug resistance strains of pathogens to current antimicrobial agents has generated an urgent need for new antibiotics from medicinal plants. Herbal formulations can provide an option for safe and long-term use.4

Among the various naturally occurring agents, Cinnamon is one of the most popular herbal ingredients in traditional oriental medicine. The term cinnamon commonly refers to the dried bark of Cinnamommum zeylanicum and C. aromaticum used for the preparation of different types of chocolate, beverages, spicy candies and liquors.5

Chemical constituents of cinnamon make it rich in many health beneficial properties like anti-oxidative, antimicrobial, insulin sensitivity, anti-ulcer, anti-diabetic, anti-inflammatory etc. Cinnamon bark contains essential oils, resinous compounds, cinnamate, cinnamic acid and cinnamaldehyde. All these components of cinnamon are considered useful in the treatment of diseases such as inflammation, gastrointestinal disorders and urinary infections.6

Another potential medical use of cinnamon would be with regards to its antimicrobial properties, especially antibacterial activity. A brief study by Ranasinghe et al. investigated that the ethanolic extract of stem bark C. zeylanicum exerted antibacterial activity against clinical isolates of methicillin resistant S. aureus (MRSA).7 Chaudhari et.al in 2012, investigated that cinnamon essential oil was active 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.8 On the

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otherhand Pader et.al stated that cinnamon is a very weak antiseptic and is primarily used for flavor.9

In addition, a study by Wei A et.al explained the anti-inflammatory actions of phytoconstituents in cinnamon ethanol extract and attributed its ability to inhibit lipooxygenase pathway which leads to the formation of leukotrienes responsible for initiation of inflammation.10 Contralaterally Tracey Roizman stated that some of the susceptible individuals experienced inflammation of the mouth or skin when they use cinnamon-based toothpastes or ointment.11

Although many studies were conducted, but there is paucity of studies regarding water extract of cinnamon against the periodontal pathogens like Porphyromonas gingivalis, Streptococcus mutans, Streptococcus sanguis, and Staphylococcus aureus. Also, many studies have not shown effective concentration of aqueous extract of cinnamon required to act as antibacterial, anti-inflammatory, or antioxidant against oral pathogens.

This invitro study was done to explore the effective concentration of cinnamon water extract required to inhibit oral microorganisms (Porphyromonas.gingivalis, Streptococcus mutans, Streptococcus sanguis, and Staphylococcus aureus). Also, the evaluation of the anti-inflammatory, antioxidant and antibacterial effects of Cinnamommum zeylanicum (cinnamon) under laboratory condition was conducted.

Material And Methods

Plant materials

The Cinnamommum zeylanicum (cinnamon) barks were procured from the general market and the laboratory procedures were done at Skanda Life Sciences Private Limited, Nagarbhavi, Bangalore.

Preparation of plant extract

Barks were then dried in shade and then powdered into fine particles with the help of a blender. These powdered barks were then used to prepare the plant extract using water. 15 g of the powdered extract material was placed in a thimble made of Whatman Filter paper No1. The whole apparatus was kept on a heating mantle for 4 hours maintaining the boiling point of the solvent. Finally, the pure extract obtained was cooled and stored in vials at room temperature for further usage.

Determination of Antimicrobial Activity

Screening of Cinnamommum zeylanicum (cinnamon) extract by Disc Diffusion

Method

Cell suspension prepared on Tryptic soy broth for Streptococcus mutans, Staphylococcus aureus, Streptococcus sanguis and Porphyromonas gingivalis. Porphyromonas gingivalis were cultured anaerobically in anaerobic chamber. The cell suspensions of all the cultures were adjusted to 1-2x 105cells/ml. 10µl of 100 mg/mL stock extract of Cinnamommum zeylanicum, chlorhexidine and water were impregnated on 6mm sterile Whatman No. 1 discs. The plates were then incubated at 35 0C for 24-48 hrs. After the incubation was done the agar plates were observed for zone of inhibition around the disc (fig.1). The assay was repeated thrice and mean of three experiments was recorded.

MIC determination against the bacteria by micro broth dilution technique as per National Committee for Clinical Laboratory Standard (NCCLS) method

Cell suspension prepared from bacterial cultures grown on Trypticase soya broth was adjusted to 1-2x105cells/mL. 90 µl of Cinnamommum zeylanicum (cinnamon) extract was then mixed with different test concentrations (0.00, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.00 mg/ml) with 10 µl inoculum in 96 well plates in triplicates. The bacterial cultures were then incubated at 35 0C. After the incubation process, the bacterial test plates were observed after 24-48 hrs. was completed. Optical density (OD) was

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measured in Tecan plate reader at 600 nm. MIC is determined as Minimum concentration of drug giving 50% inhibition of OD as compared with control.

Determination of Antiinflammatory Activity

Lipoxygenase inhibition assay

0.1 mL of 500-1000 units Lipoxygenase enzyme and 0.8mL of test solution / reference standard (Indomethacin, 300 µg/mL dissolved in 3% methanol) of various concentrations, were mixed and incubated at room temperature for 5 min, after which, ml of substrate solution was added and mixed well. The absorbance was then measured for 4 min at 234 nm. Control reaction was carried out without test sample (Cinnamommum zeylanicum bark extract).

The percentage inhibition of Lipoxygenase is calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (bark extract)}}{\text{Absorbance (control)}} \times 100$$

Hyaluronidase inhibition assay

4.15 mg/ml of hyaluronidase in 0.1 M acetate buffer and 6 mg/mL of sodium hyaluronate in 0.1 M acetate buffer was mixed and activated. Following activation, the enzyme mixture was pre-incubated with 200 ul of cinnamon extract and reference standard (cromolyn) at various concentratThe percentage inhibition of Hyaluronidase is calculated as follows: ions for 20 minutes at 37 0C.

