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Effects of Punica Granatum Peel on Oral Bacterial Species- An In-Vitro Study

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Abstract

Introduction: Oral diseases such as dental caries, gingival and periodontal diseases are major oral health problems worldwide, affecting the majority of the population. Microbial plaque accumulation is proven to be the main etiologic factor in the development of both gingivitis and periodontitis.

Aims and objectives: To assess the antibacterial, the anti-inflammatory, and the antioxidant activity of Punica granatum fruit peel extract by an in-vitro method.

Methodology: The antibacterial activity of punica granatum fruit peel extract was evaluated by disc diffusion method and determination of minimum inhibitory concentration (MIC) against *Streptococcus sanguis*, *Streptococcus mutans* and *Staphylococcus aureus* by micro broth dilution technique. The anti-inflammatory activity of Punica granatum fruit peel extract was determined by lipoxygenase inhibition assay and hyaluronidase inhibition assay.The antioxidant activity of punica granatum fruit peel extract was determined by 2, 2'-azino-bis ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay and 2, 2 diphenyl – 1 picrylhydrazyl (DPPH) antioxidant assay. The statistical tests used are Kruskal Wallis testandStudent unpaired t-test.

Results: The results showed that that the chlorhexidine exhibited a zone of inhibition with the diameter of 17.0±1.0, 14.3±3.2 and 12.0±1.7 whereas, the punica granatum peel extract exhibited a zone of inhibition with the diameter of 14.7±4.0, and 12.0±1.0 for *Streptococcus mutans*,*Streptococcus sanguis*, and *Staphylococcus aureus* respectively. The anti-inflammatory tests revealed that the indomethacin group showed lower percentage of lipoxygenase inhibition activity with the mean of 24.17+-19.34, as compared to punica granatum group mean of 29.44+-23, and with the mean difference of -5.27. The antioxidant assay revealed that the quercetin group had higher radical scavenging property with the mean of 37.08±34.26, as compared to Punica granatum group with the mean of 25.81±28.46 with the mean difference of 11.27. However, the results obtained were not statistically significant.

Conclusion: The results obtained from the current study concluded that the punica granatum peel extract has an

antibacterial, anti-inflammatory and antioxidant property under laboratory condition.

Keywords: Punica granatum, Periodontitis, Antioxidant, Anti-inflammatory, Antibacterial

Introduction

Periodontitis is a complex multifactorial disease in which disease expression involves intricate interactions of the biofilm with the host inflammatory response and subsequent alterations in bone and connective tissue metabolism.[1,2] Microbial plaque accumulations is proven to be the main etiologic factor in the development of both gingivitis and periodontitis. Supragingival plaque is composed mainly of gram-positive bacteria such as *Streptococcusmutans, Staphylococcus aureus, Streptococcus salivarius* etc. These organisms contribute to alter the equilibrium of oral microbiota by creating favorable condition for the adherence of more pathogenic gram-negative bacteria, fungi, and anaerobic bacteria, resulting in the formation of subgingival plaque and calculus. The subgingival microbiotas which is mainly composed of periodontal pathogens causes tissue destruction by producing the lipopolysaccharides, endotoxins, exotoxins and other virulent factors and also initiate inflammatory and immune response. The host immune cells produce various enzymes, cytokines, prostaglandins, free radicals and also there is increase in oxidative stress, resulting in tissue destruction and progression of the disease which is modified by the presence of various risk factors in an individual.[3]

Plaque control measures play an important role in preventing the onset of gingivitis and arrest its progression into periodontitis.[4,5] Plaque control plays an important role in prevention of periodontal diseases. Synthetic antimicrobial agents and antibiotics are known to cause antimicrobial resistance and emergence of opportunistic infections due to their inappropriate or widespread usage.[6,7,8] Thus, to overcome these limitations of synthetic antimicrobial agents, various naturally occurring agents has been tried in plaque control. The present study was planned to evaluate the antibacterial property against *Streptococcus mutans, Staphylococcus aureus* and *Streptococcus sanguis* and also to evaluateanti-inflammatory, and antioxidant effects of Punica granatum fruit peel.

Materials And Methodology

Punica granatum fruit (Pomegranate) was procured from the local market and laboratory procedures were conducted in Skanda Life Sciences Private Limited, Nagarbhavi, Bangalore. Punica granatum fruit peel was isolated, dried in shade and this dehydrated fruit peel was powdered into fine particles. This powdered fruit peel was used to prepare the plant extract using methanol. The pure extract obtained was further used for assessing antimicrobial activity by disc diffusion method and MIC determination against the bacteria by micro broth dilution technique, anti-inflammatory property by lipoxygenase inhibition assay and hyaluronidase inhibition assay, and antioxidant property using ABTS radical scavenging assay and DPPH assay.

