Benefits of Stevia (*Stevia rebaudiana B.*) and Sirih (*Piper betle L.*) Leaves Combination Againts Dental Care

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Abstract

Oral health is the most important part, to improve its hygiene by maintaining teeth. Cavities are a common problem. Betel leaf as an antibacterial. Stevia leaves as bacteriostatic and bactericidal also eliminate the cause of tooth decay and gingivitis. The purpose of the study was to determine the antibacterial activity of toothpaste combined with betel leaf extract with stevia leaves against the growth of Streptococcus mutans bacteria. The study used an experimental method consisting of making betel leaf and stevia leaf extracts using the maceration method. Preparation of toothpaste with a combination of betel leaf extract with stevia leaves at a concentration of 15% with the ratio of betel leaves and stevia leaves, namely formula 1 (5%: 10%), formula 2 (7.5%: 7.5%), formula 3 (10%: 5%). Testing antibacterial activity by measuring the clear zone using the disc paper method with blood agar media. Data analysis using ANOVA and Paired T test. The results of the study obtained formula (5%: 10%) clear zone of 16.42 mm, formula (7.5%: 7.5%) of 17.24mm, formula (10%: 5%) of 17.94mm, positive control of 18.66mm, negative control of 2.75mm. Conclusion 10:5 combination toothpaste has antibacterial activity.

Keywords: **s**tevia leaf, betel leaf, antibacterial, *S. mutans*, toothpaste

1. INTRODUCTION

Oral health is a component of whole body health that cannot be handled separately through other health aspects because it will worsen overall health. Efforts to improve oral hygiene include maintaining oral hygiene. For human health and safety, the role of the oral cavity is enormous. Usually, people are described as healthy not only because of their healthy bodies, but because of healthy oral cavities and teeth. Thus, oral health addresses the overall well-being of the individual. The teeth serve as a food delivery system. Before food enters the digestive tract, it must first be chewed. To prevent difficulties from bacteria on the teeth and delivered by food, it is important to maintain dental hygiene. Cavities are one of the most common dental problems (Nurhamidah et al., 2016).

The most common problem with dental health is caries. Dental caries develops as a result of food debris undergoing a fermentation process caused by oral bacteria, which causes plaque to form on the teeth. Dental caries cause teeth to calcify, which makes them porous, prone to cavities, or even broken (Widayanti, 2014). According to (Ramayanti & Purnakarya, 2013) microorganisms, the tooth itself, food intake, and time are the four basic

elements that affect dental caries. Dental caries is mostly caused by *Streptococcus mutans.*

One of the metabolic products required for bacterial life is lactate, which is produced by the gram-positive facultative anaerobic bacterium *Streptococcus mutans*. *Streptococcus mutans* produce glucans and water-insoluble polysaccharides that help detect bacteria on teeth, which gives it the ability to bind sugar to the tooth surface.

Oral pH is capable of being altered by *Streptococcus mutans* to a level that is favorable for the organism's metabolism but not for other species residing in the same environment. *Streptoccocus mutans*, a microorganism that can convert sugar into acid. The acid becomes bound to tooth enamel as a result, which results in demineralization of tooth tissue and cavities. (Simon, 2010).

The only component that has been used to clean teeth for a long time is toothpaste. In addition, toothpaste can smooth the tooth surface, minimize or eradicate bad breath, give the mouth a fresh taste, and maintain dental health. Herbal toothpastes are usually described as toothpastes with gentle effects and safe and healthy components. Natural substances that are beneficial, safe, and efficient to improve oral health can be added when producing toothpaste (Telrandhe et al., 2017).