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (cinnamon extract)}}{\text{Absorbance (control)}} \times 100$$

Determination of Antioxidant Activity

DPPH assay

90ul of DPPH solution was treated with 180 µl of various concentration of cinnamon extract. The different concentrations tested for reference standard were 0.00, 0.312, 0.625, 1.25, 2.5, 5, 10 ug/ml. The reaction mixture was mixed and incubated at 25°C for 15 minutes and the absorbance was measured at 510 nm using semi-auto analyser (fig. 2). The antioxidant property was then analysed using tecan plate reader (fig. 3).

A control reaction was carried out without the test sample (cinnamommum zeylanicum bark).

ABTS (2,2'-azinobis-ethyl-benzothiozoline-6-sulphonic acid) assay

Different concentrations of the cinnamon bark extract and the reference standard i.e. quercetin (highest volume taken was 50ul) were added to 950 ul of ABTS working solution to give a final volume of 1ml, made up by adding PBS. The absorbance was recorded immediately at 734nm. The percent inhibition was calculated at different concentrations and the IC50 values were calculated by Log-Probit analysis.

A control reaction was carried out without the test sample (cinnamommum zeylanicum bark).

Statistical Analysis

Kruskal Wallis test, Mann whitney, Post hoc Analysis test for disk diffusion method and Student unpaired t-test for all other assays.

Figures

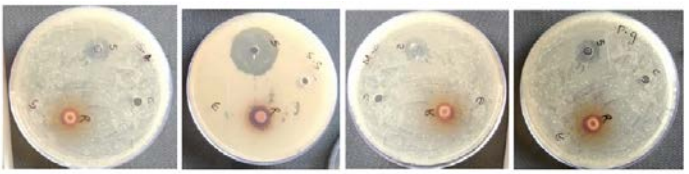

Fig. 1: Inhibitory activity of Cinnamon against Streptocoocus aureus, Streptococcus sanguis,

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Streptococcus mutans, Porphyromonas gingivalis; S- chlorhexidine, R- Cinnamon, C- Water

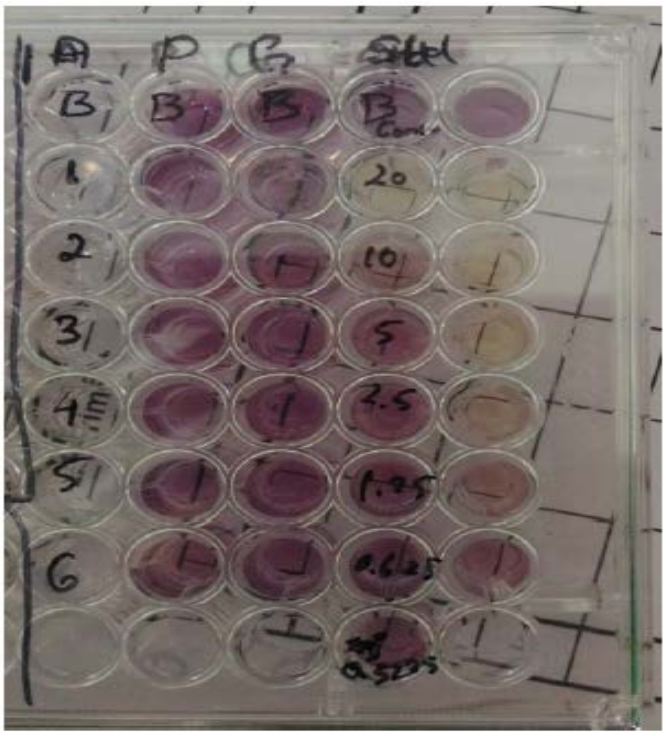

Fig.2: DPPH inhibition essay carried out on a 96 well plate

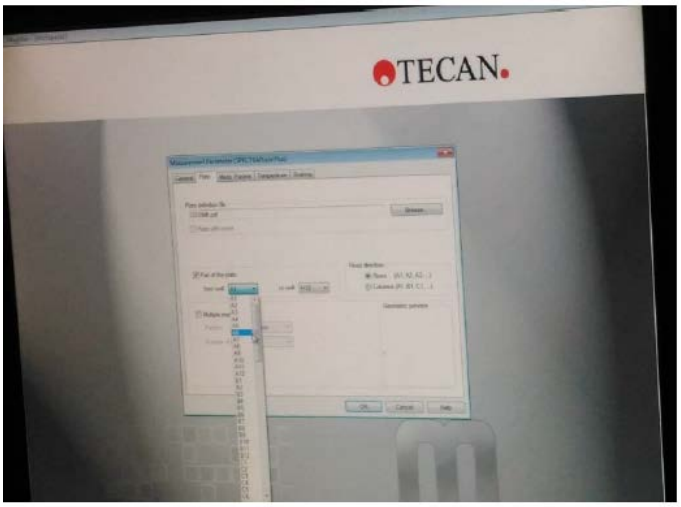

Fig. 3: Tecan plate reader

Results

Assessment of Antimicrobial activity:

The diameters of zone of inhibition (mm) obtained from disc diffusion method

In table 1a: The diameters of zone of inhibition of staphylococcus aureus against the chlorhexidine and cinnamon is described. Sample 1,2 and 3 showed the diameters of zone of inhibition (mm) of 15(15±0.0), 16.5(14.5±2.0), 20(15.1±5.0) respectively against chlorhexidine and 3(3±0.0), 5.5(3.5±2.0), 6(3.2±3.0) respectively against Cinnamon.