Screening of Punica granatum for antimicrobial activity by Disc Diffusion Method was conducted as follows: [9] Cell suspension was prepared from *Streptococcus sanguis (*ATCC 10556), *Streptococcus mutans (*ATCC 25175) and *Staphylococcus aureus (*MTCC 7443) cultures grown on Trypticase soya broth adjusted to 1-2 x 10^5cells/ml. 100µl inoculum of test cultures was then inoculated on Muller Hinton Agar plates (90 mm) for bacterial cultures. The standard disks containing the test compound (punica granatum fruit peel extract), positive and negative control were placed on Agar plates. 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 disk (as shown in figure 1).

MIC determination against the bacteria by micro broth dilution technique was performed as per National Committee for Clinical laboratory Standard (NCCLS) method.[9]

Cell suspension prepared from bacterial cultures grown on Trypticase soya broth were adjusted to 1-2x10^5cells/ml. 90 µl test compounds were then mixed with different test concentration (16, 32, 64, 128, 256, 512 and 1024mg/ml) with 10 µl inoculum in 96 well plates in triplicates. Chlorhexidine was used as the positive control. The bacterial cultures were then incubated at 35^0C. After the incubation process, the bacterial test plates were observed after 24-48 hrs. Optical density (OD) was 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.

The anti-inflammatory activity using lipoxygenase inhibition assay and hyaluronidase inhibition assay.[10] The percentage inhibition of lipoxygenase and hyaluronidase was calculated as follows:

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

Absorbance (Control)

Anti-oxidant activity using ABTS radical scavenging assay.[11]ABTS radical cations were produced by reacting ABTS and APS on incubating the mixture at room temperature in dark for 16 hours. The solution thus obtained was further diluted with PBS to give an absorbance of 1.000. Different concentrations of the test sample and the reference standard (highest volume taken was 50µl) were added to 950 µl 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.

DPPH assay is carried out as follows: [12] 90 µl of DPPH solution was treated with 180 µl of various concentration of test solution. The different concentrations tested for reference standard were 0.5, 1.0, 1.5, 2.0, 2.5 mcg/ml. The reaction mixture was mixed and incubated at 25°C for 15 minutes and the absorbance was measured at 510 nm using semi-autoanalyzer. A control reaction was carried out without the test sample (as shown in figure 2). The results obtained are given in the following tables.

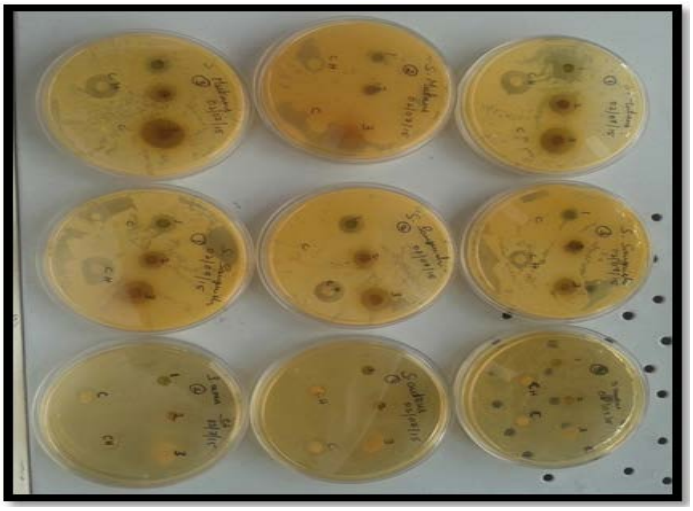


Figure 1: Antibacterial screening by disc diffusion method

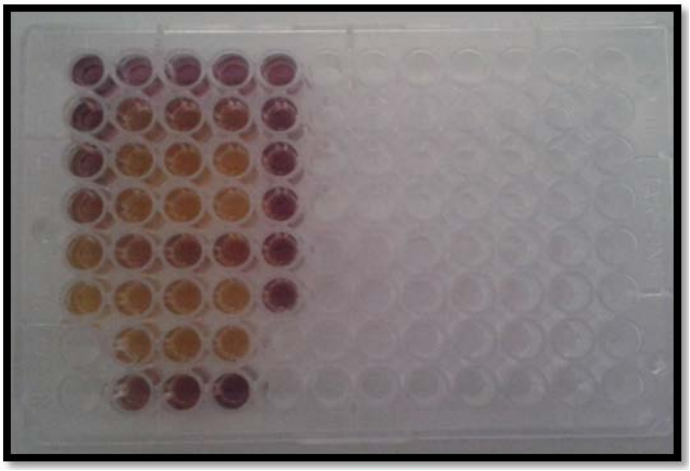


Figure 2: DPPH anti-oxidant assay by using 96 well plates

Results

Table 1a: Diameter of Zone of inhibition measured in mm against *Streptococcus mutans*

