Antibacterial potential from periodontal dressing raw propolis *Trigona itama* bee based against *Porphyromonas gingivalis*: experimental research

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Submission | 12 June 2022
Revised | 15 June 2022
Accepted | 27 April 2023
Published | 30 April 2023
DOI: 10.24198/jkg.v35i1.46229

Citation | Suryono, Yassa RM, Saputro LT, Aji RNAS. Antibacterial potential from periodontal dressing raw propolis Trigona itama bee based against Porphyromonas gingivalis. J Ked Gi. 2023;35(1):6-13
DOI: 10.24198/jkg.v35i1.46229

ABSTRACT

Introduction: One of the procedures used in dentistry to address periodontal disease is periodontal surgery, which can lead to open wounds. Periodontal dressing is a dental material that can help with this issue by accelerate the healing of wounds. The aim of the in vitro study was to investigate the potential inhibition of periodontal dressing with the basic formulated from raw propolis *Trigona itama* bees against *Porphyromonas gingivalis* bacteria at various rates. **Methods:** This in vitro study was true experimental study with *Porphyromonas gingivalis* cultured in agar plate as sample which sample size was calculated using Federer formula. Periodontal dressing with raw propolis formulations were divided into eight groups of 85, 80, 75, 70, 65, 62.5, 60 and 57%, which were then compared with the 100% raw propolis, RESO-PAC™ group, COE-PAK™, and Baer formulations. Antibacterial testing was tested using zone of inhibition test and was calculated by adding up the zone of inhibition of *Porphyromonas gingivalis*. The data results were then analyzed using one-way ANOVA ($p<0.05$). **Results:** The Shapiro-Wilk test results for the normality test show a significance level of 0.05. It means that the data is commonly distributed. the homogeneity test using Levene's statistics has a significance level of $p>0.05$. This means the data homogenous distributed. The results of statistical analysis using one-way ANOVA showed a p-value=0.001 ($p<0.05$), which means that there was a significant difference between the treatment groups. The results showed that pure raw propolis had the highest antibacterial with an inhibition zone diameter of 3.4667 mm after RESO-PAC™, followed by periodontal dressing with raw propolis 85% with an inhibition zone of 2.9167 mm, and periodontal dressing with raw propolis 80% with an inhibition zone of 2.5167 mm. However, at concentrations of 75 to 57%, no bacterial inhibition zone was found. **Conclusion:** Periodontal dressing with raw propolis formulation 85% had the highest antibacterial activity of *Porphyromonas gingivalis* after 100% raw propolis and RESO-PAC™.

Keywords

trigona itama, antibacterial assay, periodontal dressing, raw propolis

Potensi antibakteri periodontal dressing propolis mentah lebah Trigona itama terhadap Porphyromonas gingivalis: Penelitian eksperimental

