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Research Article

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Evaluation of the Effect of Rosa Damascena and Jasminum Sambac on Dental Plaque Regrowth Inhibition

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ABSTRACT

Objective: To evaluate the effect of Rosa *damascena* and *Jasminum Sambac* on dental plaque regrowth by the Plaque Glycolysis and Regrowth Method (PGRM).

Methods: Ethanolic extracts of fresh flowers of Rosa *damascena* and *Jasminum Sambac* were prepared and subject to evaluation of antimicrobial property against Streptococcus mutans and also evaluated for their antioxidant property, anti-inflammatory property and their effect on dental plaque regrowth by the PGRM.

Results: Both Rosa *damascena* and *Jasminum Sambac* showed inhibition of Growth of S. mutans, and MIC for both was found to be 30mg/ml. The antioxidant activity of both showed a dose-dependent increase, with the highest activity of 90.70% at 100 µg/mL concentration for Rosa *damascena* and 82.93 % at 100 µg/mL concentration for *Jasminum Sambac*. Both showed some inhibition of RBC haemolysis in the Membrane Protection assay, indicating anti-inflammatory activity, at the concentration of 300 µg/ml. The Percentage RBC Protection for Rosa *damascena* and *Jasminum Sambac* was 11.92% and 9.27%. The PGRM assay established that at concentration of 30mg/ml, Extracts of Rosa *damascena* and *Jasminum Sambac* showed inhibition of biofilm formation, with highest inhibition observed between 4 hours and 6 hours of incubation, and all were statistically significant.

Conclusion: Study concludes that Rosa *damascena* and *Jasminum Sambac* have an inhibitory effect on regrowth of dental plaque and has antioxidant, antibacterial and inflammatory properties.

Keywords: Rosa *Damascena*, *Jasminum Sambac*, Plaque Glycolysis and Regrowth (PGRM), Mean Optical Density (O.D), Plaque Regrowth of Extract, Minimal Inhibitory Concentration (MIC), Antioxidant, Anti-Inflammatory

Introduction

Dental caries is defined as the localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates [1]. Certain bacteria within the plaque are acidogenic, and they produce acids when they metabolize fermentable carbohydrates. These acids can dissolve the calcium phosphate mineral in the tooth enamel or dentin in a process which is known as demineralization [2]. The bacteria in the biofilm are always metabolically active, causing fluctuations in the pH. These causes loss of mineral from the tooth when the pH is dropping or a gain of mineral when the pH is increasing. The result of these demineralization and remineralization processes is a net loss of mineral, leading to dissolution of the dental hard tissues and the formation of a caries lesion [3,4]

If the biofilm is removed, partially or totally, mineral loss may be stopped or reversed toward mineral gain. Currently, the most successful antibacterial therapy against cariogenic bacteria is treatment by chlorhexidine gluconate rinse or gel. There is little evidence to either support or refute that chlorhexidine is more effective than placebo or no treatment in the prevention of caries. Among natural plant-derived products, flowers have attained high priority and found various applications. Floral extracts and essential oils are also considered to be potential natural antimicrobial agents [5].

Rosa damascena and Jasminum sambac are two locally available flower species in India, and many previous studies have proven that they possess antioxidant, anti-inflammatory and anti-microbial activities against many bacterial species. However, there are very few studies in literature which have evaluated their effect on oral bacteria, mainly Streptococcus mutans, and at present there is no scientific evidence to prove their activity against dental plaque (dental biofilm). One such method recently adopted is the use of ex-vivo oral biofilm model, which involves

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culturing of a dental plaque biofilm in the laboratory to simulate the development of dental plaque, followed by testing the products for their ability to retard biofilm formation.

Objectives

The present study aimed to evaluate the effect of Rosa damascena and Jasminum sambac on the inhibition of dental plaque, using a novel ex-vivo model called Plaque Glycolysis and Regrowth Method (PGRM).

