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EFFECT OF DEHUMIDIFICATION ON *Trigona spp.* HONEY AS AN ANTIBACTERIAL AGAINST *Streptococcus mutans*

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ABSTRACT

Honey comes from *Trigona spp*. has been known for its various health benefits and has the potential to be a natural ingredient that can be used as a preventive measure to inhibit and kill the main bacteria in dental caries (*Streptococcus mutans*). The dehumidification process using a humidifier is carried out to absorb honey water vapor so that the water content of the honey is reduced and the quality is better. The aim of this research is to determine the effect of dehumidification on *Trigona spp*. honey as an antibacterial for *S.mutans*. This research is a laboratory experiment with a post-test only control group design in vitro using the dilution method. Dehumidification is carried out on raw trigona honey to obtain dehumid trigona honey and honey water. As a control, 0.2% chlorhexidine and distilled water were used. The antibacterial activity test was carried out by calculating the total bacterial colonies using the total bacterial colony formula. The results of the study showed that the antibacterial activity of raw *Trigona spp.* honey had a significant difference against *S.mutans* with a total number of colonies of $0.4x10^6$ CFU/mL compared to *Trigona spp*. dehum honey and honey water. Kruskal-Wallis analysis showed that there was a significant difference between the control group and the treatment group in inhibiting *S.mutans*. The conclusion is that raw *Trigona spp.* honey, *Trigona spp.* dehum honey, and honey water show differences in the antibacterial activity of *S.mutans* so that their use as a preventive measure in inhibiting *S.mutans* bacteria needs to be considered.

Keywords : dehumidification, *Trigona spp*. honey, antibacterial, *Streptococcus mutans*

INTRODUCTION

Reducing the population of *S.mutans* bacteria and other bacteria in the oral cavity remains the main goal in many studies to reduce or eliminate dental and oral diseases.[1,2] The role of *S.mutans* in the caries process is to ferment carbohydrates and produce acid as a by-product. which will cause a decrease in the acidity (pH) of saliva, thereby increasing the growth of cariogenic bacteria in the mouth. This acidic environment will then diffuse into the enamel, dentin and cementum which will partially destroy mineral crystals and cause the release of minerals in the form of calcium and phosphate from the tooth layer. If this process continues as the pH of the oral cavity decreases, it will cause enamel decalcification and form white spot lesions which indicate the start of the caries process.[3]

Natural herbal ingredients are known to have antibacterial power with minimal side effects.[4] The quality of honey depends on the quality of the nectar and pollen. Honey has anti-inflammatory properties, plays a role in the healing process, antioxidant, and antineoplastic effects. The production of hydrogen peroxide (H2O2), bee's defensin-1, high osmolarity and low pH influence honey's effectiveness as an antibacterial. The results of phytochemical tests, especially phenolic compounds, and flavonoids, are important elements in providing antibacterial potential to honey.[5]

Water content affects the quality of honey. The water content of honey in Indonesia is generally higher than the required standards so that the use of a dehumidifier can be used to reduce the water content of honey to comply with SNI (maximum 22%). Using a dehumidifier will separate the thick honey (dehumidified trigona honey) and the dehumidified honey water.[6] The advantage of dehumidified honey is that there is no difference in antioxidant capacity, total phenolic content, flavonoids, taste, color, and the

honey dehumidification process does not damage the sensory quality.[7]

Research on the use of natural ingredients that have antibacterial activity has been widely carried out in an effort to avoid side effects from the use of chemicals.[8] Antibacterials are drugs or compounds that are used to kill or inhibit bacteria that are detrimental to human health. Antibacterial test using the dilution method, namely determining the minimum inhibitor concentration (MIC) value to determine the ability of natural ingredients to inhibit bacterial growth.[9]

Based on research conducted by A. Apriantini, et al[6] regarding the effect of the length of time the water content decreases on the physicochemical quality of kapok honey and rambutan honey. This is supported by previous research conducted by M. Ma'ruf, et al[10] regarding kelulut bee honey (*Trigona spp.*) in its activity against resistant *Staphylococcus aureus* bacteria.

Several studies above have described reducing water content on the quality of honey and the antibacterial activity of kelulut bee honey (*Trigona spp*.), but research that examines the effect of reducing water content on *Trigona spp.* honey as an antibacterial against *S.mutans* has never been studied, so this research aims to determine the effect of dehumidification on *Trigona spp.* honey as an antibacterial against *S.mutans* which is expected to be used as a source for studies originating from plants in alternative efforts to maintain healthy teeth and oral cavity by utilizing the contents therein.

MATERIALS AND METHOD

This research is a laboratory experiment using a post-test only control group design. Ethical approval has been obtained from the Research Ethics Commission of the Faculty of Dentistry, Trisakti University (715/S1/KEPK/FKG/9/2023).

Research was conducted at the Microbiology Center of Research and Education (MiCORE) Laboratory at Trisakti University, the Chemical Applications and Services Laboratory at the Faculty of Mathematics and Natural Sciences, Padjadjaran University, the Dental Material and Testing Center of Research (DMT Core) Laboratory at Trisakti University, and the Trisakti University's Basic Physics Laboratory.

