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Effectiveness of Tamarillo Skin Extract (*Solanum betaceum* Cav.) with Sealer Combination in Inhibiting Growth of *Enterococcus faecalis*

Siregar E.Y[1*], Novelya[1], Susanto C[1]

[1]Dentist Education, Faculty of Dentistry, Universitas Prima Indonesia.

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***Corresponding author:**
Ervina Yulia Siregar

E-mail address:
ervinayulia8@gmail.com

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ABSTRACT

Introduction : Caries is a growth medium for pathogenic micro-organisms an inflammatory process can occur which can cause damage to the tooth pulp. Enterococcus faecalis one of the Gram-positive facultative anaerobes which causes failure after endodontic treatment. In the field of dentistry, herbal ingredients can be used as alternative medicinal ingredients because the ingredients is tamarillo skin which contains flavonoids, terpenoids, saponins and phenol compounds. In addition to herbal alternatives, endodontic filling also uses ingredients that have antibacterial potential that can kill and prevent reproduction in the root canals, one of which is the zinc oxide eugenol sealer which is usually applied together with gutta percha. Research objective: to determine the effectiveness of the extract of tamarillo (Solanum betaccum Cav.) skin sealer combination in inhibiting the growth of Enterococcus faecalis. **Method:** The research was conducted in an experimental laboratory post test only control group design. **Results :** a sealer made from zinc oxide-eugenol had the largest diameter compared to the combination group of 100%, 75%, 50%, 25% tamarillo skin extract with sealer, and aquadest. Based on the One Way Anova test, there was a significant difference in the mean diameter of the inhibition zone ($p < 0.05$) and the Post Hoc LSD analysis test showed that the sealer was the best group to kill the Enterococcus faecalis bacteria. **Conclusion :** sealers have a good effect on inhibiting Enterococcus faecalis bacteria as a cause of failure in endodontic treatment.

1. Introduction

Enterococcus faecalis is the persistent bacterium that most often causes failure after root canal treatment[1]. Endodontic treatment aims to maintain the teeth or heal the periapical tissue so that it can restore the function of the teeth properly, do not feel pain and have good aesthetics.[2] The success of root canal treatment is at the irrigation stage which plays a role in removing the smear layer, dissolving necrotic tissue and bacteria3.[3]

The skin of tamarillo is one type of plant that can be used as an antimicrobial because of its content. Based on the phytochemical screening test on the n-butanol fraction of tamarillo skin extract, it is known to have compounds of the flavonoid, terpenoid, phenol and saponin groups.[4] The antibacterial properties of flavonoid compounds can inhibit the development of bacteria, one of which is E. faecalis. Saponin compounds can also inhibit the development of bacteria by reducing glucose levels in microorganisms, affecting growth and propagation of microorganisms, minimizing enzymes during metabolism and in microorganism protein synthesis[5].

Based on research on saponin and flavonoid content in starfruit, it has antimicrobial properties in E. faecalis, the higher the concentration of the extract the better its antimicrobial properties[6]. Other studies have shown that the flavonoids, saponins, alkaloids from the leaves extract of kedondong bangkok also have antimicrobial properties against E. faecalis with a

minimum inhibitory concentration of 12.5% and a minimum kill concentration of 15% [7]. Research on lemongrass stem extract with a concentration of 25%, 50%, 75%, 100% has antibacterial activity that can inhibit E. faecalis bacteria.[8]

A good sealer is a sealer that has antibacterial potential that can kill and reject bacteria from multiplying in the root canal and prevent bacteria from getting into the tissue outside the apical tissue[2]. Sealers commonly used are zinc oxide based sealers, resin based, calcium hydroxide and others[9]. Eugenol-based sealers known to inhibit bacteria are the right choice compared to calcium hydroxide bases and resin-based ones[10]

Zinc Oxide-Eugenol-based sealers combined with herbal extracts appear to have the highest zone of inhibition followed by resin-based sealers and calcium hydroxide-based sealers[11]. This study aims to determine the effectiveness of the combination of tamarillo (Solanum betaccum Cav.) Rind extract with Zinc Oxide-Eugenol-based sealer in inhibiting the growth of Enterococcus faecalis.