In table 1b: The diameters of zone of inhibition of Porphyromonas gingivalis against the chlorhexidine and cinnamon is described. Sample 1,2 and 3 showed the diameters of zone of inhibition (mm) of 15(15±0.0), 20(15±5), 17(14.9±3.0) respectively against chlorhexidine and 3(3±0.0), 5.5(3.5±2.0), 6(3.2±3.0) respectively against Cinnamon.

In table 1c: The diameters of zone of inhibition of Streptococcus mutans against the chlorhexidine and cinnamon is described. Sample 1,2 and 3 showed the diameters of zone of inhibition (mm) of 8(8±0.0), 8.5(8.5±3.0), 7(7.8±4.0) respectively against chlorhexidine and 5(5±0.0), 4(4.7±1.0), 6(4.9±3) respectively against Cinnamon.

In table 1d: The diameters of zone of inhibition of Streptococcus sanguis against the chlorhexidine and cinnamon is described. Sample 1,2 and 3 showed the diameters of zone of inhibition (mm) of 7(7±0.0), 9(7.2±2), 7(6.9±1.0) respectively against chlorhexidine and 2(2±0.0), 6(1.8±3.0), 4(2.1±2.0) respectively against Cinnamon.

A Kruskal Wallis test was used to compare the mean zone of inhibition, followed by post-hoc analysis between the study groups

Mean zone of inhibition of Staphylococcus aureus

On comparison the test results revealed that chlorhexidine group showed higher value of mean zone of inhibition against Staphylococcus aureus with a mean of 14.87 ± 0.32 as compared to Cinnamon water extract group with a

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mean of 3.23 ± 0.25. The water group, however, showed no zone of inhibition. On post-hoc analysis, the mean difference seen between all the three groups i.e. water and chlorhexidine, water and cinnamon water extract and cinnamon water extract and chlorhexidine was statistically significant with a P-value of 0.01. [Table 2, Graph 1]

Mean zone of inhibition of Porphyromonas gingivalis

On comparison the test results revealed that chlorhexidine group showed higher value of mean zone of inhibition against Porphyromonas gingivalis with a mean of 14.97 ± 0.06 as compared to cinnamon water extract group with a mean of 2.07± 0.21. The water group, however, showed no zone of inhibition. On post-hoc analysis, the mean difference seen between all the three groups i.e. water and chlorhexidine, water and cinnamon water extract and cinnamon water extract and chlorhexidine is statistically significant with a P-value of 0.01. [Table 2, Graph 1]

Mean zone of inhibition of streptococcus mutans

On comparison the test results revealed that chlorhexidine group showed higher value of mean zone of inhibition against Streptococcus mutans with a mean of 8.10 ± 0.36 as compared to Cinnamon water extract group which exhibited a mean zone of inhibition of 4.87 ± 0.15 in the present study. The Water group showed no zone of inhibition. On post-hoc analysis, the mean difference seen between all the three groups i.e. water and chlorhexidine, water and cinnamon water extract and cinnamon water extract and chlorhexidine was statistically significant with a P-value of <0.01. [Table 2, Graph 1]

Mean zone of inhibition of streptococcus sanguis

On comparison the test results revealed that chlorhexidine group showed higher value of mean zone of inhibition against Streptococcus sanguis with a mean of 7.03 ± 0.15 as compared to Cinnamon water extract group with a mean of 1.97 ± 0.15. The water group, however, showed no zone of inhibition. On post-hoc analysis, the mean difference seen between all the three groups i.e. water and chlorhexidine, water and cinnamon water extract, and cinnamon water extract and chlorhexidine was statistically significant with a P-value of 0.01. [Table 2, Graph 1]

Determination of MIC using micro broth dilution technique

The test results of micro-broth dilution technique for MIC determination revealed that chlorhexidine demonstrated MIC for Streptococcus sanguis at a concentration of 1µg/mL, for Streptococcus mutans and Staphylococcus aureus at a concentration of 0.5µg/mL and for Porphyromonas gingivalis at a concentration of 2 µg/mL whereas Cinnamon water extract demonstrated MIC for Streptococcus sanguis at a concentration of 1 µg/mL, for Streptococcus mutans and Staphylococcus aureus at a concentration of 0.5 µg/mL and for Porphyromonas gingivalis at a concentration of 2 µg/mL. [Table 3a and 3b]

A student t-test was used to compare the mean percentage of inhibition of Streptococcus sanguis, Streptococcus mutans, Staphylococcus aureus and Porphyromonas gingivalis bacteria between two study groups. (Chlorhexidine and Cinnamon water extract)

The test results revealed that chlorhexidine group showed a higher mean percentage of Staphylococcus aureus inhibition with a mean percentage of 65.88±32.65 as compared to the Cinnamon water extract group with a mean percentage of 31.13±28.82; with a mean difference of 34.75. This mean difference was statistically significant with a P-value= 0.04. [Table 4, Graph 2]

The test results revealed that chlorhexidine group showed a higher mean percentage of Porphyromonas gingivalis inhibition with a mean percentage of 48.59 ±28.21 as compared to the Cinnamon water extract group with a mean percentage of 23.64±22.67; with a mean difference

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of 24.95. This mean difference was statistically significant with a P-value= 0.07. [Table 4, Graph 2]