One of the herbs often used in traditional medicine is *betel* leaf *(Piper betle* L.*)*. Antibacterial properties are found in betel leaves *(Piper betle* L.*)*. According to (Putri efendi et al., 2020). *Flavonoid, polyphenol, steroid* and *tannin* compounds are the content of betel leaves *(Piper betle* L.*)*. Based on previous research conducted (Anas et al., 2018) proves that betel leaf extract (*Piper betle* L.) has antibacterial activity against *Streptococcus mutans.* The results of the study stated that concentrations of 0.1%, 1%, 10% and 100% were able to inhibit the growth of *Streptococcus mutans* bacteria . At a concentration of 0.1% the inhibition zone was 13.08 mm, at a concentration of 1% the inhibition zone was 18.46 mm, at a concentration of 10% the inhibition zone was 24.02 mm, and at a concentration of 100% the inhibition zone was 30.95 mm.\

Stevia (*Stevia rebaudiana B*.) promotes oral health, has antibacterial and anti-fungal characteristics, and combats the causes of gingivitis and tooth decay (Widarsih & Mahdalin, 2017). Stevia is a natural sweetener with high nutritional value that can be used to treat dental caries. Due to the presence of bioactive substances such as *stevioside, flavonoids, and tannins,* stevia leaves have antimicrobial properties (Wenda et al., 2017).

Based on previous research conducted (Putri et al., 2017) proved that stevia leaf extract *(Stevia rebaudiana* B.) has antibacterial activity against *Streptococcus mutans.* The results of the study stated that concentrations of 5%, 10%, 20%, 40% and 80% were able to inhibit the growth of *Streptococcus mutans* bacteria . The zone of inhibition obtained was 1.53 mm; 2.44 mm; 2.74 mm; 2.96 mm; the novelty of this research focuses on the activity of toothpaste with a combination of betel leaf and stevia leaf extracts that have been tested for antibacterial activity in previous studies.

2. METHOD

Making toothpaste of betel leaf extract (*Piper betle L*) combined with stevia leaf (*Stevia rebaudiana B*):

1. Weighing all ingredients according to the calculation.
2. Develop Na-CMC sorted using hot water as much as 20x the amount of CMC grind until homogeneous (mass 1).
3. Dissolving calcium carbonate with glycerin sorted until homogeneous (mass 2).
4. Adding nipagini and sodium lauryl sulfate into mass 2 slowly grind so that no foam is formed.
5. Adding extracts of betel leaf (*Piper betle L*.) and stevia leaf (*Stevia rebaudiana B*.).
6. Then mass 2 is added to mass 1 and crushed until a homogeneous toothpaste is formed.
7. Put in a container, then evaluate the preparation.

Preparation of *Betel* Leaf Simplisia (*Piper betle* L.)

Betel leaves that have been collected as much as 2 kg, then carried out wet sorting, washing with running water, then knotting the betel leaves. After that, drying the betel leaves was carried out, the leaves that had been dried were sorted dry, then the powder was made by blending. The simplified powder was stored in a clean and tightly closed container.

Table 1. Formulation of toothpaste in each sample to be tested

Material	Usefulness	Formula (%)				Requirements (%)
		X_1	X_2	X_3	K(-)	
Betel leaf extract	Active substance	5	7,5	10	-	-
Stevia leaf extract	Active substance	10	7,5	5	-	-
Na-CMC	Fastener	1,5	1,5	1,5	1,5	1-5
Kalsium karbonat	Abrasif	40	40	40	40	30-40
Gliserin	Humektan	25	25	25	25	10-30
Na lauril sulfat	Surfaktan	1	1	1	1	1-2
Nipagin	Preservatives	0,1	0,1	0,1	0,1	0,1-0,2
Aquadest ad	Solvent	100	100	100	100	-

Description:
X1 : Toothpaste combination of betel leaf extract (*Piper betle L*) with Stevia leaf (*Stevia rebudiana B*) combination 5:10
X2 : Toothpaste combination of betel leaf extract (*Piper betle L*) with Stevia leaf (*Stevia rebudiana B*) combination 7.5:7.5
X3: toothpaste combination of betel leaf extract (*Piper betle L*) with Stevia leaf (*Stevia rebudiana B*) combination 10:5
K- : negative control (toothpaste base without extract)

Formulation and mode of action Preparation Extracts of *Betel* Leaf (*Piper betle* L.) and Stevia Leaf (*Stevia rebaudiana* B.)

Betel Leaf (*Piper betle* L.), prepare tools and materials, weigh 200 grams of betel ethanol solvent, let stand for 7 x 24 hours, while occasionally stirring, after 7

days do filtering using flannel cloth. The filtrate obtained is then concentrated extract using *italic*, the difference in weight per weight (b / b) between the results obtained

and the weight of the simplisia powder used.