2. Method

This research is a post-test only control group design laboratory experimental conducted at the Pharmacy Laboratory of Prima Indonesia University. Enterococcus faecalis ATCC 29212 is a pathogenic test bacterium used. The sample in this study extracted the skin of tamarillo with maceration method to extract the skin of tamarillo. The sample size in this study used Federer's formula: (t-1) (n-1) ≥15. This study used 6 groups. So the number of samples needed for each treatment was 4 with a total of 28 samples. The total sample (n) used was 4, it was carried out 4 times.

Making dutch eggplant bark extract

With the maceration process, the skin of Dutch eggplant is washed under running water and then dried using the oven. After the skin is dry then weigh 500 grams, grind it using a blender, then put it in a maceration glass and add ethanol until the simplicia is submerged. Simplisa that has been soaked is left for 5 days and then stirs the soaking occasionally. After 5 days filter it with filter paper and place it in another glass to produce a liquid extract of the tamarillo skin that has been dissolved with ethanol. The liquid extract is then put into a rotary tool to evaporate the solvent from a mixture consisting of solvents and dissolved substances, then a concentrated liquid extract is obtained and evaporated in a waterbath for 7 hours so that a thick extract of the skin of Dutch eggplant is obtained, after which a gradual dilution is obtained until each is obtained. concentrations of 25%, 50%, 75% and 100% respectively.

Culture and inhibition measurement of enterococcus faecalis bacteria

The culture of Enterococcus faecalis was taken with a cotton swab and then put it in a solution of 10 ml of liquid BHI media. Incubated for 24 hours at 37 ^{o}C, so that the Enterococcus faecalis suspension was obtained. Dilute the culture of Enterococcus faecalis and add sterile aquadest. This study used the diffusion method carried out with treatment concentrations namely 25%, 50%, 75%, 100%, paper discs were inserted into each extract. Rub Enterococcus faecalis isolate on each MHA medium (Mueller Hinton Agar) incubated for 24 hours at 37 ^{o}C, then the inhibition zone formed is measured by the calipers in millimiter units.

Blending dutch eggplant bark extract with a sealer

The enterococcus faecalis bacteria that have been diluted are placed in each agar medium and ensure an even distribution. Zinc Oxide-eugenol sealer is added to the tamarillo skin extract and stir until well blended. To ensure the diffusion of the sealer mixed with the herbal extracts, use agar stored for two hours at room temperature. Incubate the agar at 37 ^{o}C.

Antibacterial test of dutch eggplant fruit skin extract with zinc oxide-eugenol sealer

Sterilized tools and materials are provided. Enterococcus faecalis bacteria inoculum has been

prepared by taking it with a cotton swab which is put into a 10 ml sterile aquadest solution. The culture of Enterococcus faecalis was diluted with sterile distilled water to produce turbidity according to Mc Farland standards or a comparable number of bacteria $3x10^8$ CFU / ml.

In this study, the disc diffusion method was used by providing 30 paper discs which were divided into six groups of 25%, 50%, 75%, 100% Dutch eggplant peel extract, sealer, and aquadest. Then input the disc paper into the six treatment groups. Enterococcus faecalis isolate was rubbed into MHA (Mueller Hinton Agar) media. The plates can be viewed for 24 hours to 48 hours at a temperature of 37 ^{0}C to calculate the zone of inhibition. The whole experiment was repeated four times and the zone mean zone of drag was then calculated by the shear term in millimeters.

Data analysis

Data analysis used SPSS version 23 software and the normality test was performed using Shapiro-Wilk. Furthermore, the homogeneity test used the Levene test, the parametric statistical test used One Way ANOVA with a confidence level of 95% (α = 0.05%) and to see the significance value continued with the Post Hoc LSD test.

3. Results

The skin of Dutch eggplant (Solanum betaccum Cav.) Has five main active compounds, including phenolics, flavonoids, alkaloids, tannins and terpenoids. The skin of tamarillo also contains saponin compounds, but the amount is less than the five compounds. The results of the phytochemical test of tamarillo skin extract can be seen in table 1.