ABSTRAK

Pendahuluan: *Salah satu prosedur yang dapat digunakan untuk merawat penyakit periodontal adalah bedah periodontal yang akan mengakibatkan luka terbuka pada daerah operasi.* Periodontal dressing *adalah suatu material kedokteran gigi yang dapat membantu dalam proses penyembuhan luka pasca bedah periodontal. Tujuan dari penelitian* in vitro *ini adalah mengetahui potensi inhibisi* periodontal dressing dari formulasi propolis mental lebah *Trigona Itama pada Porphyromonas gingivalis dalam beberapa konsentrasi.* ***Metode:*** *Studi ini merupakan penelitian eksperimental murni dengan sampel penelitian bakteri Porpohyromonas gingivalis yang dikultur pada plat agar dengan perhitungan sampel dilakukan menggunakan rumus Federer. Studi ini membagi periodontal dressing propolis mentah menjadi 8 kelompok: 85, 80, 75, 70, 65, 62,5, 60, dan 57% yang dibandingkan dengan propolis mentah 100%, RESO-PAC™, COE-PAK™, dan* Baer Formulation. *Pengukuran antibakteri dilakukan menggunakan uji zona inhibisi Porphyromonas gingivalis yang dianalisa menggunakan* one-way ANOVA ($p<0.05$). ***Hasil:*** *Hasil uji normalitas dengan menggunakan uji Shapiro-Wilk menunjukkan nilai signifikansi p>0,05. Hal ini berarti data berdistribusi normal, Hasil uji homogenitas dengan* levene's statistic *menunjukkan nilai signifikansi p>0,05, hal ini menunjukan bahwa data homogen. Hasil analisis statistik dengan menggunakan* one way ANOVA *menunjukkan* p-value=0,001 ($p<0,05$) yang berarti terdapat perbedaan yang bermakna antar kelompok perlakuan**.** Hasil penelitian menunjukkan *propolis mentah memiliki potensi antibakteri paling tinggi dengan diameter zona inhibisi 3.4667 mm setelah RESO-PAC™k, diikuti propolis mentah 85% dengan diameter 2.9167 mm dan propolis mentah 80% dengan diameter 2.5167 mm. Tidak ditemukan zona inhibisi bakteri pada konsentrasi 57% hingga 75%.* ***Simpulan:*** Periodontal dressing *menggunakan konsentrasi 85% memiliki aktivitas antibakteri tertinggi pada Porphyromonas gingivalis setelah propolis mentah 100% dan RESO-PAC™.*

Kata kunci

trigona itama, pengukuran antibakteri, periodontal dressing, propolis mentah

INTRODUCTION

One of the procedures used in dentistry to address periodontal disease is periodontal surgery, which can lead to open wounds like gingivectomy and depigmentation.[1] The process of healing a wound includes a number of steps and the return of normal cellular biology.[2] This process has three stages: restructuring, proliferative, and inflammatory. During these three phases, a complicated and coordinated sequence of the process takes place. It was believed that protecting the surgical site with periodontal dressing materials can prevents microbial infections by decreasing plaque accumulation.[3,4] A periodontal dressing is a material that can optimize the wound healing process by protecting the tissue from the possibility of infection, reducing bleeding, and avoiding trauma during the masticatory process.[5]

By protecting the tissue from bacterial infection such as *Porphyromonas gingivalis*, reducing bleeding, and avoiding trauma during the masticatory process, a periodontal dressing can accelerate the healing of wounds.[5] Today's periodontal treatments still have some limitations. Eugenol-based periodontal dressings frequently result in tissue necrosis, allergic responses, inflammation, and delayed wound healing. Numerous studies have discovered that eugenol-containing periodontal dressings frequently result in inflammation, tissue necrosis, allergic responses, and delays in wound healing.[6]

The human oral area is host to both pathogenic and opportunistic microbes. To prevent periodontal surgery failure, adequate microbial activity control is necessary.[7] Alternative materials need to be developed that can start wound healing without having any negative side effects. Various types of synthetic antimicrobial materials have been tried to be added to periodontal dressing materials, but this may result in the occurrence of sensitization, hypersensitivity, candidiasis, and resistance.[8]

There have been attempts at putting various manufactured antimicrobial materials into periodontal dressing material, but performing thereby can lead to sensitization, hypersensitivity, candidiasis, and resistance. As a result, alternative components that facilitate wound healing without having any negative side effects are needed.[8] Under controlled conditions, modifications and adds to active ingredients can be used as antibacterial agents, wound healing agents, and products that are simple to use and economical to produce.[9]

Propolis is a resinous yellow-brown to dark brown compound collected by bees *(Apis mellifera*) from tree buds; sap flows, shrubs, or other vegetable sources. It is a potent antimicrobial, antioxidant, and anti-inflammatory agent.[10] The bee species Trigona Sp. *(Trigona itama)* does not sting and can produce very high amounts of propolis.[11] Propolis is safe to use and can increase the cure rate of periodontal disease therapy, and can be used as an alternative therapy option in the treatment of periodontal disease and supportive periodontal therapy.[12] Therefore, the purpose of this study was to analyze the antibacterial potential of *Trigona itama* bee propolis on the growth of *Porphyromonas gingivalis*, which is used as the primary material for making periodontal dressings.