Then it is used to calculate the percentage of extract yield calculated by the formula:

$$Yield\ (\%) = \frac{Bobot\ ekstrak}{Bobot\ serbuk\ simplisia}\ 100\%$$

Stevia leaves (*Stevia rebaudiana* B.), prepare tools and materials, weigh 200 grams of stevia leaf powder, then put it in a macerator. Then add 2000 ml of 70% ethanol solvent, let it stand for 7 x 24 hours, while occasionally adding a few drops of ethanol. Stirring was carried out, after 7 days filtering using flannel cloth. The filtrate obtained then concentrates the extract using a water bath, the difference in weight per weight (b / b) between the results obtained and the weight of the simplisia powder used.

Then used to calculate the percentage yield. The extract yield was calculated by the formula:

$$Yield\ (\%) = \frac{Bobot\ ekstrak}{Bobot\ serbuk\ simplisia}\ 100\%$$

Phytochemical Screening of *Betel* Leaf (*Piper betle* L.) and Stevia Leaf (*Stevia rebaudiana* B.) Extracts

Flavonoid Test

As much as 0.5 g of extract is put into a test tube, adding 0.5 mg of magnesium powder, adding 3 drops of concentrated HCL, yellow, red, or orange color indicates positive *flavonoids*.

Tannin Test

As much as 0.5 g of extract was put into a test tube, adding 3 drops of FeCl3, blackish green color showed catechol tannin and blackish blue showed pyrogallol *tannin*.

Steroid Test

As an extract of 0.5 g was put into a test tube and added with 2 mL of concentrated H2SO4, the purple color indicates the presence of steroid compounds.

Stevia leaf (*Stevia rebaudiana* B.)

Flavonoid Test

The extract of 0.5 g is put into a test tube, adding 0.5 mg of magnesium solution, adding concentrated HCL 3 drops, yellow, red, or orange color indicates positive *flavonoids*.

Tannin Test

As much as 0.5 g of extract was put into a test tube, adding 3 drops of FeCl3, blackish green color showed catechol tannin and blackish blue showed pyrogallol *tannin*.

One-Way ANOVA Test

Tests using the anova test with a significance level = 5%. Showing the significant level of the test carried out so that this observation is stated that the extract has antibiotics.

The way to read the sig value is as follows: If the value of $_{Fcount>Ftable}$ then $_{H0}$ is rejected and $_{H1}$ is accepted. If the value of $_{Fcount}$ $<_{Ftable}$ then $_{H0}$ is accepted and $_{H1}$ is rejected.

T - Test

One of the tests used in parametric statistical testing is the T-test. The T-test is a test that shows the extent to which one independent variable can explain the dependent variable alone. A significance level of 0.05 (= 5%) is used for the T-test

statistical test (Magdalena & Angela Krisanti, 2019).

Determining the t value (t count) is by comparing with the t table. The decision making is as follows:

If the significant value > 0.05, then $_{H0}$ is accepted and $_{H1}$ is rejected. If the significant value <0.05 then $_{H0}$ is rejected and $_{H1}$ is accepted.

3. RESULT AND DISCUSSION

Collection Results of Betel (*Piper betle L.*) and Stevia (*Stevia rebaudiana* B.) Plant Materials

Betel leaf (*Piper betle* L.) in fresh, green color as much as 2 kg used was obtained from Dukuhlor Village, Sindangagung District, Kuningan Regency, West Java. Stevia leaves (*Stevia rebaudiana B*.) as much as 500 grams were taken directly in the form of dry simplisia obtained from Yogyakarta City. *Streptococcus mutans* bacteria and other materials were obtained from the Microbiology Laboratory of the Faculty of Pharmacy, YPIB University, Cirebon.

Simplisia Preparation Results of *Betel* Leaf (*Piper betle L.*) and Stevia Leaf (*Stevia rebaudiana B.*) Betel Leaf (*Piper betle* L.)

The weight of betel leaves used is 2kg. After making simplisia, the results were obtained in the form of brownish leaves with a distinctive aromatic smell of betel leaves with a dry simplisia weight of 253 grams.