Materials and Methods

The study was an ex-vivo study conducted on 15 subjects, selected from the Outpatient Department of Paediatric and Preventive Dentistry, JSS Dental College and Hospital, JSS Academy of Higher Education and Research, Mysuru. Informed consent and assent were obtained from all the subjects and their parents. Institutional Ethical Committee approval was obtained prior to commencement of the study.

The fresh flowers of Rosa damascena and Jasminum sambac were purchased from the local market,at Mysore,India and were identified and authenticated by a Botanist. Pure strain of Streptococcus mutans (MTCC 890) was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh (Customer number: 14734; HSN Code: 30029030). It was done following the MTCC guidelines [6].The antimicrobial activity was evaluated on Mitis Salivarius Bacitracin agar plates prepared using MS agar powder (Himedia labs M259), Potassium tellurite 1% vials (Himedia FD052) and Bacitracin (Himedia CMS 208) [7].The Refrigerated Centrifuge (Eppendorf, North America), UV/Vis Spectrophotometer (Eppendorf, North America), Optical Cuvettes (Eppendorf, North America) required for evaluating the antioxidant and anti-inflammatory activity, and for the PGRM Method were utilised from the facilities at the Department of Biochemistry.

Preparation of the Extracts of Rosa Damascena and Jasminum Sambac

Ethanolic extract of Rosa damascena and Jasminum sambac was prepared according to the Refluxing method using a glass condenser. The petals were then filtered from the round bottom flask using Whatman filter paper. The filtrate was then subjected to distillation. In this process, the vapours of the ethanol were made to pass through the distillation apparatus, which condensed the vapours and collected pure ethanol in a conical flask at the other end of the apparatus. The resultant concentrated extract was collected in a china dish and further concentrated on a water bath until all the ethanol evaporated, leaving behind pure extract. The extract was further dried in a concentrator for 4 hrs.

Mic Determination- Agar Dilution Method

The agar dilution method was carried out according to the CLSI guidelines,2004. Antimicrobial activity of the extract was evaluated against Streptococcus mutans using the agar dilution method. Pure strains of Streptococcus mutans (MTCC 890) were revived on Blood agar plates. Six different concentrations of each extract were made and incorporated into molten MSB agar. Streptococcus mutans were subcultured onto agar dilution plates. The plates were then incubated anaerobically at 370C for 72 hours. The lowest concentration of Rosa damascena and Jasminum sambac extracts that showed no growth was chosen as the Minimum Inhibitory Concentration (MIC).

The antimicrobial activity was also evaluated against the dental biofilm using agar dilution method. The plates were inoculated with 2 bacterial log dilutions: 103 and 106, which were prepared by serial dilution of the plaque with saline. This was followed by inoculation onto the agar dilution plates by the spread plate method. 30 mg/ml concentration was used for both Rosa damascena and Jasminum sambac.

Determination of Antioxidant Activity- DPPH Assay

The antioxidant activity was evaluated using DPPH Free radical scavenging assay. A volume of 1400µl of DPPH reagent was added to all samples and standards and incubated for 30 minutes in Dark at room temperature.

Determination of Anti-Inflammatory Activity Activity- Rbc Membrane Stabilisation Assay

The anti-inflammatory activity was evaluated using the RBC membrane protection assay. The protocol followed was similar to the one followed by Anosike et al [8]. Absorbance (OD) of the haemoglobin content of the supernatant was estimated at 540 nm using UV/V is spectrophotometer.

Determination of Plaque Regrowth Inhibition Efficacy- Plaque Glycolysis and Regrowth Method (PGRM)

The Plaque regrowth inhibition efficacy was evaluated using the Plaque Glycolysis and Regrowth Method (PGRM). The protocol followed was a modified version of the original method introduced by White et al [9]. 15 caries free subjects were selected and were provided with a washout toothpaste for brushing 1 week prior to the study. On the day of the study, Pre-brushing Pooled plaque was collected, dispersed in 0.03% Tryptic Soy broth and standardised to 0.2 O.D.

