# The effect of trigona spp. Propolis extract to saliva substitute ph as xerostomia therapy

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**ABSTRACT**

***Background:*** *Patients with xerostomia generally have an acidic salivary pH. Low oral pH increases the occurrence of tooth caries and development of oral lesions such as recurrent aphthous stomatitis (RAS) which are prone to occur in patients with xerostomia. Trigona spp. propolis extract with antimicrobial compounds was used as an additional natural ingredient in saliva substitute to inhibit the growth of Streptococcus mutans and prevent the decrease of salivary pH in patients with xerostomia. This study aimed to determine the effect of Trigona spp. propolis extract to saliva substitute pH as therapy for xerostomia with antimicrobial activity.*

***Method:*** *This study was experimental laboratory, namely Post-test only Control Group Design. The propolis Trigona spp. extract used in this study was extracted using maceration method with 70% ethanol as solvent which was then diluted into 5 concentrations 1,25%, 2,5%, 5%, 10%, 20%. This study used Streptococcus mutans (ATCC 25175) and saliva substitute with pH of 6.8. The research includes phytochemical test, absorbance measurement and pH test.*

***Result:*** *There is a significant difference in the pH value between saliva substitute induced with Streptococcus mutans with Trigona spp. propolis extract and the control group. Data analysis using One-way ANOVA test showed a significant value of <0.001 (p<0.05).*

***Conclusion:*** *Trigona spp. propolis extract is able to increase the pH of saliva substitute as xerostomia therapy, as the higher the concentration of Trigona spp. propolis extract, the higher the pH of saliva substitute. Samples with Trigona spp. propolis extract has the highest average pH value.*



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## INTRODUCTION

Xerostomia or the subjective sensation of dry mouth is often associated with decreased saliva production due to changes in saliva quantity (reduced salivary flow rate or hyposalivation) or changes in saliva quality (composition, viscosity and acidicity or salivary pH).[1,2] Salivary pH normally varies around 5.75 to 6.5.[3] In general, patients with xerostomia experience hyposalivation and have an acidic salivary pH resulting in an increase of bacteria in the oral cavity.[4] *Streptococcus mutans* is an acidogenic oral bacteria that is able to produce acid and lower salivary pH.[5,6] Low oral pH can increase the occurrence of caries dan oral lesions such as recurrent aphthous stomatitis (RAS).[7]

*Trigona* spp. propolis has been widely studied and has potential in bio-dental applications because of its antimicrobial, antioxidant, antifungal, antiviral and anti-inflammatory properties.[8] Propolis is rich in phenolic acids and flavonoid compounds which play a major role in the antibacterial activity of propolis.[9,10] The bitter taste of propolis is known to neutralized acids and has the potential to inhibit the growth of microorganisms such as *Streptococcus mutans*.[11]

Artificial saliva or saliva substitute with the addition of natural active ingredients and a neutral pH is needed to modulate the oral microflora. However, until now, there is no data regarding artificial saliva with antimicrobial components similar to human saliva.

So, this study aimed to determine the effect of *Trigona* spp. propolis extract on changes in the pH of saliva substitute, as this research is expected to support the utilization of *Trigona* spp. propolis for further development of oral hygiene products such as artificial saliva as xerostomia therapy with antimicrobial activity.

## RESEARCH METHOD

The research conducted was a laboratory experimental research (true experimental) in vitro with post-test only control group design. Three stages of testing were carried out, namely the phytochemical test, absorbance measurement and degree of acidicity (pH) test.

The samples used in this study were *Trigona* spp. propolis extract from Garut, Indonesia, artificial saliva with a pH of 6.8 and suspension of *Streptococcus mutans* (ATCC 25175) in *brain heart infusion broth* (BHI-B) media. The research sample was divided into 7 treatment groups, namely: Negative control, Treatment 1 (*Trigona* spp. propolis 1.25%), Treatment 2 (*Trigona* spp. propolis 2.5%), Treatment 3 (*Trigona* spp. propolis 5%), Treatment 4 (*Trigona* spp. propolis 10%), Treatment 5 (*Trigona* spp. propolis 20%), and positive control (CHX 0.2%). All sample tests were repeated 4 times.