Resuts of Making Sirih Leaf (*Piper betle L.*) and Stevia Leaf (*Stevia rebaudiana B.*) Extracts

The results of making betel leaf extract *(Piper betle* L.) are as follows:

Yield Formula:

$$\text{Yield (\%)} = \frac{Bobot\ ekstrak}{Bobot\ serbuk\ simplisia}\ x\ 100\%$$

$$\text{Yield (\%)} = \frac{28{,}82}{200}\ x\ 100\% = 14{,}4\%$$

Requirements for betel leaf yield *(Piper betle* L.) > 5% (Herbal Pharmacopoeia Edition II, 2017)

The results of making stevia leaf extract *(Stevia rebaudiana B*.) are as follows:

$$\text{Yield (\%)} = \frac{64{,}7}{200}\ x\ 100\% = 32{,}2\%$$

The requirement for stevia leaf yield *(Stevia rebaudiana B*.) > 10% (Herbal Pharmacopoeia Edition II, 2017)

Result of Phytochemical Screening of *Betel* Leaf (*Piper betle* L.) and Stevia Leaf (*Stevia rebaudiana* B.) Extracts

Phytochemical screening results of betel leaf extracts (*Piper betle* L.) and stevia leaves (*Stevia rebaudiana* B.) as follows:

Table 2. Phytochemical Screening Results of Betel Leaf Extract (*Piper* betle *L*.) and stevia leaf (*Stevis rebaudiana B*.)

	Test	Reagent	Result	Description
Betle leaf (*Piper betle L.*)	*Flavonoid*	Serbuk Mg + HCL Pekat	Yellow color	Positif
	Tannin	FeCl3	Bllackish green color	Positif
	Steroid	H2SO 4	Purple color	Positif
Stevia leaf (*Stevia rebaudiana B.*)	*Flavonoid*	Serbuk Mg + HCL Pekat	Yellow color	Positif
	Tannin	FeCl3	Bllackish green color	Positif

Results of Making Toothpaste Combination of *Betel* Leaf Extract (*Piper betle* L.) with Stevia Leaf (*Stevia rebaudiana* B.)

Making toothpaste combination of betel leaf extract *(Piper betle L.*) with stevia leaves *(Stevia rebaudiana B.*) made at a concentration of 15% with a combination of X_1 (5:10),X_2 (7.5:7.5) and X_3 (10:5) each 100 grams. Weighing all the ingredients according to the calculation, developing Na-CMC sorted using hot water as much as 20x the amount of CMC grind until homogeneous (mass 1), dissolving calcium carbonate with glycerin sorted grind ad homogeneous (mass 2), nipagin and sodium lauryl sulfate added slowly into mass 2 and grind slowly so that no foam is formed, then mass 2 is poured into mass 1 and grind until a homogeneous toothpaste is formed, adding betel leaf extract *(Piper betle L.*) and leaves (*Stevia rebaudiana B.*), put it in a container, then evaluate the preparation.

Evaluation Results of Toothpaste Combination of *Betel* Leaf Extract (*Piper betle L.*) with Stevia Leaf (*Stevia rebaudiana B.*)

The results of the evaluation of toothpaste combination of betel leaf extract *(Piper betle L.*) and stevia leaf *(Stevia rebaudiana B.*) are as follows:

Table 3. Physical Characteristics of Preparations

Formula	Test	Cycle to-					
	characteri	1	2	3	4	5	6
	Color	Green	Green	Green	Green	Green	Green
	pH	7	7	7	7	7	7
X_1	Foam	1,5	1,5	1,5	1,5	1,5	1,5
	Spreadabil	5,2	5,3	5,2	5,1	5,2	5,3
	Color	Green	Green	Green	Green	Green	Green
	Shape	Pasta	Pasta	Pasta	Pasta	Pasta	Pasta
	Odor	Betel leaf	betel leaf	betel leaf	betel leaf	betel leaf	betel leaf
X_2	Flavor	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
	pH	7	7	7	7	7	7
	Foam	1,5	1,5	1,5	1,5	1,5	1,5
	Spreadabil	5,1	5,1	5,2	5,1	5,2	5,1
	Color	Green	Green	Green	Green	Green	Green
	Shape	Pasta	Pasta	Pasta	Pasta	Pasta	Pasta
X_3	Odor	betel leaf	betel leaf	betel leaf	betel leaf	betel leaf	betel leaf
	Flavor	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
	pH	7	7	7	6	6	6
	Foam	1,5	1,5	1,5	1,5	1,5	1,5
	Spreadabil	5,3	5,2	5,2	5,3	5,3	5,3
	Color	White	White	White	White	White	White
	Shape	Pasta	Pasta	Pasta	Pasta	Pasta	Pasta
K-	Odor	No	No	No	No	No	No
	Flavor	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless
	pH	7	7	7	7	7	7
	Foam	1,5	1,5	1,5	1,5	1,5	1,5
	Spreadabil	5	5,2	5,1	5,1	5	5