Table 1a: Diameter of Zone of inhibition measured in mm against *Streptococcus mutans*

Punica granatum	Chlorhexidine	Methanol
17(17±1)	16(16±1)	N.S
10(8±2)	18(10±8)	N.S
17(18±1)	17(19±2)	N.S

NOTE: N.S- Not shown

Table 1b: Diameter of Zone of inhibition measured in mm against *Streptococcus sanguis*

Punica granatum	Chlorhexidine	Methanol
13(13±1)	18(16±2)	N.S
12(14±2)	13(13±1)	N.S
11(10±1)	12(11±1)	N.S

NOTE: N.S- Not shown

Table 2:Comparison of mean zone of inhibition between Punica granatum extract, negative and positive Controls

Bacteria	Group	N	Mean	SD	Std. Error	Min	Max	H	P-Value	Sig. Diff	P-Value
SM	PG	3	14.7	4.0	2.3	10	17	6.000	0.04*	NC Vs PG	0.03*
	NC	3	0.0	0.0	0.0	0	0			NC Vs PC	0.03*
	PC	3	17.0	1.0	0.6	16	18				
SG	PG	3	12.0	1.0	0.6	11	13	6.269	0.04*	NC Vs PG	0.03*
	NC	3	0.0	0.0	0.0	0	0			NC Vs PC	0.04*
	PC	3	14.3	3.2	1.9	12	18				
SA	PG	3	0.0	0.0	0.0	0	0	7.714	0.02*	PC Vs PG	0.04*
	NC	3	0.0	0.0	0.0	0	0			PC Vs NC	0.04*
	PC	3	12.0	1.7	1.0	10	13				

NOTE: PG-punica granatum, NC- negative control, PC-positive control, *SM-Streptococcus mutans, SG-Streptococcus sanguis, SA- Staphylococcus aureus,*- statistically significant

Table 3a: Determination of Minimum Inhibitory concentration (MIC) of Chlorhexidine against test cultures

Chlorhexidine Concentration (µg/mL)	*% inhibition*		
	Streptococcus mutans	***Streptococcus sanguis***	***Staphylococcus aureus***
0.00	0.00	0.00	0.00
4.00	38.52	28.89	21.22
8.00	**58.25**	35.95	33.57
16.00	77.95	**66.52**	**54.25**

32.00	85.25	88.21	75.85
64.00	95.25	95.98	89.64
128.00	99.28	100	100
256.00	100	100	100
MIC	**8**	**16**	**16**

Table 3b: Determination of Minimum Inhibitory concentration of Punica granatum extract against test cultures

Punica granatum extract Concentration (µg/mL)	***% inhibition***	
	Streptococcus mutans	***Streptococcus sanguis***
0.00	0.00	0.00
16	33.57	7.91
32	47.52	13.68
64	**52.64**	23.88
128	66.52	36.81
256	78.89	**50.93**
512	99.20	78.25
1024	100.21	85.52
MIC	**64**	**256**

Table 4: Results of Lipoxygenase inhibition activity

Conc. µg/ml	**Indomethacin**	**Punica granatum extract**
0.00	0.00	0.00
1.25	5.33	5.66
2.50	11.23	11.20
5.00	18.77	16.77
10.00	24.33	29.88
20.00	33.23	36.77
40.00	45.66	44.66
80.00	54.79	51.22
160.00		68.77

Table 5: Comparison of Lipoxygenase inhibition activity by two groups

Group	N	Mean	SD	S.E.M	Mean Diff	95% CI of the Diff		t	P-Value
						Lower	Upper		
IM Group	8	24.17	19.34	6.84	-5.27	-27.40	16.86	-0.507	0.62
PG Group	9	29.44	23.00	7.67					

NOTE: IM- Indomethacin, PG- Punica granatum, , SD- Standard deviation, SEM- Standard error of mean, CI- Confidence interval

Table 6: Hyaluronidase inhibition activity

Conc. µg/ml	Cromolyn, Std	Punica granatum extract
0.00	0.00	0.00
1.25	5.78	5.77
2.50	10.78	13.45
5.00	18.09	17.88
10.00	23.06	26.33
20.00	35.66	31.22
40.00	51.22	39.88
80.00	61.21	44.56
160.00		69.11

Table 7: Comparison of mean percentage of Hyaluronidase inhibition activity by two groups

Group	N	Mean	SD	S.E.M	Mean Diff	95% CI of the Diff		t	P-Value
						Lower	Upper		
CML Group	8	25.73	21.90	7.74	-1.85	-24.33	20.63	-0.176	0.86
PG Group	9	27.58	21.53	7.18					

NOTE: CML- Cromolyn, PG- Punica granatum, SD- Standard deviation, SEM- Standard error of mean, CI- Confidence interval