The average diameter of the inhibition zone of the combination of tamarillo (Solanum betaccum cav.) Skin extract combination of 100%, 75%, 50%, and 25% with sealer was 16.95 ± 3.39 mm, 14.35 ± 1.02 mm, 13.71 ± 0.96 mm and 12.49 ± 1.02 mm. In this study, there were two positive controls used were sealers, while the negative control was aquadest. The results showed that the diameter of the sealer inhibition zone was 18.10 ± 2.86 mm, and the aquadest had no resistance, which can be seen in Table 2.

It can be seen that the p value = 0.000 ($p < 0.05$) in which there is a difference in the inhibition zone diameter of the combination of tamarillo (Solanum betaccum cav.) Skin extract combination of 100%, 75%, 50%, and 25% with sealer, positive control (sealer), and negative control (aquadest) in inhibiting the growth of Enterococcus faecalis can be seen in Table 3.

There was a significant difference in the effectiveness of the inhibitory power between groups I with groups III, IV, and VI, groups II with V, and VI, groups III and V, and VI, groups IV with VI, and groups V with VI. While the results of the study, which means there is no significant difference in the effectiveness of the inhibition between groups I and II and V, groups II with III and IV, groups III and IV can be seen in table 4.

Table 1. The dutch eggplant skin extract compound group (Solanum betaccum Cav.)

Phytochemical screening			
No	**Secondary metabolite compounds**	**Testing method**	**Result**
1	Phenolic	$FeCl_3$	+++
2	Flavonoids	Pb $(CH_3COO)_2$	+++
		NaOH	+++
3	Alkaloids	Mayer	+++
		Dragendoff	++
4	Saponins	Foam Test	+
5	Tannins	$FeCl_3$ 5 %	+++
6	Terpenoids	L Bourchad	+++

Table 2.Average diameter of the inhibition zone combination of 100%, 75%, 50%, and 25% Dutch eggplant peel extract with sealer, positive control (sealer), and negative control (aquadest)

Group	Inhibition zone diameter (mm)				
	1	2	3	4	$\bar{X} \pm$ SD
Extract 100% + sealer	21.9	16.0	14.2	15.7	16.95 ± 3.39
Extract 75% + sealer	14.1	14.1	13.4	15.8	14.35 ± 1.02
Extract 50% + sealer	13.85	12.8	13.2	15.0	13.71 ± 0.96
Extract 25% + sealer	13.8	12.45	11.3	12.4	12.49 ± 1.02
Control + sealer	22.3	17.5	16.3	16.3	18.10 ± 2.86
Control - (aquadest)	0	0	0	0	0.00 ± 0.00

Table 3.The effectiveness of the inhibition of the combination of tamarillo skin extract combination of 100%, 75%, 50%, and 25% with sealer in inhibiting the growth of Enterococcus faecalis

Group	**$\bar{X}$ ± SD**	**P value**
Extract 100% + sealer	16.95 ± 3.39	0.000*
Extract 75% + sealer	14.35 ± 1.02	
Extract 50% + sealer	13.71 ± 0.96	
Extract 25% + sealer	12.49 ± 1.02	
Control + sealer	18.10 ± 2.86	
Control - (aquadest)	0.00 ± 0.00	

Note: * there is a significant difference

Table 4.Differences in the effectiveness of the combination of 100%, 75%, 50%, and 25% Dutch eggplant peel extract with a sealer in inhibiting the growth of Enterococcus faecalis

Group	**I**	**II**	**III**	**IV**	**V**	**VI**
I	-	0.054	0.019*	0.002*	0.000*	0.000*
II	0.054	-	0.622	0.158	0.008*	0.000*
III	0.019*	0.622	-	0.347	0.002*	0.000*
IV	0.002*	0.158	0.347	-	0.000*	0.000*
V	0.377	0.008*	0.002*	0.000*	-	0.000*
VI	0.000*	0.000*	0.000*	0.000*	0.000*	-

Note : * P <0.05 there is a significant difference

Group I : 100% extract combination + sealer

Group II : 75% extract combination + sealer

Group III : 50% extract combination + sealer

Group IV : 25% extract combination + sealer

Group V : positive control (sealer)

Group VI : negative control (aquadest)

4. Discussion

The results of the phytochemical test in this study found that the extract of tamarillo skin contains several components of active compounds including phenolics, flavonoids, alkaloids, tannins, terpenoids and saponins. This is in line with the screening research that the ethanol extract of tamarillo skin has chemical compounds including flavonoids, terpenoids, steroids, saponins, tannins and alkaloids[12]. Also supported by research which also states that flavonoids and phenols are active compounds that are very much found in the skin of Dutch eggplant[13].

