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COMPARATIVE EVALUATION OF ANTI-MICROBIAL EFFICACY OF PROPOLIS AND CHLORHEXIDINE MOUTHWASH ON P. GINGIVALIS, T. FORSYTHIA, P. INTERMEDIA AND AGGREGATIBACTOR ACTINOMYCETEMCOMITANS PERIODONTAL PATHOGENS – AN IN-VITRO STUDY.

Dental Science

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ABSTRACT

Introduction: The clinical use of antibiotics and other antimicrobial agents, as adjuvant for the treatment of periodontitis, has been extensively investigated in the past decade. Recently, special attention has been paid to natural medication, and propolis has been reported to possess certain medicinal properties. Chlorhexidine has proved to be the most effective agent for reducing plaque and gingivitis. Aim of this study is to comparatively evaluate antimicrobial efficacy of Propolis mouthwash with 0.2 % chlorhexidine on P.Gingivalis, T. Forshythia, P. Intermedia and A.a. periodontal pathogens.

Materials and methods: Sterilized nutrient agar plates were inoculated by rubbing sterile cotton swabs dipped into bacterial suspensions of P.Gingivalis, T. Forshythia, P. Intermedia and A.a. (over night cultures grown at 37° on nutrient agar) over the entire surface of the plate. The micro-organisms used were cultured and made available from the strains present with the institute. The plates were allowed to culture over night at 37°. After inoculation, 10 mm diameter five wells were cut into the surface of the agar using a sterile cork borer for each sample. Propolis and 0.2% chlorhexidine, were added into wells in four different plates containing above mentioned four different bacterias

Results: Result of the study showed that 100% and 75% propolis concentration showed significant antimicrobial property against P.gingivalis, T.forsythia, P.Intermedia and A.a, equal to that of chlorhexidine.

Conclusion: This study showed that propolis has significant antimicrobial efficacy against periodontopathogens, in which 100% and 75% propolis showed significant antimicrobial efiicacy, which is equal to that of 0.2% chlorhexidine.

KEYWORDS

Propolis, Chlorhexidine, P.gingivalis, T.forsythia, Aggregatibector actinomycetemcomitans, P.intermedia

INTRODUCTION:

Human periodontal disease has been associated with a complex microbiota. The development of destructive periodontitis seems to be the result of a specific infection. Gram positive coccoid bacteria have been related to periodontal health, while periodontal disease was associated with Gram negative rods and spirochetes.[1]

Many authors suggest that the presence of A.a, P. gingivalis and P. intermedia is related to active periodontal disease. Other species like F. nucleatum and Capnocytophaga sp. were also associated with disease. According to the Consensus report of the World Workshop on Clinical Periodontics (1996), human periodontitis is initiated and perpetuated by a small group of bacteria that colonize the subgingival region, mainly Gram negative anaerobic bacteria.

The periodontal pathogens mainly, Porphyromonas Gingivalis (Red Complex) which is aggressive periodontal pathogen with series of virulence factors like proteases, haemolysin and collagenase. It also has ability to inhibit migration of PMNs across the epithelial barrier and also can penetrate the soft tissue. T. forshythia (Red Complex) produces several proteolytic enzymes that are able to destroy immunoglobulins and can also induce cell death. Prevotella intermedia (Orange Complex) are less virulent and less protelytic as compared to P. gingivalis and T. forsythia, however it utilizes growth factors for the nutrition.[2]

Numerous oral active antimicrobial substances have been discovered and used to prevent and reduce the formation of dental plaque. Chlorhexidine gluconate has proved to be the most effective agent in numerous studies and is still examined intensely. It has been used either combined with other ingredients or as a positive control. Duration of its use is limited due to its undesirable effects like taste disturbances, tooth discoloration and mucosal erosions but because of its outstanding substantivity, the active ingredient chlorhexidine has proved to be the most effective agent for reducing plaque and gingivitis. 0.2% chlorhexidine solution is therefore the gold standard and generally used in studies as a positive control.[2]

Various antimicrobial agents and antibiotics have been investigated clinically as an adjunct in the treatment of periodontitits. Recently, special attention has been paid to natural medication and propolis has been reported to possess certain medicinal properties.