Table 8: Absorbance and % activity of Quercetin and Punica granatum extract by DPPH assay

Sample	Conc. µg/ml	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Mean absorbance	% radical Scavenging	IC50 µg/ml
Quercetin	0	0.495	0.493	0.496	0.495	0.00	
	0.6	0.466	0.47	0.468	0.468	5.45	
	1.25	0.442	0.439	0.441	0.441	10.98	
	2.5	0.342	0.337	0.334	0.338	31.78	52.93
	5	0.235	0.231	0.232	0.233	53.00	

	10	0.147	0.149	0.145	0.147	70.30	
	20	0.059	0.061	0.057	0.059	88.08	

Sample	Conc. µg/ml	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Mean absorbance	% radical Scavenging	IC50 µg/ml
Punica granatum Extract	0	0.495	0.493	0.496	0.495	0.00	58.92
	3.125	0.478	0.48	0.475	0.478	3.47	
	6.25	0.465	0.46	0.463	0.463	6.52	
	12.5	0.432	0.435	0.433	0.433	12.48	
	25	0.357	0.352	0.358	0.356	28.18	
	50	0.200	0.203	0.199	0.201	59.43	
	100	0.142	0.146	0.148	0.145	70.62	

Table 9a: Absorbance and percentage activity of Quercetin and Punica granatum extract by using DPPH assay

Variables	Group	N	Mean	SD	S.E.M	Mean Diff	95% CI of the Diff		t	P-Value
							Lower	Upper		
AT1	QCT Group	7	0.312	0.169	0.064	-0.055	-0.237	0.127	-0.655	0.53
	PG Group	7	0.367	0.142	0.054					
AT2	QCT Group	7	0.311	0.169	0.064	-0.056	-0.236	0.125	-0.671	0.52
	PG Group	7	0.367	0.140	0.053					
AT3	QCT Group	7	0.310	0.171	0.064	-0.057	-0.239	0.125	-0.683	0.51
	PG Group	7	0.367	0.140	0.053					
Mean. Abs	QCT Group	7	0.312	0.170	0.064	-0.056	-0.237	0.126	-0.668	0.52
	PG Group	7	0.367	0.141	0.053					

NOTE: QCT- quercetin, PG- punica granatum, SD- Standard deviation, SEM- Standard error of mean, CI- Confidence interval

Table 9b: Comparison of the mean percentage of radical scavenging between Quercetin and Punica granatum groups

Variables	Group	N	Mean	SD	S.E.M	Mean Diff	95% CI of the Diff		t	P-Value
							Lower	Upper		
%_RS	QCT Group	7	37.08	34.26	12.95	11.27	-25.41	47.95	0.669	0.52
	PG Group	7	25.81	28.46	10.76					

NOTE: QCT- quercetin, PG- punica granatum, SD- Standard deviation, RS-Radical scavenging, SEM- Standard error of mean, CI- Confidence interval

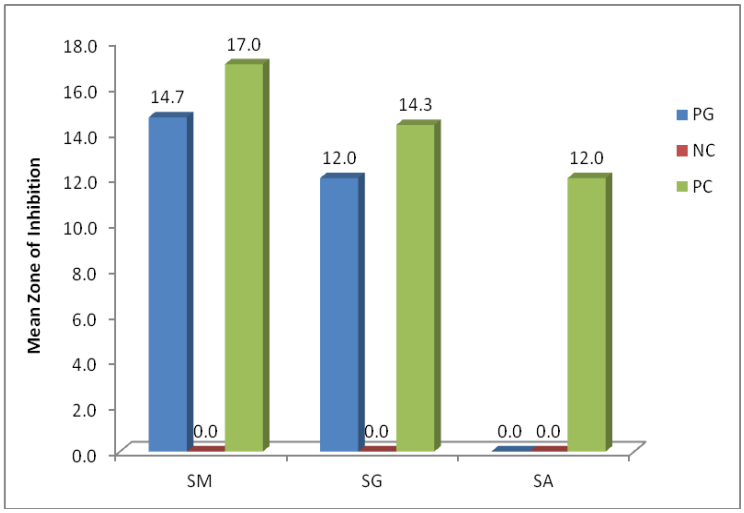
Table 10: Absorbance and percentage activity of Quercetin and Punica granatum extract by ABTS assay

Sample	Conc. µg/ml	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Mean absorbance	% radical Scavenging	IC50 µg/ml
Quercetin	0	0.519	0.518	0.516	0.518	0.00	
	0.6	0.511	0.509	0.509	0.510	1.55	
	1.25	0.462	0.472	0.458	0.464	10.39	
	2.5	0.419	0.406	0.431	0.418	19.16	8.299
	5	0.321	0.327	0.337	0.328	36.59	
	10	0.199	0.195	0.207	0.200	61.34	
	20	0.122	0.131	0.118	0.124	76.11	