METHODS

This in vitro study is true experimental research. For three months (July–October 2021), this study was conducted in *Laboratorium Riset Terpadu*, Faculty of Dentistry, *Universitas Gadjah Mada* for making the sample of Propolis; Microbiology Laboratory Research Center, Faculty of Dentistry, *Universitas Airlangga* for supply and DOIng observations on the activity of these bacteria (July– October 2021). The research was conducted using a simple random sampling method. Inclusion criteria for the antibacterial assay were sterilized periodontal dressing with formulations of raw propolis, COE-PAK™, RESO-PAC™, and a periodontal dressing Baer formulation and bacteria *Porphyromonas gingivalis* (ATCC 33277). The bacteria used were not contaminated with other strains. The criteria for sampling are calculated using the Federer formula. Based on the total of the treatment, it is obtained that the sample size of each treatment is three.

Tools made of glass and metal are washed and sterilized with a dry heat oven for 15 minutes with a temperature of 110 °C, while tools which are made of plastic are washed, dried, and smeared with alcohol of 70%. The raw propolis harvesting stage of *Trigona itama* is located in Godean, Sleman, Yogyakarta. This raw propolis is taken from the honeycomb box using sterile medical gloves. Then, scrape the knife to separate the bees still attached to the honeycomb, put it into a jar for further labeling. Samples of honey and propolis collected from the field are then processed in the laboratory, with the propolis separated from the honey and then weighed, and then the vacuum process is used to sterilize raw propolis.

Cellulose-based periodontal dressing such as RESO-PAC™ was manipulated in the *Laboratorium Riset Terpadu* of Faculty of Dentistry*, Universitas Gadjah Mada* by removing the material from the tube and pressing it until it comes out on moist medical gloves, then applied by evenly spreading the material on the wound area. The material was then shaped like bars 5 mm long for three pieces, then used as a positive control.

The making process was conducted in *Laboratorium Riset Terpadu* FKG UGM, with powder composition: 2.85 grams of rosin and zinc oxide of 2.15 grams mixed until homogeneous. Negative control for periodontal dressing was made by mixing 4.75 grams of hydrogenated fat and 0.25 grams of zinc oxide until homogeneous.[2] The periodontal dressing was shaped as bars for 5 mm long for three pieces, then used as a negative control.

Trigona itama raw propolis was mixed with zinc oxide in *Laboratorium Riset Terpadu* FKG UGM. The concentrations used were 57%:43%, which means 5.7 grams of propolis and 4.3 grams of zinc oxide, 60%:40%

that was 6 grams of propolis and 4 grams of zinc oxide, 62.5: 37.5% that was 6.25 grams of propolis and 3.75 grams of zinc oxide, 65%:35% that was 6.5 grams of propolis and 3.5 grams of zinc oxide, 70%:30% that was 7 grams of propolis and 3 grams of zinc oxide, 75%:25% that was 7.5 grams of propolis and 2.5 grams of zinc oxide, 80%:20% that was 8 grams of propolis and 2 grams of zinc oxide, 85%:15% that was 8.5 grams of propolis and 1.5 grams of zinc oxide, and 100% raw propolis with several treatment samples of each concentration were three samples, so the total sample were 27.

The bacterial agar plate was partitioned for each sample form. Bacterial agar plates that have been given a sample are marked according to the sample applied, closed, and put in a desiccator and incubated under anaerobic conditions at 37 °C for 24 hours. After 24 hours bacterial agar plate is taken out and measured according to inhibition zone measurement formula. Microbiology Laboratory Research Center, Faculty of Dentistry, *Universitas Airlangga* provided the bacteria used in this study. Observations on the activity of these bacteria were carried out in the Microbiology Laboratory Research Center, Faculty of Dentistry, *Universitas Airlangga* in order to minimize the possibility of bacteria being damaged due to shipping process.