Propolis is a natural composite balsam, produced by honey bees (*Apis melifera*) from the gum of various plants. Bees collect vegetal exudates and form pellets with their mandibles, mixing the exudates with wax and products of their salivary glands.[3]

Among the various natural products, propolis has received greater attention due to its broad-spectrum antimicrobial activity against wide range of pathogenic microorganism. It is suggested that propolis extract could be same as a new source of natural antimicrobial and antioxidant with potential applications for reducing the levels of periopathogens.[4]

Insufficient evidences are available to support antimicrobial property of different concentrations of propolis mouthwash in context to red, orange and green complexes of periodontal pathogens and even comparison of same with clinically used and accepted 0.2% chlorhexidine.

Insufficient evidences are available to support antimicrobial property of different concentrations of propolis mouthwash in context to red, orange and green complexes of periodontal pathogens and even comparison of same with clinically used and accepted 0.2% chlorhexidine.

This in-vitro study was planned to evaluate antibacterial efficacy of Propolis mouthwash 100%, 75%, 50%, 25% and 12.5% and to compare that with 0.2 % chlorhexidine on P.Gingivalis, T. Forshythia (Red complex), P. Intermedia (Orange complex) and A.a. (Green Complex) periodontal pathogens.

METHODOLOGY:

The Present study was conducted in the Department of Periodontics, K. M. Shah Dental College, Pipariya, Vadodara. after Institutional Ethics Committee approval was obtained. I. E.C. approval no. is SVIEC/ON/DENT/SRP/15118 (Annexure I). Microbiological analysis was done at Maratha Mandal's Nathajirao G. Helgekar Institute of Dental Science Department of Microbiology, Belgaum.

A total of 112 samples were included in the study. There were 4 groups of organisms, tested on 7 different reagents: 100% propolis, 75% propolis, 50% propolis, 25% propolis, 12.5% propolis liquid forms

and 0.2% chlorhexidine mouthwash and distilled water. Each sample was repeated 4 times to obtain a mean value.

Sterilized nutrient agar plates were inoculated by rubbing sterile cotton swabs dipped into bacterial suspensions of Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Aggregatibacter actinomycetemcommitans (over night cultures grown at 37° on nutrient agar) over the entire surface of the plate. The micro-organisms used were cultured and made available from the strains present with the institute. Strict precautions were followed in order to avoid any contamination of the surrounding area. The inoculation of the plates was performed in the laminar air flow unit. The strains were allowed to grow in strict anaerobic condition. The plates were allowed to culture over night at 37°. After inoculation, 10 mm diameter five wells were cut into the surface of the agar using a sterile cork borer for each sample.

The concentration of propolis was obtained volume by volume dilution. 100% propolis had 1ml of propolis. 75 % propolis had 0.75 ml propolis and 0.25ml distilled water. 50% propolis had 0.50ml of propolis and 0.50ml of distilled water. Similarly 25% propolis had 0.25 ml of propolis and 0.75 ml of distilled water. 12.5% propolis had 0.125 ml of propolis and 0.875 ml of distilled water.

Different concentration of Propolis and 0.2% chlorhexidine, was added into wells in four different plates containing above mentioned four different bacterias. Plates were then incubated at 37° for 24 hours. The diameter of zones of inhibition was measured using Vernier calliper on all the plates. The mean score of zones of inhibition were calculated. (Figure 1, 2, 3 and 4).