The test results revealed that chlorhexidine group showed a higher mean percentage of Streptococcus mutans inhibition with a mean percentage of 65.02±31.26 as compared to the Cinnamon water extract group with a mean percentage of 31.76 ±28.16; with a mean difference of 33.26. This mean difference was statistically significant with a P-value= 0.04. [Table 4, Graph 2]

The test results revealed that chlorhexidine group showed a higher mean percentage of Streptococcus sanguis inhibition with a mean percentage of 60.93±32.47 as compared to the Cinnamon water extract group with a mean percentage of 23.77±21.99; with a mean difference of 37.15. This mean difference was, however, statistically significant with a P-value= 0.02. [Table 4, Graph 2]

Assessment of anti-inflammatory activity

1. Lipoxygenase inhibition assay

The results obtained from the Lipoxygenase inhibition assay revealed that indomethacin showed 58.47% of lipoxygenase inhibition activity at a concentration of 10 µg/mL whereas cinnamon water extract showed 51.35% of lipoxygenase inhibition activity at a concentration of 20 µg/mL. [Table 5]

An independent student t-test was used to compare the mean percentage of Lipoxygenase inhibition activity between Indomethacin and Cinnamon water extract groups.

The test results revealed that indomethacin group showed a higher mean percentage of Lipoxygenase inhibition activity with a mean percentage of 38.42±25.06 as compared to the Cinnamon water extract group with a mean percentage of 23.55±19.22 with a mean difference of 14.87. The mean difference did not show any statistically significant difference (P- value = 0.27). [Table 6, Graph 3]

2. Hyaluronidase inhibition assay

The results obtained from the hyaluronidase inhibition assay revealed that cromolyn showed 51.22% of hyaluronidase inhibition activity at a concentration of 40 µg/mL whereas Cinnamon water extract did not show any hyaluronidase inhibition activity. [Table 5 and 7]

An independent student t-test was used to compare the mean percentage of Hyaluronidase inhibition activity between Cromolyn and Cinnamon water extract groups.

The test results revealed that cromolyn group showed a higher mean percentage of hyaluronidase inhibition activity with a mean percentage of 29.40±20.82 as compared to the Cinnamon water extract group with a mean percentage of 11.21±10.50 with a mean difference of 18.19. The mean difference did not show any statistically significant difference (P- value = 0.09). (Table 8 and Graph 4)

The results obtained from anti-inflammatory activity through lipoxigenase assay, has shown that the positive control has higher anti-inflammatory activity compared to Cinnamon water extract. Whereas the results obtained from hyaluronidase assay, Cinnamon water extract has shown less activity compared to positive control.

Assessment of antioxidant activity

1. DPPH assay

The percentage of radical scavenging capacity obtained from DPPH assay revealed that Quercetin showed 80.03% of radical scavenging capacity at a concentration of 10µg/mL whereas Cinnamon water extract showed 55.28% of radical scavenging capacity at a concentration of 10µg/mL. [Table 9]

An independent student t-test was used to compare percentage of radical scavenging between quercetin and Cinnamon water extract groups using DPPH assay.

The test results showed that the quercetin had higher percentage of radical scavenging with a value of 46.53±

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31.16 as compared to Cinnamon water extract with a mean of 24.78±19.46 with a mean difference of 21.75. However, this mean difference was not statistically significant (P-value = 0.20). [Table 10, Graph 6]

2. ABTS assay

The percentage of radical scavenging capacity obtained from ABTS assay showed that Quercetin showed 95.85% of radical scavenging capacity at a concentration of 10µg/mL whereas Cinnamon water extract showed 84.77% of radical scavenging capacity at a concentration of 10µg/mL. [Table 11]

An independent student t-test was used to compare percentage of radical scavenging between quercetin and Cinnamon water extract groups using ABTS assay.

The test results showed that the quercetin had higher percentage of radical scavenging with a mean of 54.65± 33.54 as compared to Cinnamon water extract with a mean of 52.59±25.86 with a mean of 2.06. However, this mean difference was not statistically significant (P-value = 0.75). [Table 12, Graph 5]

1. DPPH assay

The percentage of radical scavenging capacity obtained from DPPH assay revealed that Quercetin showed 80.03% of radical scavenging capacity at a concentration of 10µg/mL whereas Cinnamon water extract showed 55.28% of radical scavenging capacity at a concentration of 10µg/mL. [Table 9]

An independent student t-test was used to compare percentage of radical scavenging between quercetin and Cinnamon water extract groups using DPPH assay.

The test results showed that the quercetin had higher percentage of radical scavenging with a value of 46.53± 31.16 as compared to Cinnamon water extract with a mean of 24.78±19.46 with a mean difference of 21.75. However, this mean difference was not statistically significant (P-value = 0.20). [Table 10, Graph 6]

2. ABTS assay

The percentage of radical scavenging capacity obtained from ABTS assay showed that Quercetin showed 95.85% of radical scavenging capacity at a concentration of 10µg/mL whereas Cinnamon water extract showed 84.77% of radical scavenging capacity at a concentration of 10µg/mL. [Table 11]

An independent student t-test was used to compare percentage of radical scavenging between quercetin and Cinnamon water extract groups using ABTS assay.

The test results showed that the quercetin had higher percentage of radical scavenging with a mean of 54.65± 33.54 as compared to Cinnamon water extract with a mean of 52.59±25.86 with a mean of 2.06. However, this mean difference was not statistically significant (P-value = 0.75). [Table 12, Graph 5]

Tables

Table 1a: Diameter of Zone of inhibition measured in (mm) against Staphylococcus aureus.