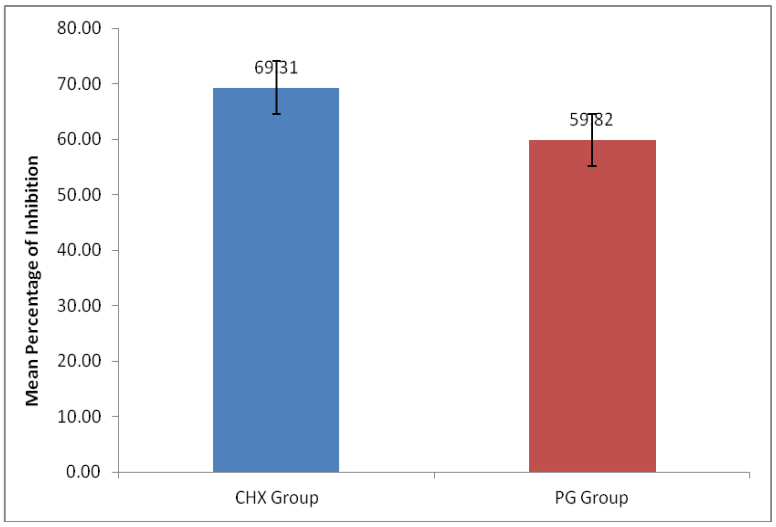
Sample	Conc. µg/ml	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Mean absorbance	% radical Scavenging	IC50 µg/ml
Punica granatum Extract	0	0.519	0.518	0.516	0.518	0.00	53.38
	3.125	0.500	0.497	0.496	0.498	3.87	
	6.25	0.480	0.474	0.470	0.474	8.36	
	12.5	0.439	0.425	0.432	0.432	16.56	
	25	0.358	0.357	0.356	0.357	31.02	
	50	0.272	0.275	0.267	0.271	47.58	
	100	0.185	0.188	0.176	0.183	64.65	

Graphs:

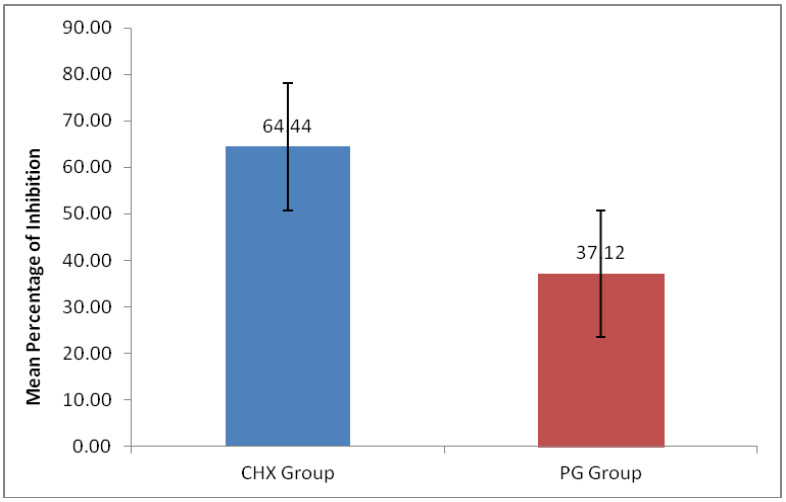
Graph I: Comparison of zone of inhibition between Punica granatum extract (PG), positive (PC) and negative controls (NC)



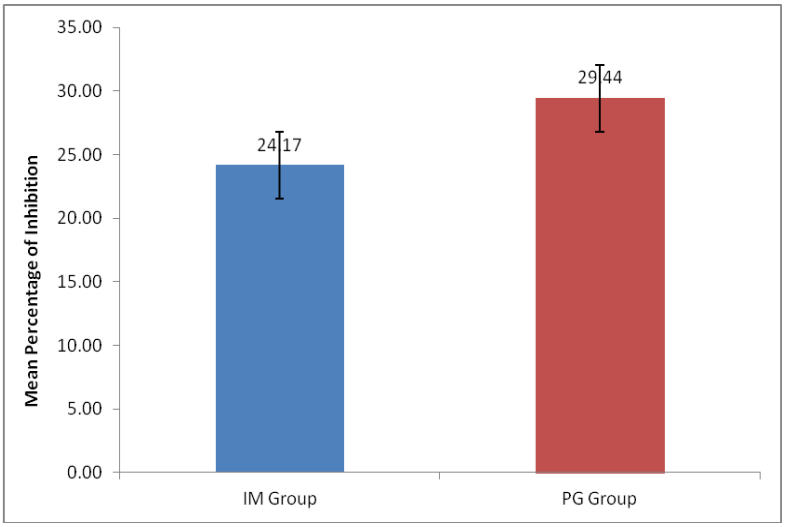
Graph II: Minimum inhibitory concentration against *Streptococcus mutans*