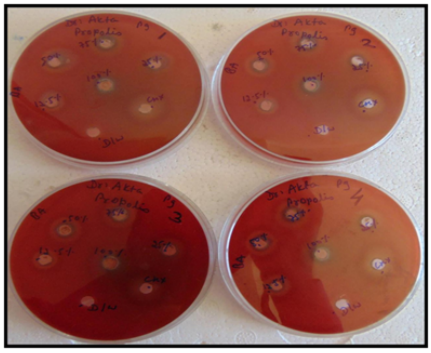

figure - 1

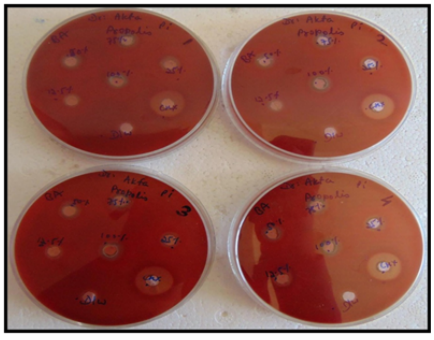

figure - 2

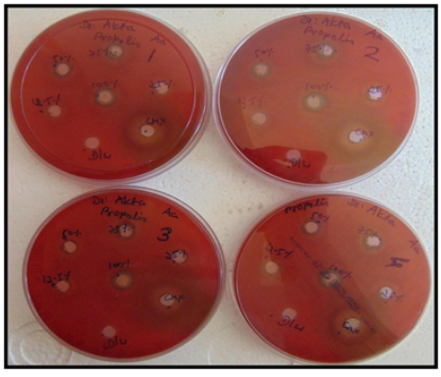

figure - 3

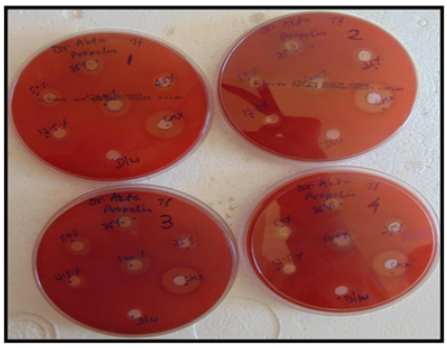

figure - 4

Groups: 28 such plates will be prepared for the following groups:

I a	II a	III a	IV a
I b	II b	III b	IV b
I c	II c	III c	IV c
I d	II d	III d	IV d
I e	II e	III e	IV e
I f	II f	III f	IV f
I g	II g	III g	Iv g

RESULT:-

Table -1 is showing inter-group comparison of different concentration of propolis liquid form and chlorhexidine mouthwash. ANOVA test was used for inter-group comparison and significant difference found between groups in their antimicrobial activity against periodontal pathogens (p-value, Pg, 0.007, Pi <0.001, Aa 0.008, Tf 0.033). (Table – 1)

TABLE – 1 Inter-group Comparison Of Different Concentration Of Propolis And 0.2% Chlorhexidine Against Periodontal Pathogens

		Sum of Squares	Df	Mean Square	F	p-value
Pg	Between Groups	87.11	5	17.42	4.91	0.007
	Within Groups	53.16	15	3.54		
	Total	140.28	20			
Pi	Between Groups	163.50	5	32.70	26.16	<0.001
	Within Groups	22.50	18	1.25		
	Total	186.00	23			
Aa	Between Groups	45.98	3	15.32	6.54	0.008
	Within Groups	25.75	11	2.34		
	Total	71.73	14			
Tf	Between Groups	28.58	3	9.52	4.86	0.033
	Within Groups	15.66	8	1.95		
	Total	44.25	11			

p-value <0.05 significant.

Table – 2 is showing multiple comparisons among different concentration of propolis and chlorhexidine group in their antimicrobial efficacy against P. gingivalis. Result showed significantly equal antimicrobial efficacy of propolis 100% (p-value – 0.354), propolis 75% (p value - 0.668), 50% (p-value 0.373) and 0.2% chlorhexidine (p-value 0.354) while propolis 25% (p-value 0.529) and 12.5% (p-value 0.206) concentrations showed significantly less antimicrobial efficacy compared to others.

TABLE -2 Post Hoc Tests Was Used For Multiple Comparisons Between Different Concentration Of Propolis And 0.2% Chlorhexidine Against P. Gingivalis

Dependent Variable	Group		Mean Difference	Std. Error	p-value	95% Confidence Interval	
						Lower Bound	Upper Bound
Pg	Propolis 100%	Propolis 75%	2.00	1.33	0.66	-2.32	6.32
		Propolis 50%	2.91	1.43	0.37	-1.75	7.58
		Propolis 25%	5.25*	1.43	0.02	0.57	9.92