The antibacterial assay was measured and the data were analyzed statistically using the Statistical Package for the Social Sciences (SPSS) software for Windows, version 24.0 (SPSS, Chicago, Illinois) for One-way analysis of variance (ANOVA) with a significance level of 0.05 for antibacterial effect followed by a post hoc least significant difference (LSD).

RESULTS

The results of the descriptive analysis test showed that the treatment group against *Porphyromonas gingivalis* had the highest inhibitory, namely RESO-PAC™ with a mean zone of inhibition of 9,6500 mm, followed by a concentration of 100% raw propolis with a mean zone of inhibition of 5,1000 mm, followed by a concentration of raw propolis. Eighty Five percents with an inhibition zone of 4,1333 mm, for a concentration of raw propolis 80% has the lowest inhibition zone of 3,5333 mm. Concentrations of raw propolis 57, 60, 62.5, 65, 70, and 75% had no inhibition zones for *Porphyromonas gingivalis bacteria* at all as seen in Table 1.

Table 1. Descriptive analysis

Group	n	Mean	Std. Deviation
Raw Propolis 57%	3	0.00	0.00
Raw Propolis 60%	3	0.00	0.00
Raw Propolis 62.5%	3	0.00	0.00
Raw Propolis 65%	3	0.00	0.00
Raw Propolis 70%	3	0.00	0.00
Raw Propolis 75%	3	0.00	0.00
Raw Propolis 80%	3	3.5333	0.30551
Raw Propolis 85%	3	4.1333	0.30551
Raw Propolis 100%	3	5.1000	0.13229
COE-PAK™	3	0.00	0.00
RESO-PAC™	3	9.6500	0.27839
Baer Formulation	3	0.00	0.00

Table 2. One-way ANOVA test results of significance differences between each treatment group tested

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	320.477	11	29.134	1241.225	0.001*
Within Groups	0.563	24	0.023		
Total	321.041	35			

* = Statistically significant

Table 3. Tests of normality

		Kolmogorov-Smirnov[g]			Shapiro-Wilk		
	Perlakuan	Statistic	df	Sig.	Statistic	df	p-value
Inhibitory P. gingivalis	Raw propolis 80%	0.253	3	.	0.964	3	0.637
	Raw propolis 85%	0.253	3	.	0.964	3	0.637
	Raw propolis 100%	0.314	3	.	0.893	3	0.363
	RESO-PAC™k	0.238	3	.	0.976	3	0.702

The average inhibition zone diameter against *Porphyromonas gingivalis* bacteria. RESO-PAC™ has the most significant inhibition compared to raw propolis 100%, formulation of 85% raw propolis, and 80%. On the other side, the negative control, which is coe-pack and periodontal dressing of Baer formulation, does not have

antibacterial strength. The results of statistical analysis using one-way ANOVA showed a p-value=0.001 (p<0.05), which means that there was a significant difference between the treatment groups. The Shapiro-Wilk test results for the normality test show a significance level of p>0.05. It means that the data is normally distributed.

Table 4. Test of homogeneity of variances

P.Gingivalis inhibitation zone			
Levene Statistic	**df1**	**df2**	**p-value**
1,737	11	24	0.175

The table above shows the results of the homogeneity test using Levene's statistics has a significance level of p > 0.05. This means the data homogenous distributed.