Cinnamon zeylanicum	Chlorhexidine	Water
3(3±0.0)	15(15±0.0)	-
5.5(3.5±2.0)	16.5(14.5±2.0)	-
6(3.2±3.0)	20(15.1±5.0)	-

Table 1b: Diameter of Zone of inhibition measured in (mm) against Porphyromonas gingivalis

Cinnamon zeylanicum	Chlorhexidine	Water
2(2±0.0)	15(15±0.0)	-
3 (2.3±4)	20(15±5)	-
2(1.9±1.0)	17(14.9±3.0)	-

Table 1c: Diameter of Zone of inhibition measured in (mm) against *Streptococcus mutans*

Cinnamon zeylanicum	Chlorhexidine	Water
5(5±0.0)	8(8±0.0)	-
4(4.7±1.0)	8.5(8.5±3.0)	-
6(4.9±3)	7(7.8±4.0)	-

Table 1d: Diameter of Zone of inhibition measured in (mm) against Streptococcus sanguis

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Cinnamon zeylanicum	Chlorhexidine	Water
2(2±0.0)	7(7±0.0)	-
6(1.8±3.0	9(7.2±2)	-
4(2.1±2.0)	7(6.9±1.0)	-

Table 2: Comparison of mean zone of inhibition between *Cinnamon* extract, negative and positive Controls using One-way ANOVA test followed by Tukey's Post hoc Analysis test.

Bacteria	Group	N	Mean	SD	Min	Max	P-Value	Sig. Diff	P-Value
S. aureus	CM	3	3.23	0.25	3.0	3.5	<0.001*	CM Vs NC	<0.001*
	NC	3	0.00	0.00	0.0	0.0		CM VS PC	<0.001*
	PC	3	14.87	0.32	14.5	15.1		NC Vs PC	<0.001*
P. gingivalis	CM	3	2.07	0.21	1.9	2.3	<0.001*	CM Vs NC	<0.001*
	NC	3	0.00	0.00	0.0	0.0		CM VS PC	<0.001*
	PC	3	14.97	0.06	14.9	15.0		NC Vs PC	<0.001*
S. mutans	CM	3	4.87	0.15	4.7	5.0	<0.001*	CM Vs NC	<0.001*
	NC	3	0.00	0.00	0.0	0.0		CM VS PC	<0.001*
	PC	3	8.01	0.36	7.8	8.5		NC Vs PC	<0.001*
S. sanguis	CM	3	1.97	0.15	1.8	2.1	<0.001*	CM Vs NC	<0.001*
	NC	3	0.00	0.00	0.0	0.0		CM VS PC	<0.001*
	PC	3	7.03	0.15	6.9	7.2		NC Vs PC	<0.001*

NOTE: CM-cinnamon, NC- negative control, PC-positive control, SM-Streptococcus mutans, SG-Streptococcus sanguis, SA- Staphylococcus aureus, PG-Porphyromonas gingivalis *- statistically significant.

Table 3a: Determination of the percentage (%) of minimum inhibitory concentration (MIC) of Chlorhexidine against test cultures

Concentration (µg/mL)	*% inhibition*			
	S.sanguis	*S. aureus*	*S.mutans*	*P.gingivalis*
0.000	0.00	0.00	0.00	0.00
0.250	33.17	39.94	45.30	28.21
0.500	48.56	**58.03**	**56.05**	32.56
1.000	**62.30**	70.52	68.89	46.16
2.000	74.32	81.54	79.33	**55.93**
4.000	82.72	88.80	87.06	61.67
8.000	90.78	93.11	89.87	76.41
16.000	95.56	95.11	93.63	87.76
MIC (µg/mL)	**1.0**	**0.5**	**0.5**	**2.0**

Table 3b: Determination of the percentage (%) of minimum inhibitory concentration of Cinnamon zeylanicum extract against test cultures

Concentration (µg/mL)	*% inhibition*			
	S.sanguis	*S. aureus*	*S.mutans*	*P.gingivalis*
0.00	0.00	0.00	0.00	0.00
0.250	5.51	5.97	5.64	3.24
0.500	8.72	11.48	10.54	5.19
1.000	15.64	15.79	21.29	11.84
2.000	21.23	27.55	31.73	23.56
4.000	30.45	45.91	42.90	36.19
8.000	42.55	**62.44**	**63.99**	48.32
16.000	**66.09**	79.89	77.97	**60.74**
MIC (µg/mL)	16	8	8	16

Table 4: Comparison of percentage (%) of minimum inhibitory concentration of inhibition between CHX and *cinnamon* extracts for different organisms using student t Test.

Organisms								
S.Sanguis	Groups	N	Mean	SD	S.E.M	Mean Diff	t	P-Value
S.Sanguis	CHX	8	60.93	32.47	11.48	37.15	2.679	0.02*
S. Aureus	Cinnamon	8	23.77	21.99	7.78			
S. Aureus	CHX	8	65.88	32.65	11.54	34.75	2.257	0.04*
S.Mutans	Cinnamon	8	31.13	28.82	10.19			
S.Mutans	CHX	8	65.02	31.26	11.05	33.26	2.236	0.04*
P.Gingivalis	Cinnamon	8	31.76	28.16	9.95			
P.Gingivalis	CHX	8	48.59	28.21	9.98	24.95	1.950	0.07

NOTE: CHX- Chlorhexidine, SD- Standard deviation, SEM- Standard error of mean

***statistically significant**

Table 5: Lipoxygenase inhibition activity

Concentration (µg/mL)	% inhibition	
	Indomethacin	Cinnamon extract
0	0.00	0.00
1.25	9.90	2.24
2.50	20.04	16.81
5.00	34.53	25.51
10.00	58.47	36.11
20.00	69.13	51.35