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		Propolis 12.5%	6.25*	1.43	0.00	1.57	10.92
		CHX	2.75	1.33	0.35	-1.57	7.07
	Propolis 75%	Propolis 100%	-2.00	1.33	0.66	-6.32	2.32
		Propolis 50%	0.91	1.43	0.98	-3.75	5.58
		Propolis 25%	3.25	1.43	0.26	-1.42	7.92
		Propolis 12.5%	4.25	1.43	0.08	-0.42	8.92
		CHX	0.75	1.33	0.99	-3.57	5.07
	Propolis 50%	Propolis 100%	-2.91	1.43	0.37	-7.58	1.75
		Propolis 75%	-0.91	1.43	0.98	-5.58	3.75
		Propolis 25%	2.33	1.53	0.65	-2.66	7.32
		Propolis 12.5%	3.33	1.53	0.30	-1.66	8.32
		CHX	-0.16	1.43	1.00	-4.83	4.50
	Propolis 25%	Propolis 100%	-5.25*	1.43	0.02	-9.92	-0.57
		Propolis 75%	-3.25	1.43	0.26	-7.92	1.42
		Propolis 50%	-2.33	1.53	0.65	-7.32	2.66
		Propolis 12.5%	1.00	1.53	0.98	-3.99	5.99
		CHX	-2.5	1.43	0.52	-7.17	2.17
	Propolis 12.5%	Propolis 100%	-6.25*	1.43	0.00	-10.92	-1.57
		Propolis 75%	-4.25	1.43	0.08	-8.92	0.42
		Propolis 50%	-3.33	1.53	0.30	-8.32	1.66
		Propolis 25%	-1.00	1.53	0.98	-5.99	3.99
		CHX	-3.50	1.43	0.20	-8.17	1.17
	CHX	Propolis 100%	-2.750	1.33	0.35	-7.07	1.57
		Propolis 75%	-0.75	1.33	0.99	-5.07	3.57
		Propolis 50%	0.16	1.43	1.00	-4.50	4.83
		Propolis 25%	2.50	1.43	0.52	-2.17	7.17
		Propolis 12.5%	3.50	1.43	0.20	-1.17	8.17

Pg – Porphyromonas gingivalis, (p-value <0.05 significant)

Table – 3 is showing multiple comparisons among different concentration of propolis and chlorhexidine group in their antimicrobial efficacy against P. intermedia. Result showed significantly equal antimicrobial efficacy of propolis 100% (p-value – 0.799), propolis 75% (p-value – 0.095 and 0.2% chlorhexidine (p value -0.799) while propolis 50% (p-value – 0.00), 25% (p value 0.00) and 12.5% (p-value 0.00) concentrations showed significantly less antimicrobial efficacy compared to others.

TABLE – 3 Post Hoc Tests Was Used For Multiple Comparisons Between Different Concentration Of Propolis And 0.2% Chlorhexidine Against P. Intermedia

Dependent Variable	Group		Mean Difference	Std. Error	p-value	95% Confidence Interval	
						Lower Bound	Upper Bound
Pi	Propolis 100%	Propolis 75%	2.25	0.79	0.09	-0.26	4.76
		Propolis 50%	3.75*	0.79	0.00	1.23	6.26
		Propolis 25%	5.75*	0.79	0.00	3.23	8.26
		Propolis 12.5%	5.75*	0.79	0.00	3.23	8.26
		CHX	-1.00	0.79	0.79	-3.51	1.51
	Propolis 75%	Propolis 100%	-2.25	0.79	0.09	-4.76	0.26
		Propolis 50%	1.50	0.79	0.43	-1.01	4.01
		Propolis 25%	3.50*	0.79	0.00	0.98	6.01
		Propolis 12.5%	3.50*	0.79	0.00	0.98	6.01
		CHX	-3.25*	0.79	0.00	-5.76	-0.73
	Propolis 50%	Propolis 100%	-3.75*	0.79	0.00	-6.26	-1.23
		Propolis 75%	-1.50	0.79	0.43	-4.01	1.01
		Propolis 25%	2.00	0.79	0.16	-0.51	4.51
		Propolis 12.5%	2.00	0.79	0.16	-0.51	4.51
		CHX	-4.75*	0.79	0.00	-7.26	-2.23
	Propolis 25%	Propolis 100%	-5.75*	0.79	0.00	-8.26	-3.23
		Propolis 75%	-3.50*	0.79	0.00	-6.01	-0.98
		Propolis 50%	-2.00	0.79	0.16	-4.51	0.51
		Propolis 12.5%	0.00	0.79	1.00	-2.51	2.51
		CHX	-6.75*	0.79	0.00	-9.26	-4.23
	Propolis 12.5%	Propolis 100%	-5.75*	0.79	0.00	-8.26	-3.23
		Propolis 75%	-3.50*	0.79	0.00	-6.01	-0.98
		Propolis 50%	-2.00	0.79	0.16	-4.51	0.51
		Propolis 25%	0.00	0.79	1.00	-2.51	2.51
		CHX	-6.75*	0.79	0.00	-9.26	-4.23
	CHX	Propolis 100%	1.00	0.79	0.79	-1.51	3.51
		Propolis 75%	3.25*	0.79	0.00	0.73	5.76
		Propolis 50%	4.75*	0.79	0.00	2.23	7.26
		Propolis 25%	6.75*	0.79	0.00	4.23	9.26
		Propolis 12.5%	6.75*	0.79	0.00	4.23	9.26