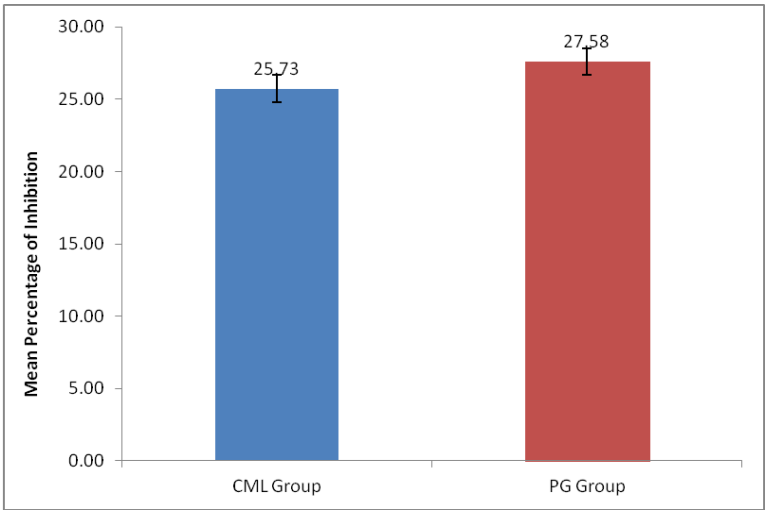
Graph III: Minimum inhibitory concentration against *Streptococcus sanguis*



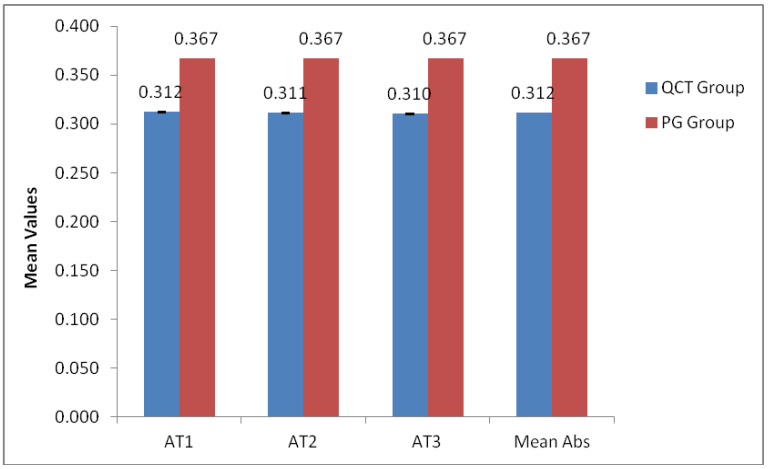
Graph IV: Comparison of mean percentage of Lipoxygenase inhibition activity by Indomethacin (IM) and Punica granatum(PG) groups



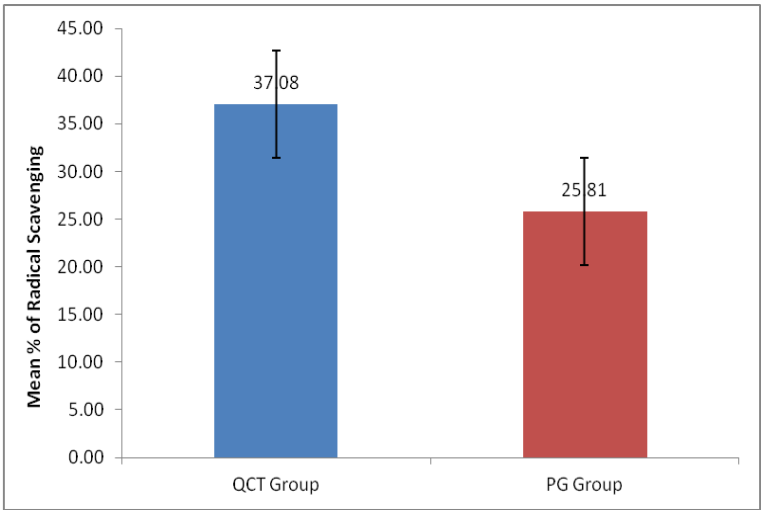
Graph V: Comparison of mean percentage of Hyaluronidase inhibition activity by Cromolyn (CML) and Punica granatum (PG) groups