Table 6: Comparison of Lipoxygenase inhibition activity between Indomethacin & Cinnamon group using Mann Whitney U Test

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Group	N	Mean	SD	Mean Diff	Z	P-Value
Indomethacin	6	38.42	25.06	14.87	-1.095	0.27
Cinnamon	6	23.55	19.22			

NOTE: IMC- Indomethacin, SD- Standard deviation, SEM- Standard error of mean

Table 7: Hyaluronidase inhibition activity

Concentration (µg/mL)	%inhibition	
	Chromolyn std	Cinnamon extracts
0.00	0.00	0.00
1.25	5.78	5.72
2.50	10.78	13.21
5.00	18.09	17.84
10.00	23.06	26.56
20.00	35.66	31.23
40.00	51.22	39.18
80.00	61.21	44.67

Table 8: Comparison of the Hyaluronidase inhibition activity Activity between Cromolyn Std. & *Cinnamon* group using Mann Whitney U Test

Group	N	Mean	SD	Mean Diff	Z	P-Value
Cromolyn Std.	7	29.40	20.82	18.19	-1.714	0.09
Cinnamon	7	11.21	10.50			

NOTE: SD- Standard deviation, SEM- Standard error of mean.

Table 9: % radical scavenging activity of Quercetin and *Cinnamon* zeylanicum extract by DPPH assay

Concentration (µg/mL)	% radical Scavenging	
	Quercetin standard	Cinnamon extract
0.00	0.00	0.00
0.312	8.81	4.89
0.625	18.01	8.95
1.25	26.86	16.98
2.50	38.48	21.99
5.00	67.07	40.56
10.00	80.03	55.28

Table 10. Comparison of mean percentage of radical scavenging by DPPH assay between Quercetin and *Cinnamon* extracts using Mann Whitney U Test

Group	N	Mean	SD	Mean Diff	Z	P-Value
Quercetin	7	46.53	31.16	21.75	-1.286	0.20
Cinnamon	7	24.78	19.46			

NOTE: SD- Standard deviation, SEM- Standard error of mean

Table 11: Percentage radical scavenging activity of Quercetin and *Cinnamon* zeylanicum extract by ABTS assay

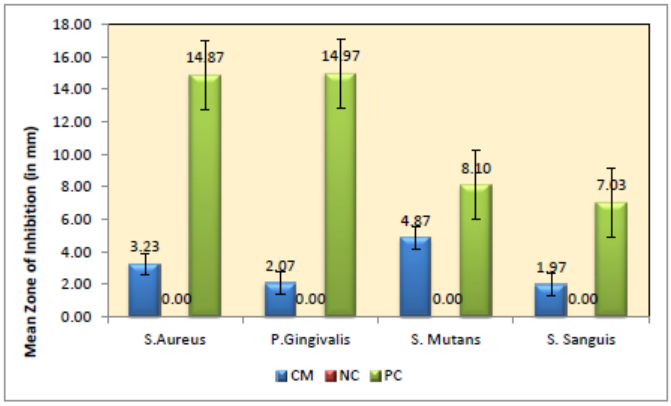
Concentration (µg/mL)	% radical Scavenging	
	Quercetin	Cinnamon extract
0.00	0.00	0.00
0.312	12.06	19.77
0.625	26.77	26.29
1.25	40.46	49.99
2.50	66.67	60.39
5.00	86.09	74.31
10.00	95.85	84.77

Table 12: Comparison of mean percentage of radical scavenging by ABTS assay between Quercitin and *Cinnamon* extracts using Mann Whitney U Test

Group	N	Mean	SD	Mean Diff	Z	P-Value
Quercetin	7	54.65	33.54	2.06	-0.320	0.75
Cinnamon	7	52.59	25.86			

NOTE: SD- Standard deviation, SEM- Standard error of mean.

Graphs



Graph 1: Comparison of mean zone of inhibition between *cinnamon* water extract(CWE), negative (NC) and positive control (PC)