The honey samples used in this research were raw trigona honey (RTH), dehumidified trigona honey (DTH) and dehumidified honey water (DHW) which were obtained directly from the Garut area. Raw trigona honey (RTH) is light brown in color which is produced directly from *Trigona spp.* bees, Dehumidified trigona honey (DTH) is dark brown in color which is produced from separating part of the raw honey in the dehumidifier process and dehumidified honey water (DHW) will produce water from the dehumidifier process.

The materials used in this research were *S.mutans* ATCC 25175 bacterial preparation, mueller hinton broth (MHB) media, bacteriological agar media, distilled water, phosphate buffered saline (PBS), chlorhexidine 0.2%, buffer solution pH 6.86, buffer solution pH 9.18 and acid pH 4.00.

The tools used in this research were a digital pH meter, glass beaker, Ostwald viscometer, balance scale, glass tube, stopwatch, Erlenmeyer tube, autoclave, petri dish, hot plate, microtube, anaerobic jar, incubator, vortex mixer, microplate reader, 96-well-plate-flat-bottom microplate, micropipette.

a. Bacterial Activity Test

Antibacterial activity tests were carried out on the five test groups (RTH, DTH, DHW, Control +, Control -). MHA media and *S.mutans* bacterial suspension were prepared using appropriate sterilization and manufacturing procedures. The test was carried out using the dilution method in the wells of a 96-well plate, with the addition of honey and positive control (chlorhexidine 0.2%) and negative control (distilled water). After incubation for 48 hours at 37°C, dilution and evaluation of the antibacterial activity of honey were carried out.

The results of the dilution treatment into agar media from each well on the well plate were evaluated by taking 10 µL of sample using a micropipette and spreading it in a petri dish containing agar media. The petri dishes were then incubated for 24 hours at 37°C. The decrease in the number of bacterial colonies after incubation shows the Minimum Inhibitory Concentration (MIC) value. The results of the bacterial colonies formed are obtained using the formula for the number of bacterial colonies in CFU/mL units.

b. Phytochemical Test

Phytochemical tests were carried out on RTH, DTH, DHW qualitatively. Several reagents are used to assist the process of identifying active compounds, namely phenolics, triterpenoids, flavonoids, alkaloids, steroids, tannins, and saponins.

c. Stability Testing (Color and pH Test)

Stability tests are carried out to ensure that the test materials, namely RTH, DTH, and DHW can maintain their characteristics in the long term. Nine glass tubes were filled with the three test materials and stored at room temperature around 27°C for 1 month. Observations of the color of honey are carried out periodically using organoleptic tests by the human sense of sight on days 0, 7, 14, 21, and 28. Next, pH stability is tested using a pH meter that has been calibrated with a buffer solution of pH 6.86, pH 9 .18, and a pH of 4.00. The pH value was measured on the same day during the storage period.

d. Viscosity Test

The viscosity test was carried out to determine the viscosity of RTH, DTH, and DHW. Specific gravity is measured first, followed by the use of an Ostwald viscometer. The 10 mL preparation is put into tube B, sucked, then allowed to flow from boundary "a" to "b". Flow time is calculated using a stopwatch. Viscosity measurements were carried out for 1 month on days 0, 7, 14, 21, and 28 to monitor changes.

RESULT

a. Antibacterial Activity Test

The results of antibacterial activity testing using the dilution method in the five test groups are presented in **Table 1** below.

Table 1. Total Colonies of *Streptococcus mutans* bacteria

No.	Concentration	Total Bacterial Colonies (CFU/mL)
1	RTH	0,4 x 10^6
2	DTH	1,8 x 10^6
3	DHW	2 x 10^6
4	Control (+)	7,2 x 10^6
5	Control (-)	1,096 x 10^6

b. Phytochemical Test

The results of phytochemical testing using qualitative methods in the RTH, DTH, and DHW groups are presented in **Table 2** below.

Table 2. Results of Phytochemical Content

Active Compounds	Metode Uji	Test Results		
		RTH	DTH	DHW
Phenolic	$FeCl_3$ 5%	-	-	-
Tanin	$FeCl_3$ 1%	-	-	-
Flavonoid	a.Concentrated HCl + Mg	-	-	-
	b. H_2SO_4 2n	-	-	-
	c. NaOH 10%	+	+	-
Saponin	HCL 2N	+	+	-
Triterpenoid	Concentrated	+	+	-
Steroid	H_2SO_4	-	-	-
Alkaloid	Wagner	+	+	-

c. Color Test

Color test results during the 1 month storage period in the RTH, DTH, and DHW groups (**Figure 1**).