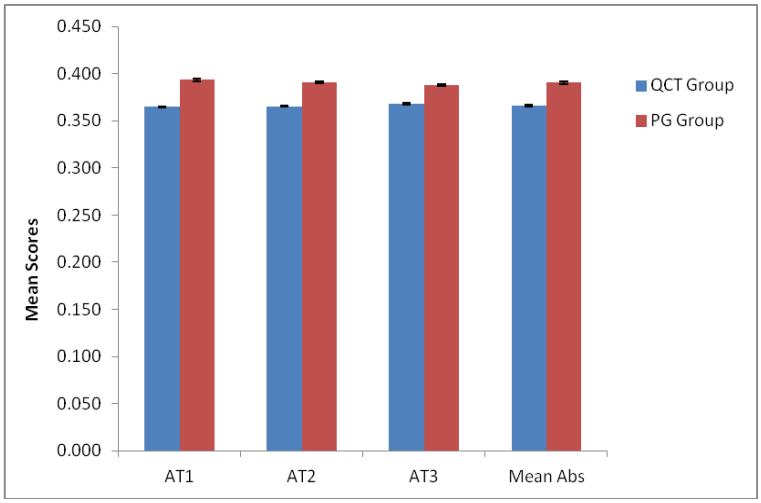
Graph VI: Comparison of the absorbance between Quercetin (QCT) and Punica granatum (PG) groups at different time intervalsby using DPPH assay



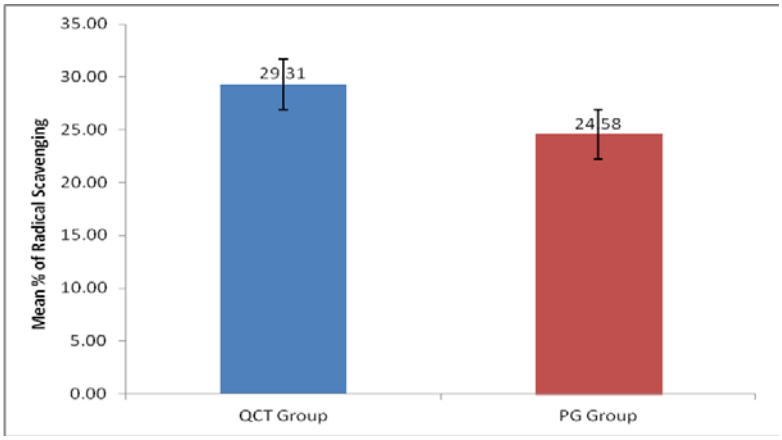
Graph VII: Comparison of the mean percentage of radical scavenging between Quercetin (QCT) and Punica granatum (PG) groups by using DPPH assay



Graph VIII: Comparison of the absorbance between Quercetin (QCT) and Punica granatum (PG) groups at different trials by using ABTS assay



Graph IX: Comparison of the mean percentage of radical scavenging between Quercetin (QCT) and Punica granatum (PG) groups



The results of screening test for the antimicrobial activity carried out by disc diffusion method revealed that the chlorhexidine exhibited a zone of inhibition with the diameter of 17.0±1.0, 14.3±3.2 and 12.0±1.7 for *Streptococcus mutans*, *Streptococcus sanguis* and *Staphylococcus aureus* respectively. The Punica granatum peel extract exhibited a zone of inhibition with the diameter of 14.7±4.0, and 12.0±1.0 for *Streptococcus mutans*, and *Streptococcus sanguis* respectively but did not exhibited inhibitory effect on the growth of *Staphylococcus aureus* in the present study. However, the results obtained were statistically significant (p-value <0.05). (Table 1a, 1b, 2 and Graph I). A student unpaired t-test was used to compare the mean percentage of inhibition of *Streptococcus mutans*, and *Streptococcus sanguis* between two study groups i.e. chlorhexidine and Punica granatum fruit peel extract.

The test results revealed that the chlorhexidine group showed the higher mean percentage of *Streptococcus mutans* inhibition with a mean percentage of 69.31±35.29, as compared to the Punica granatum group with a mean percentage of 59.82±33.93, with the mean difference of

9.49. This mean difference was, however did not yield a statistically significant result with p-value=0.59 (Table 3a,3b, 4a and Graph II). The test results revealed that, the chlorhexidine group showed the higher mean percentage of *Streptococcus sanguis* inhibition with a mean percentage of 64.44±38.40, as compared to the Punica granatum group with mean percentage inhibition of 37.12±32.03, with a mean difference of 27.32. This mean difference was, however did not yield a statistically significant result with p-value=0.59 (Table 3a, 3b, 4b and Graph III). Punica granatum fruit peel extract did not elicit inhibitory effect on the growth of *Staphylococcus aureus* hence, minimum inhibitory concentration(MIC) determination was not carried out against Staphylococcus aureus (Table 1c. 2 and Graph I).