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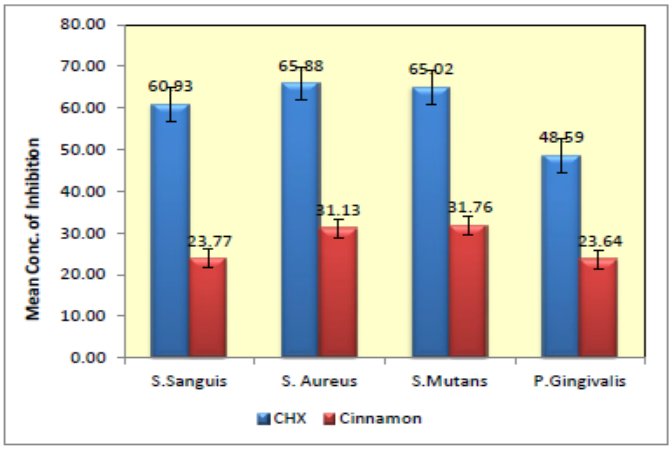
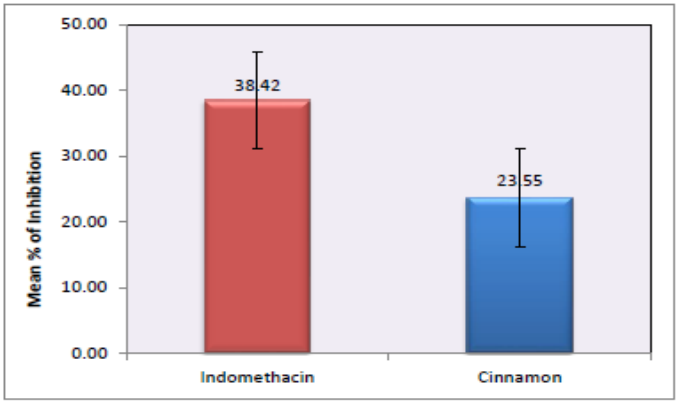
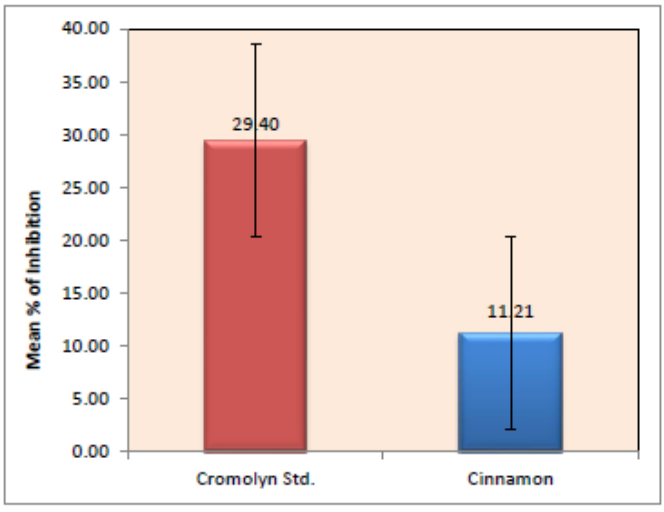


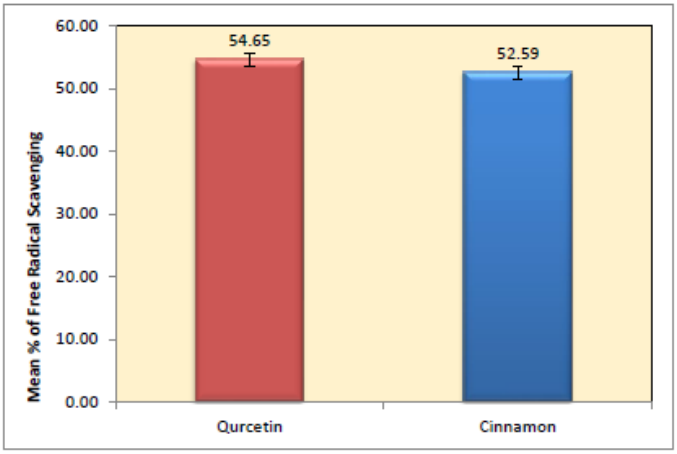
Graph 2: Comparison of mean % of inhibition between chlorhexedine and *cinnamon* water extract (CWE) for *S. sanguis, S. aureus, S. mutans and P.gingivalis*



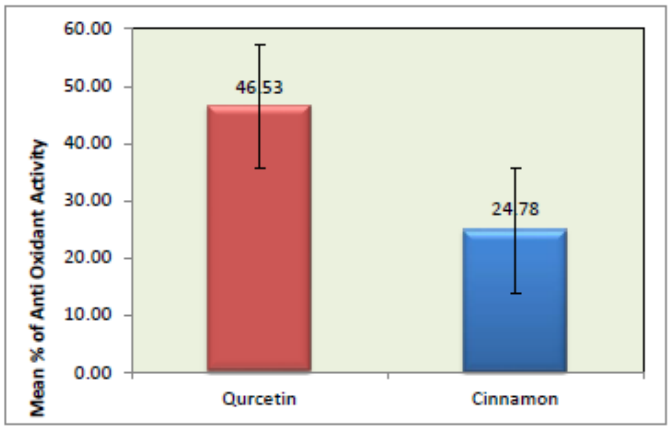
Group 3: Comparison of mean percentage of Lipoxygenase inhibition activity by Indomethacin (IMC) and *Cinnamon* extract groups.



Group 4: Comparison of mean percentage of Hyaluronidase inhibition activity by Indomethacin (IMC) and Cinnamon extract groups



Graph 5: Comparison of the mean % of radical scavenging activity between Quercetin (QCT) Cinnamon extract groups at different trials by using ABTS assay



Group 6: Comparison of the mean % radical scavenging action between Quercetin (QCT) and *Cinnamon* extract groups at different time intervals by using DPPH assay.

Discussion

Overall, approximately 250 species have been identified among the cinnamon genus, with trees being scattered all over the world. Out of which four species have great economic importance for their multiple culinary uses as common spices worldwide:

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1. Cinnamommum zeylanicum Blume
2. Cinnamommum loureiroi Nees
3. Cinnamommum burmann
4. Cinnamommum aromaticum Nees

Among these cinnamon zeylanicum is the species most commonly used due to its enhanced antimicrobial and anti-inflammatory properties (Palombo EA, 2009)12.

Vangalapati et al. in 2012 elaborated the chemical constituents found in different parts of cinnamon as follows13 -

1. Leaves: leaves contain cinnamaldehyde (1- 5%) and eugenol (70 – 95%)
2. Bark: contains cinnamaldehyde (65- 80%) and eugenol (5 – 10%)
3. Root bark: contain camphor (60%)
4. Fruit: contains trans- cinnamyl acetate (42-54%) and caryophyllene (9-14%)
5. Cinnamommum zeylanicum buds: contain terpene hydrocarbons (78%^), alpha-bergamotene (27.38%), alpha-copaene (23.05%), oxygenated terpenoids (9%).
6. Cinnamommum zeylanicum flowers: contain trans-cinnamyl acetate (41.98%), alpha-bergamotene (9.7%), carophylline oxide.