Organoleptic Test Results

Formulations 1, 2 and 3 have a green color. All three formulations are paste-shaped and have a sweet taste, the aroma of formulas 1 and 2 is typical of betel leaves, while formula 3 is typical of strong betel leaves. In the negative control, the color is white, paste-shaped, tasteless and odorless.

Homogeneity Test Results

All of the formulations and the negative control were homogeneous preparations.

pH Test Results

The pH of formulas 1, 2 and the negative control were at 7, while formula 3 had an initial pH of 7 and a final pH of 6.

Foamability Test Results

Formula 1, 2, 3 and the negative control had a foam power of 1.5 cm.

Results of Spreadability Test

Formula 1 wide spreadability: 5.03 cm, formula 2: 5.5 cm, formula 3: 5.7 cm, and negative control: 5.1 cm

Observation Results of Toothpaste Activity Test Combination of Betel Leaf Extract (*Piper betle L.*) with Stevia Leaf (*Stevia rebaudiana B.*)

Measurement Results of Clear Zone Diameter (mm) of Toothpaste Combination of *Betel* Leaf Extract (*Piper betle L.*) with Stevia Leaf *(Stevia rebaudiana B.*) on the First Day

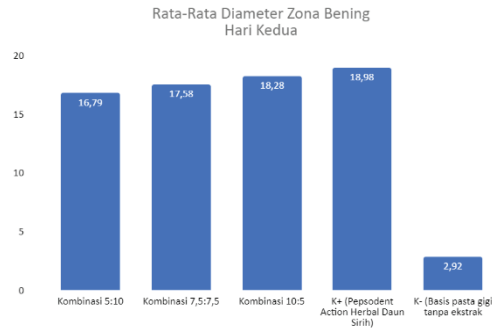

Diagram 1. Clear zone measurement results of toothpaste extract combination of betel leaf and stevia leaf on the first day of observation

Clear Zone Diameter Measurement Results (mm) Toothpaste Combination of Betel Leaf Extract (*Piper betle L.*) With Stevia Leaf *(Stevia rebaudiana B.*) on the Second Day.

The following are the results of the calculation of the clear zone on the 2nd day of observation of toothpaste extracts of betel leaf and stevia leaf combinations

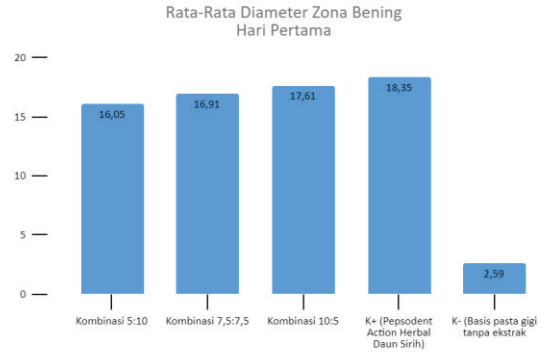

Diagram 2. Clear zone measurement results of toothpaste extracts of betel leaf and stevia leaf combination on the 2nd day of observation.