The anti-inflammatory activity test results revealed that the Indomethacin group showed lower percentage of lipoxygenase inhibition activity with the mean of 24.17+-19.34, as compared to Punica granatum group mean of 29.44+-23, and with the mean difference of -5.27. However, this mean difference did not yield a statistically significant difference (P-value=0.62). (Table 5, 6, and Graph IV)

In hyaluronidase inhibition assay, the test results revealed that the Cromolyn group showed lower percentage of hyaluronidase inhibition activity with the mean of 25.73+-21.90, as compared to Punica granatum group with mean of 27.58+-21.53, and with the mean difference of -1.85. However, this mean difference did not yield a statistically significant difference (P-value=0.86). (Table 7, 8, and Graph V)

The results obtained from anti-inflammatory activity assay, has shown that the Punica granatum peel extract exhibited slightly higher anti-inflammatory activity when compared to positive control group.The mean percentage of radical scavenging activity was compared between quercetin and the Punica granatum group. The test results revealed that the quercetin group had higher radical scavenging property with the mean of 37.08±34.26, as compared to Punica granatum group with the mean of 25.81±28.46 with the mean difference of 11.27. However, the results obtained were not statistically significant (P-value=0.52).(Table 9, 10b and Graph VII)

The mean percentage of radical scavenging activity was compared between quercetin and the Punica granatum group. The test results revealed that the quercetin group had higher radical scavenging property with the mean of 29.31±29.89, as compared to Punica granatum group with the mean of 24.58±24.28 with the mean difference of 4.73. However, the results obtained were not statistically significant (P-value=0.75).(Table 11, and Graph IX)

Discussion

According to the results obtained by this study, the methanolic extract of Punica granatum peel extract had inhibitory effect on the growth of *Streptococcus mutans* and *Streptococcus sanguis*. The zone of inhibition shown by the Punica granatum fruit peel extract for *Streptococcus mutans* and *Streptococcus sanguis* is 14.7±4.0 and 12±0.1 respectively. The positive control chlorhexidine showed the maximum zone of inhibition of 17.0±0.1 for *Streptococcus mutans* and 14.3±3.2 for *Streptococcus sanguis*. The negative control methanol did not show any inhibitory effects on all the three organisms. The Punica granatum peel extract did not show any effect on the growth of *Staphylococcus aureus*.The results obtained from this study were similar to the results of the study conducted by Pereira JVet al.[13]

The study conducted by Braga et al. had shown that the Punica granatum extract had strong inhibitory effect on the growth of *Staphylococcus aureus*. Whereas, in the present study there was no any inhibitory effect exhibited on the growth of *Staphylococcus aureus* by the Punica

granatum peel extract. This may be because of difference in the composition of the different parts of the plant. The composition of extract is also influenced by the geographical location of the plant, age of the plant, season of harvesting, growth stage, and methods of drying and extraction technique. Also, the different parts of the plant have varying level of antimicrobial activity.[14, 15]

The results of the lipoxygenase inhibition assay of the present study revealed that the Indomethacin group showed lower percentage of lipoxygenase inhibition activity with the mean of 24.17+-19.34, as compared to Punica granatum group mean of 29.44+-23, and with the mean difference of -5.27. However, this mean difference did not yield a statistically significant difference (P-value=0.62). The hyaluronidase inhibition assay results revealed that that the Cromolyn group showed lower percentage of hyaluronidase inhibition activity with the mean of 25.73+-21.90, as compared to punica granatum group with mean of 27.58+-21.53, and with the mean difference of -1.85. However, this mean difference did not yield a statistically significant difference (P-value=0.86). The results obtained in this study are similar to the results obtained by the study conducted by Rajan S. et al. and Mahajan DC et al.[14, 16]

The results obtained in this study for the antioxidant property of Punica granatum peel extract reveal that in DPPH assay, a higher mean absorbance was observed with punica granatum group with mean of 0.367±0.141 as compared to 0.312±0.170 of quercetin group with a mean difference of -0.056. But, this mean difference was not statistically significant (P-value=0.52).

The test results of ABTS assay revealed that the quercetin group had a higher percentage of radical scavenging with a mean of 37.08±32.26, as compared to punica granatum group with a mean of 25.81±28.46, yielding a mean difference of 11.27. However, this mean difference was not statistically significant with the p-value 0.52. The results obtained in this study are similar to the results obtained by the study conducted by Rajan S. et al. and Mahajan DC et al.[14, 16]

Conclusion

Methanolic extract of Punica granatum fruit peelhad a significant antibacterial effect on common oral bacteria namely, *Streptococcus mutans* and *Streptococcus sanguis,* but didnot have any antibacterial effect on *Staphylococcus aureus*. The antimicrobial efficacy was as close as that of the chlorhexidine which is the positive control and considered as gold standard for its antimicrobial property.The anti-inflammatory property of Punica granatum peel extract has slightly higher anti-inflammatory activity comparing to the positive control Indomethacin and Cromolyn.The antioxidant property of Punica granatum peel extract has similar free radical scavenging property as that of the positive control Quercetin.Hence, it can be concluded that Punica granatum peel extract has an antimicrobial, anti-inflammatory and antioxidant property under laboratory condition.

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