Plaque regrowth was evaluated by taking 300 microliters of the standardized dispersed plaque solution in a 2 ml Eppendorf vial with 0.5 ml of 6% TSB, 100 microliters of sterile water and adding 50 microliters of 40% sucrose solution. To this mixture, Flower Extracts (Extract Test) or 0.2% chlorhexidine (positive control) or 0.03% TSB (Plaque Control) were added. This was followed by incubating at 370 in Eppendorf Thermomixer at 1200 rpm for 6 hours. Final OD was noted using a spectrophotometer. The readings were noted between 0-8hours.

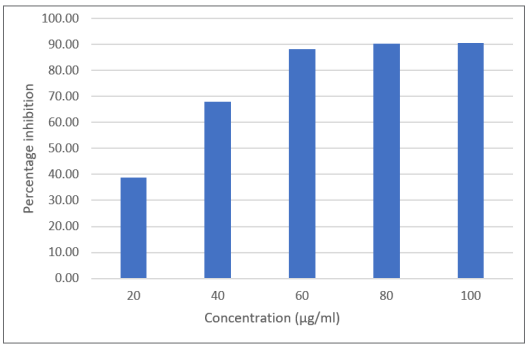

Figure 1: The Percentage of Free Radical Scavenging Activity of Varying concentrations of Rosa damascena extract

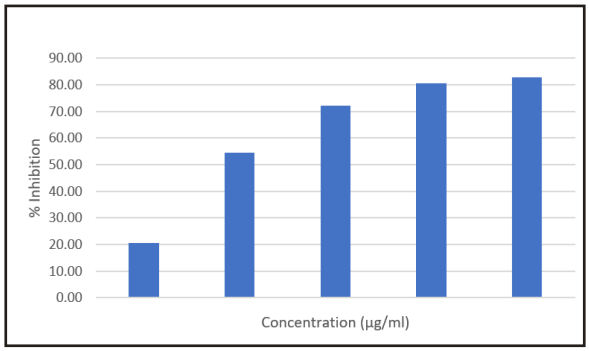

Figure 2: The Percentage of Free Radical Scavenging Activity of varying concentrations of Jasminum sambac extract

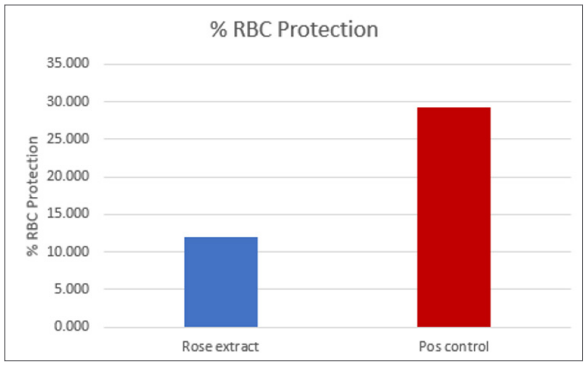

Figure 3: Percentage Protection of RBC Membrane by Rosa damascena extract

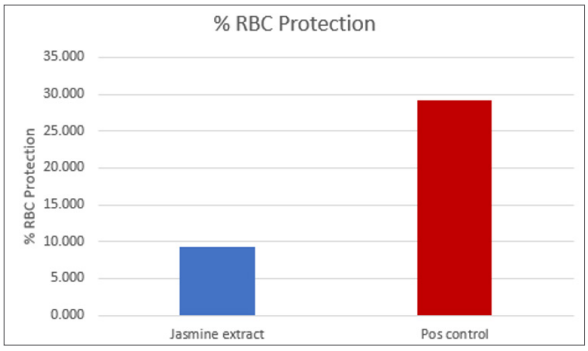

Figure 4: Percentage Protection of RBC Membrane by Jasminum sambac Extract

Results and Discussion

The yield was calculated to be 18.2% for Rosa damascena and 1.65 % for Jasminum sambac (Table 1).