Recapitulation of Average Clear Zone (mm) of Toothpaste Combination of Betel Leaf Extract (*Piper betle L.*) with Stevia Leaf (*Stevia rebaudiana B.*) on Day One and Two

From diagrams 1 and 2, a recapitulation was then carried out to find the average of how many creepage areas were obtained from the Toothpaste Combination of Betel Leaf Extract *(Piper betle L.*) with Stevia Leaf *(Stevia rebaudiana B.*). And below are the recapitulation results:

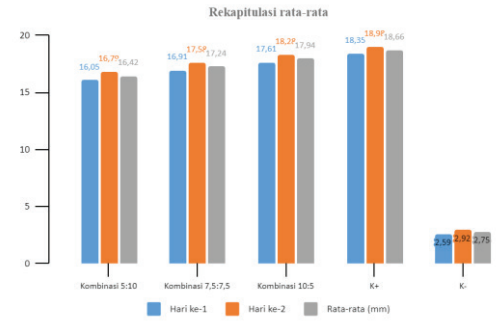

Diagram 3. Recapitulation of clear zone measurements of toothpaste with a combination of betel leaf and stevia leaf extracts from day 1 and 2.

DATA ANALYSIS

Normality Test

Based on the observation data, the antibacterial activity test data of toothpaste combination of betel leaf extract *(Piper betle L.*) with stevia leaf *(Stevia rebaudiana B.*) against *Streptococcus mutans* was obtained. The normality test carried out aims to determine whether all data are normally distributed or not. As for how to make a decision:

If the significance value> 0.05 then the data is said to be normal. If the significance value <0.05 then the data is said to be abnormal.

Based on the table above, with a confidence level of 95% = 0.05, the significance value (sig) in the Shapiro-Wilk test (sig) > 0.05 is obtained. In X_1 (0.944 > 0.05), X_2 (0.959 > 0.05), X_3 (0.435 > 0.05), K+ (0.219 > 0.05) and K- (0.389 > 0.05). This means that all data is normally distributed.

One-Way ANOVA Test

Testing using the anova test with a significance level = 5%. Shows the significant level of the test conducted so that it can directly determine whether H_0 is accepted or rejected.

The way to read the sig value is as follows:

If the $F_{count} > F_{table}$ value then H_0 is rejected and H_1 is accepted. If the value of $F_{count} < F_{table}$ then H_0 is accepted and H_1 is rejected.

The following are the results of anova test of toothpaste combination of *betel* leaf extract *(Piper betle L.*) with stevia leaf *(Stevia rebaudiana B.*).

T - Test

One of the tests used in parametric statistical testing is the T-test. The T-test is a test that shows the extent to which one independent variable can explain the dependent variable alone. A significance level of 0.05 (= 5%) is used for the T-test statistical test (Magdalena & Angela Krisanti, 2019).

Determining the t value (t count) is by comparing with the t table. The decision making is as follows:

If the significant value > 0.05, then H0 is accepted and H1 is rejected. If the significant value <0.05, then H0 is rejected and H1 is accepted.

The results of the study proved that the combination of betel leaf and stevia provides bacterial activity against streptococcus mutans. In line with previous research that betel leaves, which are known as antiseptics, are indeed able to inhibit the growth of streptococcus mutans bacteria carried out in vitro (Willia, 2017). This inhibitory ability is due to the content of betel leaf (piper betle L), namely polyphenol compounds, piperine, and other flavonoids. In addition, betel leaf content is essential oil which has the ability as an antibacterial (Hilma, 2022).

Stevia is a plant used as a natural sweetener with high safety limits (Marianan, 2020). In this toothpaste, the use of stevia is not only a sweetener but also has the ability as an antibacterial. The results of this study are in line with previous research (Andryana, 2017).

4. CONCLUSION

Based on the data from the research entitled "Test of Antibacterial Activity of Toothpaste Combination of Betel Leaf Extract (*Piper betle L.*) with Stevia Leaf (*Stevia rebaudiana B.*) against *Streptococcus mutans"* it can be concluded that the toothpaste combination of betel leaf

extract (*Piper betle L.*) with stevia leaf (*Stevia rebaudiana B.*) combination (10:5) has antibacterial activity against *Streptococcus mutans* has antibacterial activity against *Streptococcus mutans*, toothpaste combination of betel leaf extract (*Piper betle L.*) with stevia leaves (*Stevia rebaudiana B.*) combination (10: 5) which has the greatest antibacterial activity against *Streptococcus mutans*, toothpaste combination of betel leaf extract (*Piper bet* le L.) with stevia leaves (*Stevia rebaudiana* B.) meets the requirements and is stable at storage temperature.

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6. LITERATURE

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