*Trigona* spp. propolis was extracted using maceration method with 70% ethanol solvent.[12] In the first stage, a phytochemical test was carried out on propolis extract to determine the presence of active compounds. Identification that was carried out include terpenoid test, flavonoid test, alkaloid test, steroid test, and tannin test. The terpenoid test was carried out by adding ether, acetic anhydride, and sulfuric acid. A positive result in the terpenoid test will show sample changes color to red or purple. The flavonoid test of the sample using ammonia and sulfuric acid will show color changes to red if the sample contains flavonoids. The tannin test was carried out by adding 0.1% ferric chloride and the positive result was indicated by the formation of a bluish-black or green color in the sample.[13] Alkaloid test with the addition of Dragendorff or Meyer reagents, will show positive results with the formation of a red (Dragendorff) or yellow (Meyer) precipitate. The steroid test was

carried out using chloroform and sulfuric acid. The sample will change color to green if the result was positive.[14]

*Trigona* spp. propolis extract (100%) then was diluted using artificial saliva with the formula (V1.M1=V2.M2), to be divided into 5 concentrations of 20%, 10%, 5%, 2.5% and 1.25% ($^{w}/_{v}$). Sample preparation begins by placing 28 sterile test tubes on the test tube rack. After that, the tubes in the row of A-D and column 5 were given 2 ml of *Trigona* spp. propolis extract (1.25%), while column 4 was given 2 ml of *Trigona* spp. propolis extract (2.5%), column 3 was given 2 ml of *Trigona* spp. propolis extract (5%), column 2 was given 2 ml of *Trigona* spp. propolis extract (10%), column 1 was given 2 ml of *Trigona spp*. propolis extract (20%), column 9 was given 2 ml of artificial saliva as negative control and column 10 was added with 2 ml CHX 0.2%. For the next step, 2 ml of *Streptococcus mutans* (ATCC 25175) suspension was then distributed in each test tube with a micropipette. After that, incubation was carried out for 24 hours at 37℃ and followed by absorbance measurement of each sample.

Absorbance measurement using the broth microdilution method, in which 100 µL of each sample was taken and distributed into the well plates holes (row A-D, column 1-5), while the positive control (CHX 0.2%) was distributed into the four well-plate holes (column 9, row A-D), and negative control were distributed into the four well plate wells (column 10, row A-D) on the 96-well plate. The measurement was carried out with a spectrophotometer with McFarland standard 0.5 ($1.5x10^8$ CFU/mL), a wavelength of 600 nm and an absorbance value or optical density (OD) = 0.132. Each sample was then measured by a pH meter for pH measurements and each concentration was repeated four times.[5]

Data analysis in this study used the SPSS program. The normality test was carried out to determine whether the research data is normally distributed or not. The normality test uses the Shapiro-Wilk test because the number of samples is below 50. If the data is normally distributed (p>0.05), the data analysis test used is the parametric One-way Analysis of Variance (ANOVA) test could be carried out to find out whether there is a significant difference between test group. If the ANOVA test show a significant value or difference (p <0.05), then the analysis test can be continued with *Least Significant Difference* (LSD) Post-Hoc test to show whether there is a significant difference between samples.

## RESULTS

The results of the phytochemical tests attached in Table 1 prove that the *Trigona* spp. propolis extract contains various active compounds such as terpenoids, alkaloids, tannins, flavonoids, and steroids.

**Table 1.** Results of phytochemical test of *Trigona* spp. propolis extract with qualitative testing methods

| Extract | Type | Results |
|---|---|---|
| **Extract of Propolis *Trigona* spp.** | Phytochemical test: | |
| | Terpenoids | + |
| | Flavonoids | + |
| | Alkaloids | + |
| | Steroids | + |
| | Tannins | + |

Measurement of the pH value of artificial saliva induced by *Streptococcus mutans* with *Trigona* spp. propolis extract with concentrations of 1.25%, 2.5%, 5%, 10%, and 20% were carried out after an incubation process for about 24 hours at 37℃. Based on the results of the pH test shown in Table 2, the pH of artificial saliva increases along with the concentration of *Trigona* spp. propolis extract. Sample with *Trigona* spp. propolis extract concentration of 20% had the highest average pH

(6.08), while the sample with *Trigona* spp. propolis extract of 1.25% had the lowest average pH (5.03).