Table 5. LSD Multiple Comparisons

(I) Periodontal Dressing	(J) Periodontal Dressing	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Raw propolis 57%	Raw propolis 60%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 62.5%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 65%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 70%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 75%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 80%	-3.53333*	0.12509	0.000	-3.7915	-3.2752
	Raw propolis 85%	-4.13333*	0.12509	0.000	-4.3915	-3.8752
	Raw propolis 100%	-5.10000*	0.12509	0.000	-5.3582	-4.8418
	COE-PAK™	0.00000	0.12509	1.000	-0.2582	0.2582
	RESO-PAC™k	-9.65000*	0.12509	0.000	-9.9082	-9.3918
	Baer formulation	0.00000	0.12509	1.000	-0.2582	0.2582
Raw propolis 60%	Raw propolis 57%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 62.5%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 65%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 70%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 75%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 80%	-3.53333*	0.12509	0.000	-3.7915	-3.2752
	Raw propolis 85%	-4.13333*	0.12509	0.000	-4.3915	-3.8752
	Raw propolis 100%	-5.10000*	0.12509	0.000	-5.3582	-4.8418
	COE-PAK™	0.00000	0.12509	1.000	-0.2582	0.2582
	RESO-PAC™k	-9.65000*	0.12509	0.000	-9.9082	-9.3918
	Baer formulation	0.00000	0.12509	1.000	-0.2582	0.2582
Raw propolis 62.5%	Raw propolis 57%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 60%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 65%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 70%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 75%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 80%	-3.53333*	0.12509	0.000	-3.7915	-3.2752
	Raw propolis 85%	-4.13333*	0.12509	0.000	-4.3915	-3.8752
	Raw propolis 100%	-5.10000*	0.12509	0.000	-5.3582	-4.8418
	COE-PAK™	0.00000	0.12509	1.000	-0.2582	0.2582
	RESO-PAC™k	-9.65000*	0.12509	0.000	-9.9082	-9.3918
	Baer Formulation	0.00000	0.12509	1.000	-0.2582	0.2582
Raw propolis 65%	Raw propolis 57%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 60%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 62.5%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 70%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 75%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 80%	-3.53333*	0.12509	0.000	-3.7915	-3.2752
	Raw propolis 85%	-4.13333*	0.12509	0.000	-4.3915	-3.8752
	Raw propolis 100%	-5.10000*	0.12509	0.000	-5.3582	-4.8418
	COE-PAK™	0.00000	0.12509	1.000	-0.2582	0.2582
	RESO-PAC™k	-9.65000*	0.12509	0.000	-9.9082	-9.3918
	Baer Formulation	0.00000	0.12509	1.000	-0.2582	0.2582
Raw propolis 70%	Raw propolis 57%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 60%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 62.5%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 65%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 75%	0.00000	0.12509	1.000	-0.2582	0.2582