Table 1: Yield of Rosa damascena and Jasminum Sambac Extracts

Flower	Weight of petals (gm)	Volume of Solvent (L)	Weight of Extract (gm)	Yield (% w/w)
Rosa Damascene	100	2.3	18.2	18.2
Jasminum Sambac	310	1.4	5.1	1.65

The plate with lowest concentration of the extract to show inhibition of growth of S.mutans was considered to be the Minimum Inhibitory Concentration. Among the 6 concentrations of the extracts used, both Rosa damascena and Jasminum sambac showed inhibition of bacterial growth over 7 days of incubation (Table 2). Hence, the Minimum Inhibitory Concentration (MIC) for both Rosa damascena and Jasminum sambac was assessed to be 30mg/ml (3%).

Table 2: Results for MIC Against S. Mutans - 3 Days

Colony Growth: 3 Days		
Sample	**Rosa damascena**	**Jasminum sambac**
Dose		
30 mg/ml	NG	NG
15 mg/ml	Confluent Growth	Growth
7.5 mg/ml	Confluent Growth	Growth
1.87mg/ml	Confluent Growth	Growth

NG: No growth

Colony Growth: 3 Days		
Sample	**CHX**	**Inoculum**
Dose		
1µg/ml	NG	Growth
400 µg/ml	NG	

CHX: Chlorhexidine, NG: No Growth

The results for inhibition of biofilm formation at 3 and 5 days showed that at a 30 mg/ml concentration, both Rosa damascena and Jasminum sambac extracts completely inhibited growth of the biofilm at 106 log dilution (Table 3,4) . However, at the 106 dilution, only Rosa damascena extracts showed complete inhibition of growth of the biofilm, while Jasminum sambac extract showed colony growth on the plate, indicating inability to inhibit biofilm growth at a higher bacterial load.

Table 3: Results for Antimicrobial Activity Against Dental Biofilm - 3 Days

Subject	Rosa damascena 30mg/ml			Jasminum Sambac 30mg/ml		CHX		DMSO	
	10^3 Dil	10^6 Dil	10^3 Dil	10^6 Dil	190 µg/ml	400 µg/ml	0.41 ml	0.624 ml	1.25 ml
1	NG	NG	3 CFU	NG	NG	NG	149 CFU	51 CFU	15 CFU
2	NG	NG	NG	NG					

Table 4: Results for Antimicrobial Activity Against Dental Biofilm - 5 Days

Subject	Rosa damascena 30mg/ml			Jasminum Sambac 30mg/ml		CHX		DMSO	
	10^3 Dil	10^6 Dil	10^3 Dil	10^6 Dil	190 µg/ml	400 µg/ml	0.41 ml	0.624 ml	1.25 ml
1	NG	NG	3 CFU	NG	NG	NG	180 CFU	128 CFU	128 CFU
2	NG	NG	145 CFU	NG					

The antioxidant activity of Rosa damascena extract showed a dose-dependent increase with the highest activity of 90.7% at 100 µg/ml, and the lowest activity of 38.82% at 20 µg/ml. IC50 value for Rosa damascena extract was calculated to be 25.54 µg/ml.

The antioxidant activity of Jasminum sambac extract showed a dose-dependent increase with the highest activity of 82.86% at 100 µg/ml, and the lowest activity of 20.60% at 20 µg/ml. IC50 value for Jasminum sambac extract was calculated to be 38.53 µg/ml.

The Percentage Protection of RBC Membrane shown by Rosa damascena Extract was 11.92% and Jasminum sambac was 9.27%. The % Protection shown by the Positive Control (Standard) was 29.21%. The results show that the Rosa damascena and Jasminum sambac extracts have a lesser degree of RBC Protection compared to the Positive control (Standard).

In the Plaque Glycolysis and Regrowth Method (PGRM), The percentage inhibition values of Rosa damascena extract (Table 5,6) indicate that the inhibition of Plaque regrowth at 2 hours is lower (21.091) than the inhibition at 4 and 6 hours (37.99% and 34.70%, respectively). This shows that the percentage of inhibition of plaque regrowth is greater after 4 hours of incubation, indicating a latent period before the extract starts exerting its antimicrobial property. At 8 hours, the percentage inhibition is the least (19.70%), which could be due to degradation of the phenolic compounds and pigments by light or metabolic by-products of bacteria.