The results of disc diffusion revealed that the Chlorhexidine group showed a higher mean zone of inhibition against Streptococcus mutans, Streptococcus sanguis, Staphylococcus aureus and Porphyromonas gingivalis. The major reason devoted to the antibacterial action of chlorhexidine is its way in inactivating non sporulent bacteria.13 Cinnamon also has shown significant mean zone of inhibition against Streptococcus mutans, Streptococcus sanguis, staphylococcus aureus and Porphyromonas gingivalis. This is in accordance with the study done by Chaudhari et.al in 2012 in which cinnamon essential oil was investigated for antibacterial activity against Streptococcus mutans and concluded that the use of cinnamon essential oil contains cinnamaldehyde in more percentage and hence it can be a good alternative to other antibacterial compounds against the bacteria responsible for oral infections.14 But on comparison, chlorhexidine showed greater zone of inhibition than Cinnamon and the difference found was statistically significant.

After screening for the zone of inhibition, the minimum inhibitory concentration of Cinnamon extract was assessed and compared with that of positive control chlorhexidine. The test results revealed that chlorhexidine group showed a higher mean percentage of *Streptococcus mutans* inhibition with a mean percentage of 65.02 ±31.26, *Streptococcus sanguis* inhibition with a mean percentage of 60.93 ±32.47, *Staphylococcus aureus* inhibition with a mean percentage of 65.88 ±32.65 and *Porphyromonas gingivalis* with a mean percentage of 48.59 ±28.21 as compared to the Cinnamon with a mean percentage of 31.76 ±28.16, 23.77 ±21.99, 31.13 ±28.82, 23.64 ±22.67 for *Streptococcus mutans, Streptococcus sanguis, Staphylococcus aureus and Porphyromonas gingivalis* respectively.

The results of the lipoxygenase inhibition assay revealed that the Indomethacin group revealed a significant mean percentage of lipoxygenase inhibition activity with a mean of 38.42 ± 25.06. This is in accordance with the study done by Vajravijayan S et al in the year 2013, where indomethacin showed higher percentage inhibitory activity in lipoxygenase assay.15

The major reason devoted to the anti-inflammatory activity of indomethacin in Lipoxygenase pathway is to shunt the mechanism of arachidonic acid to 5-LOX pathway, thus reducing prostaglandins and subsequently anti-inflammatory effect. 71Cinnamon did not show any significant result.

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The results of the Hyaluronidase inhibition assay revealed that the cromolyn group revealed a significant mean percentage of hyalourinidase inhibition activity with a mean of 29.40± 20.82. This is in accordance with the study done by Tian-Xiao Meng et al where Cromolyn was used as positive control for hyalourinidase assay as it shows significant anti-inflammatory property. The major reason devoted to its anti-inflammatory activity is its potent inhibitory activity on mast cells, also it is said to inhibit macrophages, eosinophils, monocytes, and platelets that believed to play a role in the inflammatory response.16

Cinnamon did not show any significant result.

The test results of DPPH radical scavenging assay showed that the quercetin had a significant percentage of radical scavenging with a mean of 46.53 ± 31.16. This is in accordance with the study done by Dasha Mihaylova et al in the year 2013, where quercetin showed significant antioxidant property.17

The possible mechanism of action of quercetin, being, its ability to act as a free radical inhibitor, particularly with respect to the peroxy radical, which is the propagator of the autoxidation processes in fats and oils.18Cinnamon did not show any significant result.

In ABTS radical scavenging assay, the test results revealed that Quercetin group had a higher percentage of radical scavenging with a mean of 54.65 ± 33.54. This is in accordance with the study done by Montserrat Dueñas et al in the year 2011 where the author described that the hydroxyl groups of quercetin result in a decrease in the antioxidant activity with regard to the parent compound.19 Cinnamon did not show any significant result.

Limitations of the study:

• The present study evaluated the antibacterial activity of cinnamon water extract against only one anaerobic periodontopathogenic bacteria i.e. *Porphyromonas gingivalis* whereas the periodontitis results from interactions of a complex microbial community comprising of various species of periopathogens.

• It is an in-vitro study conducted to study the antimicrobial, anti-inflammatory and antioxidant property of cinnamon water extract. The outcome of the study cannot be correlated to the in vivo scenario.

Conclusion

1. Water extract of Cinnamommum zeylanicum showed significant mean zone of inhibition against Streptococcus mutans, Streptococcus sanguis, Staphylococcus aureus and Porphyromonas gingivalis but Chlorhexidine showed higher antibacterial activity as compared to the bark extract.

2. Lipoxygenase inhibition assay results revealed that the Indomethacin group showed higher percentage of lipoxygenase inhibition activity as compared to the Cinnamommum Zeylanicum and the differences obtained were statistically insignificant. Hyaluronidase inhibition assay results revealed that the Cromolyn group showed higher percentage of inhibition activity compared to Cinnamommum Zeylanicum extract with a statistically significant difference.

Therefore, Standards exhibit more anti-inflammatory activity than Water extract of Cinnamon zeylanicum.

3. DPPH assay results revealed that Cinnamommum Zeylanicum showed lower radical scavenging property as compared to quercetin group with statistically insignificant difference. ABTS assay results revealed that the Quercetin group had higher radical scavenging property as compared to Cinnamommum Zeylanicum with statistically insignificant difference.

Therefore, Standards exhibit more anti-oxidant activity than Water extract of Cinnamommum zeylanicum.

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