	Raw propolis 80%	-3.53333*	0.12509	0.000	-3.7915	-3.2752
	Raw propolis 85%	-4.13333*	0.12509	0.000	-4.3915	-3.8752
	Raw propolis 100%	-5.10000*	0.12509	0.000	-5.3582	-4.8418
	COE-PAK™	0.00000	0.12509	1.000	-0.2582	0.2582
	RESO-PAC™k	-9.65000*	0.12509	0.000	-9.9082	-9.3918
	Baer Formulation	0.00000	0.12509	1.000	-0.2582	0.2582
Raw propolis 75%	Raw propolis 57%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 60%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 62.5%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 65%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 70%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 80%	-3.53333*	0.12509	0.000	-3.7915	-3.2752
	Raw propolis 85%	-4.13333*	0.12509	0.000	-4.3915	-3.8752
	Raw propolis 100%	-5.10000*	0.12509	0.000	-5.3582	-4.8418
	COE-PAK™	0.00000	0.12509	1.000	-0.2582	0.2582
	RESO-PAC™k	-9.65000*	0.12509	0.000	-9.9082	-9.3918
	Baer Formulation	0.00000	0.12509	1.000	-0.2582	0.2582
Raw propolis 80%	Raw propolis 57%	3.53333*	0.12509	0.000	3.2752	3.7915
	Raw propolis 60%	3.53333*	0.12509	0.000	3.2752	3.7915
	Raw propolis 62.5%	3.53333*	0.12509	0.000	3.2752	3.7915
	Raw propolis 65%	3.53333*	0.12509	0.000	3.2752	3.7915
	Raw propolis 70%	3.53333*	0.12509	0.000	3.2752	3.7915
	Raw propolis 75%	3.53333*	0.12509	0.000	3.2752	3.7915
	Raw propolis 85%	-0.60000*	0.12509	0.000	-0.8582	-0.3418
	Raw propolis 100%	-1.56667*	0.12509	0.000	-1.8248	-1.3085
	COE-PAK™	3.53333*	0.12509	0.000	3.2752	3.7915
	RESO-PAC™k	-6.11667*	0.12509	0.000	-6.3748	-5.8585
	Baer Formulation	3.53333*	0.12509	0.000	3.2752	3.7915
Raw propolis 85%	Raw propolis 57%	4.13333*	0.12509	0.000	3.8752	4.3915
	Raw propolis 60%	4.13333*	0.12509	0.000	3.8752	4.3915
	Raw propolis 62.5%	4.13333*	0.12509	0.000	3.8752	4.3915
	Raw propolis 65%	4.13333*	0.12509	0.000	3.8752	4.3915
	Raw propolis 70%	4.13333*	0.12509	0.000	3.8752	4.3915
	Raw propolis 75%	4.13333*	0.12509	0.000	3.8752	4.3915
	Raw propolis 80%	0.60000*	0.12509	0.000	0.3418	0.8582
	Raw propolis 100%	-0.96667*	0.12509	0.000	-1.2248	-0.7085
	COE-PAK™	4.13333*	0.12509	0.000	3.8752	4.3915
	RESO-PAC™k	-5.51667*	0.12509	0.000	-5.7748	-5.2585
	Baer Formulation	4.13333*	0.12509	0.000	3.8752	4.3915
Raw propolis 100%	Raw propolis 57%	5.10000*	0.12509	0.000	4.8418	5.3582
	Raw propolis 60%	5.10000*	0.12509	0.000	4.8418	5.3582
	Raw propolis 62.5%	5.10000*	0.12509	0.000	4.8418	5.3582
	Raw propolis 65%	5.10000*	0.12509	0.000	4.8418	5.3582
	Raw propolis 70%	5.10000*	0.12509	0.000	4.8418	5.3582
	Raw propolis 75%	5.10000*	0.12509	0.000	4.8418	5.3582
	Raw propolis 80%	1.56667*	0.12509	0.000	1.3085	1.8248
	Raw propolis 85%	0.96667*	0.12509	0.000	0.7085	1.2248
	COE-PAK™	5.10000*	0.12509	0.000	4.8418	5.3582
	RESO-PAC™k	-4.55000*	0.12509	0.000	-4.8082	-4.2918
	Baer Formulation	5.10000*	0.12509	0.000	4.8418	5.3582
COE-PAK™	Raw propolis 57%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 60%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 62.5%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 65%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 70%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 75%	0.00000	0.12509	1.000	-0.2582	0.2582
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	Raw propolis 85%	-4.13333*	0.12509	0.000	-4.3915	-3.8752
	Raw propolis 100%	-5.10000*	0.12509	0.000	-5.3582	-4.8418
	RESO-PAC™k	-9.65000*	0.12509	0.000	-9.9082	-9.3918
	Baer Formulation	0.00000	0.12509	1.000	-0.2582	0.2582
RESO-PAC™k	Raw propolis 57%	9.65000*	0.12509	0.000	9.3918	9.9082
	Raw propolis 60%	9.65000*	0.12509	0.000	9.3918	9.9082
	Raw propolis 62.5%	9.65000*	0.12509	0.000	9.3918	9.9082
	Raw propolis 65%	9.65000*	0.12509	0.000	9.3918	9.9082
	Raw propolis 70%	9.65000*	0.12509	0.000	9.3918	9.9082
	Raw propolis 75%	9.65000*	0.12509	0.000	9.3918	9.9082