Pi – Provotella intermedia, (p value <0.05 significant)

Table – 4 is showing multiple comparisons among different concentration of propolis and chlorhexidine group in their antimicrobial efficacy against A.a. Result showed significantly equal antimicrobial efficacy of propolis 100% and Chx (p-value 0.821) while propolis 75% and propolis 50% showed significantly less antimicrobial efficacy (p-value 0.003 and 0.041)compared to other concentrations

TABLE – 4 Post Hoc Tests Was Used For Multiple Comparisons Between Different Concentration Of Propolis And 0.2% Chlorhexidine Against A.a

Dependent Variable	Group		Mean Difference	Std. Error	p-value	95% Confidence Interval	
						Lower Bound	Upper Bound
A.a	Propolis 100%	Propolis 75%	2.50	1.08	0.04	0.11	4.88
		Propolis 50%	4.50	1.16	0.00	1.92	7.07
		CHX	0.25	1.08	0.82	-2.13	2.63
	Propolis 75%	Propolis 50%	2.00	1.16	0.11	-0.57	4.57
		CHX	-2.25	1.08	0.06	-4.63	0.13
	Propolis 50%	CHX	-4.25	1.16	0.00	-6.82	-1.67

Table – 5 is showing multiple comparisons among different concentration of propolis and chlorhexidine group in their antimicrobial efficacy against T.f. Result showed significantly equal antimicrobial efficacy of propolis 100% and Chx (p-value 0.307) while propolis 75% showed significantly less antimicrobial efficacy compared to other concentrations (p-value 0.035).

TABLE – 5 Post Hoc Tests Was Used For Multiple Comparisons Between Different Concentration Of Propolis And 0.2% Chlorhexidine Against T.f

Dependent Variable	Group		Mean Difference	Std. Error	p-value	95% Confidence Interval	
						Lower Bound	Upper Bound
T.f	Propolis 100%	Propolis 75%	2.50	0.98	0.03	0.21	4.78

		CHX	-1.16	1.06	0.30	-3.63	1.29
	Propolis 75%	CHX	-3.66	1.06	0.00	-6.13	-1.20

Hence, Result of the study showed that 100% and 75% propolis concentration showed significant antimicrobial property against P.gingivalis, T.forsythia, P.Intermedia and A.a, equal to that of chlorhexidine.

DISCUSSION:-

The aim of the present study was to evaluate the antimicrobial efficacy of different concentration of propolis (100%, 75%, 50%, 25% and 12.5%) and to compare it with 0.2% chlorhexidine mouthwash against P.gingivalis, T.forsythia, P intermedia and A.a.

Antimicrobial activity of propolis is not surprising. In the hive, primary function of propolis is to act as a biocide, being active against invasive bacteria, fungi and even invading larvae. Numbers of studies have proved the biocidal functions of propolis, its extracts and constituents. Propolis has broad-spectrum of activity against Gram positive and Gram negative rods and cocci, yeast and fungi. The antimicrobial activity of propolis extract has been studied by several authors, however, few studies have investigated its activity towards oral pathogens.

The present study included red complex, orange complex and green complex periodontopathogens, which are mainly involved in various periodontal diseases. In this study, micro-oraganisms were cultured in sterilized agar and different concentration of propolis and 0.2% chlorhexidine was added into them to examine their antimicrobial activity (figure – 1,2,3 and 4).

In 2005, Feres M et al studied antimicrobial efficacy of propolis and chlorhexidine in saliva of healthy and chronic periodontitis patients. This study reported significant reduction in oral microorganism.[9]

Recently in 2014, Sanghani N used Indian propolis extract subgingivally in patients with ≥5 mm. Subgingival delivery of propolis as an adjuct to scaling and root planning showed significant improvement in both clinical and microbiological parameters ($p<0.01$).[8]

The present study showed antimicrobial efficacy of various concentration of propolis in which, 100% propolis and 75% propolis showed significant antimicrobial efficacy against P.gingivalis and P. intermedia periodontal pathogens, equal to that of 0.2% chlorhexidine. (Table – 1.2 and 3) Also Antimicrobial efficacy of propolis 100% was found significantly equal to that of Chx against A.a and T.f, compared to other concentrations. (Table – 1, 4 and 5)

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