**Table 2.** The results of the pH value of artificial saliva induced by *S. mutans* with the addition of *Trigona* spp. propolis extract.

| Group | Average pH value |
|---|---|
| **Negative control** | 4.87 |
| **Propolis 1.25%** | 5.03 |
| **Propolis 2.5%** | 5.23 |
| **Propolis 5%** | 5.80 |
| **Propolis 10%** | 5.89 |
| **Propolis 20%** | 6.08 |
| **Positive control** | 6.47 |

The absorbance value was measured to test the effect of the concentration of *Trigona* spp. propolis extract with the concentrations of 1.25%, 2.5%, 5%, 10%, and 20%, against the growth of *Streptococcus mutans* bacteria. The absorbance measurement of artificial saliva was carried out after an incubation process of around 24 hours at 37°C. Based on data shown from the average absorbance value of each sample in Table 3, there was a decrease in absorbance from samples with *Trigona* spp. propolis extract of 1.25% to 5%, but increased in samples with *Trigona* spp. propolis extract concentration of 10% and 20%.

**Table 3.** Results of absorbance measurements of artificial saliva induced by *Streptococcus mutans* with *Trigona* spp. propolis extract after incubation

| Group | Average Absorbance Value |
|---|---|
| **Negative control** | 0.603 |
| **Propolis 1.25%** | 0.562 |
| **Propolis 2.5%** | 0.494 |
| **Propolis 5%** | 0.304 |
| **Propolis 10%** | 0.642 |
| **Propolis 20%** | 1.103 |
| **Positive control** | 0.955 |

The result of normality test (*Shapiro-Wilk test*) of the pH test results in Table 4, shows that the data were normally distributed (p>0.05). While the results of the One-way ANOVA test attached in Table 5, shows a significant value (p<0.05) which indicates that there is a significant difference in meaning or change in pH between the control group and the treatment group, namely *Trigona* spp. propolis extract (1.25%, 2.5%, 5%, 10%, and 20%). The results of the LSD Post-hoc test in Table 6 shows a significant value (p<0.05) between the control group and the treatment group, so there were differences between samples, except between the groups of *Trigona* spp. propolis extract with a concentration of 5% and 10%.

**Table 4.** Results of normality (Shapiro-Wilk) test of artificial saliva pH

| | Effect of Propolis *Trigona* spp. Extract to Saliva Substitute pH | *Shapiro Wilk* | | |
|---|---|---|---|---|
| | | ***Statistic*** | ***df*** | ***Sig.*** |
| **pH** | **Negative control** | 0.840 | 4 | 0.195* |
| | ***Trigona* spp. propolis 1.25%** | 0.981 | 4 | 0.909* |
| | ***Trigona* spp. propolis 2.5%** | 0.927 | 4 | 0.577* |
| | ***Trigona* spp. propolis 5%** | 0.924 | 4 | 0.562* |
| | ***Trigona* spp. propolis 10%** | 0.852 | 4 | 0.233* |
| | ***Trigona* spp. propolis 20%** | 0.971 | 4 | 0.848* |
| | **Positive Control** | 0.902 | 4 | 0.442* |

**Description:** (*) = p>0,05, which means data are normally distributed.

**Table 5.** One-way ANOVA test results

| | | *Sum of Square* | *df* | *Mean Square* | *F* | *Sig.* |
|---|---|---|---|---|---|---|
| **pH** | *Between Groups* | 8,446 | 6 | 1,408 | 243,293 | <0,001* |
| | *Within Groups* | 0,121 | 21 | 0,006 | | |
| | *Total* | 8,567 | 27 | | | |

**Description:** (*) = p<0,05, which means there is a significant difference.

**Table 6**. LSD Post-Hoc test results

| | ***p-value*** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **K-** | **1,25%** | **2,5%** | **5%** | **10%** | **20%** | **K+** |
| **K-** | | 0.006 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **1,25%** | 0.006 | | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **2,5%** | <0.001 | 0.001 | | <0.001 | <0.001 | <0.001 | <0.001 |
| **5%** | <0.001 | <0.001 | <0.001 | | 0.092* | <0.001 | <0.001 |
| **10%** | <0.001 | <0.001 | <0.001 | 0.092* | | 0.002 | <0.001 |
| **20%** | <0.001 | <0.001 | <0.001 | <0.001 | 0.002 | | <0.001 |
| **K+** | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | |

**Description:** (*) = p>0,05, which means there is no significant difference.

## DISCUSSION

People with xerostomia often complain of a dry and sour mouth.[4] One of the symptomatic therapies for xerostomia patients with very low saliva production is the use of artificial saliva.[15] Although artificial saliva is still rarely used in Indonesia, artificial saliva is proven as an intraoral topical agent which serves as lubrication and reduces the discomfort of xerostomia symptoms.[4,16]

The results of data analysis showed that there was a significant difference between the pH of the control group and the artificial saliva added with *Trigona* spp. propolis extract (1.25%, 2.5%, 5%, 10%, and 20%) which were induced by *Streptococcus mutans* bacteria. It is shown as the pH of artificial saliva increases along with the concentration of *Trigona* spp. propolis extract. The negative control group had lower pH compared to the treatment group. This is expected to occur because there was no additional *Trigona* spp. propolis extract in the negative control, which are alkaline. Meanwhile, the pH value of the treatment group was lower than the positive control (CHX 0.2%), which has a higher pH than the *Trigona* spp. propolis extract. CHX 0.2% is a broad-spectrum antibacterial agent that has also been known to have the ability to kill *Streptococcus mutans* bacteria as it causes changes in the permeability of bacterial cell membranes and cause leakage of cytoplasm components resulting in the death of bacteria[17] so no acid formation occurs.

The absorbance measurement using a spectrophotometer has been widely used in several

studies to prove the number of bacterial colonies present in a liquid medium. The absorbance value is directly proportional to the concentration of the substance.[18] The higher the concentration, the higher the absorbance value, the more bacteria contained.[19] Absorbance measurement was carried out to support the results of pH test, as *Streptococcus mutans* bacteria is one of the factors that affect salivary pH because of its ability to lower salivary pH. If the test solution after incubation becomes cloudy and its absorbance value increases, then there is a possibility of bacterial growth.[20] Based on the data obtained, there was a decrease in the absorbance value that occurred in the treatment group with *Trigona* spp. propolis extract from the concentration of 1.25% to 5% after incubation, indicating an inhibitory effect of *Trigona* spp. propolis extract against *Streptococcus mutans* bacteria. Previous research also proved *Trigona itama* bee propolis at concentrations of 40, 60, and 80% could inhibit the growth of *Streptococcus mutans bacteria*.[21] Meanwhile, from the results of the study there was an increase in the absorbance value in the treatment group with *Trigona* spp. propolis extract with the concentration of 10% to 20% which may occur due to the influence of color and turbidity from the concentration of *Trigona* spp. propolis extract. High concentrations can affect light absorption so that the absorbance value will increase.[22] So based on the results of the study, it can be concluded that the sample with *Trigona* spp. propolis extract with a concentration of 5% showed the best results in inhibiting the growth of *Streptococcus mutans* bacteria because it had the lowest average absorbance value among the other treatment groups. In addition, the absorbance value in the positive control group (CHX 0.2%) also had a high average absorbance value, which probably occurred because CHX was able to produce protein and nucleic acid precipitates from bacterial cells thereby affecting light absorption and increasing the absorbance value. [23,24]

The results of this study indicate that the concentration of propolis *Trigona* spp. extract with concentrations of 1.25%, 2.5%, 5%, 10%, and 20% could increase the pH of artificial saliva induced by *Streptococcus mutans* as the higher the concentration of *Trigona* spp. extract, the higher the pH of artificial saliva. Clinical benefits that can be developed from the results of this study are the potential use of *Trigona* spp. propolis extract as an additional natural ingredient in therapeutic products for patients with xerostomia. Increasing the pH of artificial saliva with *Trigona* spp. propolis extract as xerostomia therapy is expected to inhibit the growth of pathogenic bacteria in the oral cavity such as *S. mutans*. This research is expected to be used as reference material for further research in the field of dentistry. There were several deficiencies in this study, namely the absence of a pH test before incubation and a measurement of bacterial colonies before and after incubation to see the antibacterial effectiveness of *Trigona* spp. propolis extract against *Streptococcus mutans.*

## CONCLUSION

Propolis extract of *Trigona* spp. has the ability to increase the pH of artificial saliva as a xerostomia therapy. The higher the concentration of *Trigona* spp. propolis extract contained, the higher the pH of artificial saliva. The sample with *Trigona* spp. propolis extract of 20% is proven to have the highest average pH value compared to the pH of the samples with other concentrations.

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