	Raw propolis 80%	6.11667*	0.12509	0.000	5.8585	6.3748
	Raw propolis 85%	5.51667*	0.12509	0.000	5.2585	5.7748
	Raw propolis 100%	4.55000*	0.12509	0.000	4.2918	4.8082
	COE-PAK™	9.65000*	0.12509	0.000	9.3918	9.9082
	Baer Formulation	9.65000*	0.12509	0.000	9.3918	9.9082
Baer Formulation	Raw propolis 57%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 60%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 62.5%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 65%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 70%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 75%	0.00000	0.12509	1.000	-0.2582	0.2582
	Raw propolis 80%	-3.53333*	0.12509	0.000	-3.7915	-3.2752
	Raw propolis 85%	-4.13333*	0.12509	0.000	-4.3915	-3.8752
	Raw propolis 100%	-5.10000*	0.12509	0.000	-5.3582	-4.8418
	COE-PAK™	0.00000	0.12509	1.000	-0.2582	0.2582
	RESO-PAC™k	-9.65000*	0.12509	0.000	-9.9082	-9.3918

*The mean difference is significant at the 0.05 level.

Furthermore, the data obtained were analyzed using multivariate comparisons with the LSD post hoc test, showing significant differences between each treatment group. Raw propolis formulation, which has the highest antibacterial strength, is 85%; this shows that raw propolis has antibacterial potential against *Porphyromonas gingivalis* bacteria.

DISCUSSION

The results showed that RESO-PAC™ had the most significant bacterial inhibition, followed by Raw Propolis 100, 85, and 80%, with significant differences between groups. Raw Propolis 75, 70, 65, 62.5, 60, and 57%, COE-PAK™™, and Baer formulations of periodontal dressing had no antimicrobial effect as indicated by the absence of the diameter of the inhibitory zone on the bacterial plate. Meanwhile, in the COE-PAK™ and the Baer formulation did not find an inhibition zone because there were no active antibacterial compounds contained in the material. Periodontal dressing raw propolis formulations of 57, 60, 62.5, 65, 70, 75% COE-PAK™, and Baer formulations of periodontal dressing had no antimicrobial effect as indicated by the absence of the diameter of the inhibitory zone on the bacterial plate. This can be explained in the periodontal dressing group with raw propolis as the base material has not reached the optimal concentration target, and with the assumption of the researcher when mixing the two periodontal dressing materials there is an area of raw propolis covered by zinc oxide so that the active compounds in raw propolis are less able to work. Periodontal dressings do not have therapeutic compounds that can accelerate wound healing but can still accelerate the wound healing process by protecting the wound while minimizing the possibility of infection and bleeding post periodontal surgical treatment.[13] An ideal periodontal dressing is soft, easy to apply, hardens after application, and is dimensionally stable, and does not cause irritation.[8] The roughness of the dressing materials causes the plaque accumulation that happens with dressings, and the often-inappropriate use of dressings allows the creation of areas of bacterial adherence. Microorganisms in plaque accumulation can cause inflammation, disrupting the wound healing process.[13] Propolis is a mixture of resinous substances, pollen, waxes, and enzymes used by bees to protect their hives, maintaining their homeostasis, reducing vibration, keeping airflow, and preventing putrefaction. People use propolis as immunostimulant due to its antibacterial and antiviral effect, skin care product due to its soothing and healing properties, drug for urinary tract itching and redness, and also to treat small ulcer and canker sores in oral cavity in many formulation form such as dentifrices, lozenges, mouth rinse, gels, tablet, and many more.[14] These usages of propolis comes from its 180 different types of chemicals which generally contains polyphenol flavonoids, phenolic acids and esters), phenolic aldehydes, and ketones.[15]

The results obtained at concentrations of 80, 85, and 100% raw propolis were significant to reduce the growth of the periodontal pathogen *Porphyromonas gingivalis* bacteria. Previous research also stated that propolis mixed with a solution in the concentration range of 10%-70% has the optimal antibacterial against gram-positive and gram-negative bacteria.[16] No studies have been found that analyze the effectiveness and antimicrobial of raw propolis samples. ZnO mixed with aquadest with a raw propolis concentration of 80% formed an inhibition zone against *Porphyromonas gingivalis* bacteria with an average diameter of 3.5333 mm, and ZnO dissolved in aqua dest then mixed with 85% raw propolis had an inhibition zone with an average diameter of 4.1333 mm, with a concentration of 100%, raw propolis without any mixing had an average inhibition zone of 9.6500 mm.