Flavonoids are one of the phenolic compounds that have a tendency to denaturate proteins which result in the not happening of bacterial cell metabolic activity. This results in these compounds being able to inhibit bacterial growth, while the presence of steroid and terpenoid compounds can cause bacteria to become lysed, namely by binding to the proteins, lipids and carbohydrates present in the cell membrane of Enterococcus faecalis[14].

The presence of alkaloid compounds present in the extract of tamarillo skin can inhibit the development of Enterococcus faecalis. According to Trease and Evans (1978) that the mechanism of inhibiting bacterial development by alkaloid compounds can damage the formation of peptidoglycan constituent components in bacterial cells which can cause bacteria to become lysis.

Tannins are compounds that belong to the polyphenol group as well as antibacterial properties. The work process of tannin compounds as antibacterials, which can inhibit the reverse transcriptase and DNA topoisomerase enzymes so that bacterial cells cannot be formed. Tannins also target cell wall polypeptides, which leads to poor cell formation[14,15,16].

Based on the results of this study, it can be stated that the extract of tamarillo skin can be used as an alternative for root canal irrigation to limit the development of the Enterococcus faecalis bacteria. The combination of tamarillo peel extract and sealer may be due to the active compound content in the extract.

The results showed that the average diameter of the inhibition zone combination of 100%, 75%, 50%, and 25% Dutch eggplant peel extract with sealer was 16.95 ± 3.39 mm, 14.35 ± 1.02 mm, 13.71. ± 0.96 mm and 12.49 ± 1.02 mm. Antibacterial activity <5 mm is in the weak category, 5-10 mm is in the moderate category, 11-20 mm is in the strong category and > 20 mm is in the very strong category[14]. The inhibitory power produced by the combination of tamarillo peel extract and sealer shows how much effect the concentration has on the growth of Enterococcus faecalis. According to Pelczar (1986) states that gram-positive bacteria have cell walls that have a lot of peptidoglycan, little lipids and cell walls store polysaccharides (teichoic acid). The destruction of bacterial cells due to the addition of tamarillo skin extract will block the biosynthesis of specific enzymes needed in a metabolic reaction[14].

In this study, the positive control used was sealer, while the negative control was aquadest. Based on the results of the inhibition test, it was found that the diameter of the sealer inhibition zone was 18.10 ± 2.86 mm, while the aquadest had no resistance. In this study, the type of sealer used was a sealer made from zinc oxide-eugenol. A good sealer is a sealer that has antibacterial properties that can kill and inhibit the growth of residual bacteria in the root canal and can prevent re-contamination of bacteria from leakage of the tooth crown[2].

In this study, it can be seen that the diameter of the sealer inhibition zone is the highest compared to the combination of tamarillo skin extract with sealer. It can be stated that the sealer antibacterial power is strongest than any other group. Sealers made from zinc oxide-eugenol have met the requirements as a good sealer for root canal treatment to inhibit the growth of Enterococcus faecalis. This is in accordance with the results of research which states that zinc oxide-eugenol-based sealers have better antibacterial power than epoxy resin and mineral trioxide aggregate against E. faecalis[2]. Based on these results, it can be stated

that the use of a combination of 100%, 75%, 50%, and 25% Dutch eggplant peel extract with a sealer has been shown to significantly inhibit the growth of Enterococcus faecalis.

5. Conclusion

There is an effective inhibition of the combination of tamarillo (Solanum betaccum Cav.) Skin extract combination of 100%, 75%, 50%, and 25% with sealers in inhibiting the growth of Enterococcus faecalis. Sealers made from zinc oxide-eugenol had the largest inhibition diameter compared to the combination group of 100%, 75%, 50%, and 25% tamarind skin extract with sealer, and aquadest.

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