Table 5: DPPH Activity of Varying Concentrations of Rosa Damascena against an Ethanol Blank

Concentration	N	Mean OD	S. D.	% inhibition
Ethanol Blank	1	0.699		
20 µg/mL	3	0.428	0.029	38.82
40 µg/mL	3	0.233	0.032	68.05
60 µg/mL	3	0.082	0.005	88.27
80 µg/mL	3	0.067	0.003	90.41
100 µg/mL	3	0.065	0.000	90.70

Table 6: DPPH Activity of Varying Concentrations of Jasminum Sambac Against an Ethanol Blank

Concentration	N	Mean OD	S.D.	% inhibition
Ethanol Blank	1	0.699		
20 µg/mL	3	0.559	0.016	20.60
40 µg/mL	3	0.317	0.013	54.54
60 µg/mL	3	0.204	0.025	72.13
80 µg/mL	3	0.149	0.036	80.61
100 µg/mL	3	0.119	0.020	82.86

The percentage inhibition values of Jasminum extract (Table 7) indicate that the inhibition of Plaque regrowth at 2 hours is lower (21.39%) than the inhibition at 4 hours (31.23%). This shows that the percentage of inhibition of plaque regrowth is greater after 4 hours of incubation, indicating a latent period before the extract starts exerting its antimicrobial property. At 6 hours, the value decreases (27.63%) and at 8 hours, the percentage inhibition is the least (17.19%), which could be due to degradation of the phenolic compounds and pigments by light or metabolic by-products of bacteria.

Table 7: Percentage Protection of RBC Membrane by Rosa Damascena and Jasminum Sambac Extract

Sample	Mean OD	% Protection
Rosa damascena (0.3 mg/ml)	1.972	**11.92**
Jasminum sambac (0.3mg/ml)	2.032	**9.27**
Positive Control (Aspirin 100 µg/ml)	1.585	**29.21**
Negative control	2.239	

The Percentage (%) inhibition values of 0.2% Chlorhexidine (Table 8-10) are 66.00%, 93.34%, 96.45% and 97.83% at 2,4,6 and 8 hours respectively. The maximum % inhibition of Plaque Regrowth by the Extract is seen at 8 hrs (97.83%) followed by 6 hrs (96.45). The least inhibition is seen at the time period of 2 hrs (66.00%). This indicates a continuous inhibition of plaque regrowth till a time period of 8 hours with chlorhexidine.

Table 8: Percentage Inhibition of Plaque Regrowth by Rosa Damascena Extract

Parameter	2 Hrs	4 Hrs	6 Hrs	8 Hrs
Mean	21.10	37.99	34.70	19.70
S.D.	17.20	13.65	10.85	10.45
S.E.	4.44	3.53	2.80	2.70

S.D -Standard deviation. S.E -Standard error

Table 9: Percentage Inhibition of Plaque Regrowth Jasminum Sambac Extract

Parameter	2 Hrs	4 Hrs	6 Hrs	8 Hrs
Mean	21.39	31.23	27.63	17.19
S.D.	32.24	17.71	12.04	12.78
S.E.	8.32	4.57	3.11	3.30

S.D -Standard deviation. S.E -Standard error

Table 10: Percentage Inhibition of 0.2% Chlorhexidine

Parameter	2 Hrs	4 Hrs	6 Hrs	8 Hrs
Mean	66.00	93.34	96.45	97.84
S.D.	13.39	3.99	3.39	2.99
S.E.	3.87	1.15	0.98	0.86

Conclusion

This study concluded that the ethanolic extracts of Rosa damascena and Jasminum Sambac, two locally available flowers in India, have an inhibitory effect on regrowth of dental plaque. Additionally, they also possess antioxidant, antibacterial and inflammatory properties. Hence further clinical studies need to be carried out to validate further as a suitable natural preventive oral antimicrobial acting locally in form of supplements added to the dietary products and oral care products for children and adults for health and disease of oral cavity.

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