Previous researches have proven propolis antibacterial effect on various bacteria such as P. aeruginosa, S. aureus, Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, and many more. This antibacterial potential of propolis can act through either direct action or affecting immune system of the host. Modification of cell membrane permeability by propolis can reduce bacteria membrane-related activity such as ATP production thus interrupting bacteria motility. Inhibition of cell division, ATPase production, and biofilm development by disrupting metabolic pathways with pH homeostasis through cinnamic acid component can also increase antibacterial effect of propolis.[17] Several components in propolis may explain why this substance has antibacterial properties. These compounds can damage bacterial cell membranes, inhibit nucleic acid synthesis, increase cell membrane

permeability, and cause lysis of these bacteria.[18] Pinocembrin and apigenin are two more flavonoids discovered in propolis. The antibacterial activity of both of these compounds against *Streptococcus mutans* was found to be higher than that of polyphenols mixture or even chlorhexidine (MICs=1.6 g/mL), with minimum inhibitory concentrations (MICs) of 1.4 g/mL and 1.3 g/mL, respectively.[19] Apigenin's antibacterial action was found to be enhanced when combined with beta-lactam medicines in the fight against methicillin-resistant *Staphylococcus aureus*.[20] Artepillin C (3,5-diprenyl-p-coumaric acid) is one of the many phenolic compounds present in propolis (prenyl derivative of p-coumaric acid). In tests against the anaerobic bacterium *Porphyromonas gingivalis*, artepillin C was found to have bacteriostatic activity with membrane blebbing.[15]

The limitation of this study is antimicrobial effect of propolis varied according to its collection region. Every specific region where propolis is collected may have different effect on the same bacteria due to different composition and concentration of phenols and flavonoid. Among different extraction methods, solvent types, and bee species, Hossain et al[17], concluded that floral diversity is most responsible for different bioactivity of propolis. Thus, study of using different region specific propolis effect on *Porphyromonas gingivalis* is further needed to find the best periodontal dressing. This finding requires further investigation to make a clinical assessment of the overall situation.

CONCLUSION

Raw propolis basic formulation, a novel periodontal dressing that was not previously investigated, has a potential to reduce the growth of the periodontal pathogen *Porphyromonas gingivalis* bacteria. The results of the investigation are significant in comparison to the RESO-PAC™k, Coe pak™, and Baer formulation periodontal dressing material. This finding requires further investigation in order to carry out a clinical evaluation of the entire situation.

Acknowledgment: This study was supported by granted funds of Hibah Dana Masyarakat, Universitas Gadjah Mada, Republic of Indonesia, in the fiscal year 2021 under contract No. 4155/UN1/FKG1/Set.KG1/LT/2021.

Author contributions: "Conceptualization, S and RMY.; methodology, S; software, RMY.; validation, APW.; LTS: RMY ; formal analysis, S; investigation, RMY. ; resources, LTS. ; APW. ; data curation, RMY.; writing— preliminary drafting, S.; RMY.; LTS.; APW.; writing-review and editing, RMY.; S.; visualization, APW. ; supervision, S.; project administration, RMY.; obtaining funding, S.; All authors have read and agree to the published version of the manuscript

Funding: This study was supported by granted funds of Hibah Dana Masyarakat, Universitas Gadjah Mada, Republic of Indonesia, in the fiscal year 2021 under contract No. 4155/UN1/FKG1/Set.KG1/LT/2021.

Institutional review board statement: Not applicable (This research was conducted on research samples that did not involve humans or animals)

Informed consent statement: This research did not involve humans

Data availability statement: Availability of research funding will be given permission by all researchers via email correspondence with due regard to ethics in research

Conflicts of Interest: The author declares no conflict of interest in the research

Ethical Approval: All the procedures were followed following the ethical standards of experimentation by the Faculty of Dentistry Ethics Committee, Universitas Gadjah Mada 00725/KKEP/FKG-UGM/EC/2021.

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