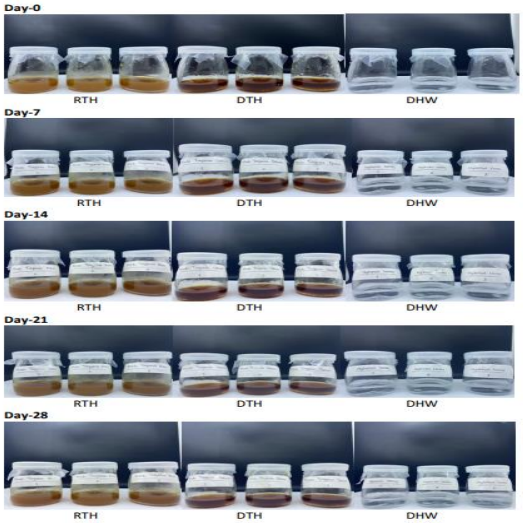

Figure 1. Color observations in RTH, DTH, and DHW for 1 month on days 0, 7, 14, 21, and 28

d. pH Test

The results of pH testing during the 1 month storage period in the RTH, DTH and DHW groups are presented in **Table 3** below.

Table 3. pH Test Results

Day-	Average pH		
	RTH	DTH	DHW
0	3.15	2.92	3.64
7	3.23	3.06	4.27
14	3.26	2.95	4.26
21	3.15	3.12	4.14
28	3.17	3.09	4.10

e. Viscosity Test

The results of viscosity testing during a storage period of 1 month in the RTH, DTH, and DHW groups are presented in **Table 4** below.

Table 4. Viscosity Results

Day-	Average Viscosity (cP)		
	RTH	DTH	DHW
0	1,792	44,372	0,902
7	1,864	49,366	0,817
14	1,922	51,871	0,796
21	1,936	54,671	0,785
28	1,951	54,786	0,764

f. Results of Antibacterial Activity Test Data Analysis

The results of the Shapiro-Wilk normality test on the total colony antibacterial activity data showed that of the 5 categories, there were 2 categories that had a significance value of $p < 0.05$ so it could be said that the data was not normally distributed and continued with the Kruskal-Wallis test. The results of the Kruskal-Wallis test show a sig value. 0.002 ($p < 0.05$) so that Ho is rejected, it can be concluded that there is a significant difference between the groups. So, the test was continued with the Mann-Whitney U test which is presented in **Table 5** below.

Table 5. Results of the Mann-Whitney U Test for Antibacterial Activity in the 5 Sample Groups

	P - *value*				
	RTH	DTH	DHW	Control (+)	Control (-)
RTH		0.288	0.026*	0.033*	0.007*
DTH	0.288		0.163	0.165	0.009*
DHW	0.026*	0.163		0.163	0.008*
Control (+)	0.033*	0.165	0.163		0.009*
Control (-)	0.007*	0.009*	0.008*	0.009*	

Based on the Mann-Whitney U test above, RTH has a difference in the average total colonies with DHW, control (+) and control (-). DTH had a difference in the average total colonies with the control group (-). DHW has a difference in the average total colony with the RTH and

control groups (-). The control group (+) had a difference in the average total colonies with RTH and control (-). The control group (-) had a difference in the average total colonies with all groups. This is because the significance value obtained is smaller than 0.05.

DISCUSSION

Honey can absorb up to 33% of its weight in humid air. If the water content increases, it will cause fermentation and will reduce the specific gravity of the honey. Damage to honey can be caused by fermentation by microorganisms during storage which is triggered by high water content.[11] The high water content of honey is also caused by the nature of honey which easily absorbs water or is hygroscopic and can also absorb humidity in the surrounding air.[6]

The water content of honey can be influenced by environmental factors during production such as weather and humidity in the hive. Apart from that, the conditions of the nectar and the treatment during honey extraction and storage can affect the water content of the honey. This can be reduced before or after extraction with a special technique.[12] A method that has been developed to reduce the water content of honey is adsorption drying.[13]

The bioactive compounds contained in honey are known to have antioxidant, anti-inflammatory, anti-microbial, anti-bacterial, and anti-cancer activities which can also be used to prevent oral diseases such as dental caries, plaque, canker sores, cavities, and gingivitis.[14] The positive control used in this study, chlorhexidine is the gold standard compound in mouthwash that is often used by the public and dental practitioners for oral antimicrobial therapy in reducing *S.mutans* and biofilm in the mouth.[15,16] Chlorhexidine's activity as an antibacterial is by destroying the cytoplasm which can cause cell lysis bacteria.[17] Chlorhexidine concentration influences its effectiveness.[18]

This research uses the microdilution method, a liquid dilution method using media, bacteria, and test compounds which will be inoculated using a 96-wells microplate.[19] Based on this research, it is proven that RTH, DTH, and DHW originating from Garut, West Java, Indonesia have antibacterial activity that can inhibit the growth of *S.mutans* bacteria. Trigona honey which has a minimum total colony can be seen in raw Trigona honey with a bacterial colony count of 0.4 x 10^6 CFU/mL compared to dehumidified honey and honey water.

The results of the phytochemical test in the RTH and DTH test groups in this study were positive for containing flavonoids, saponins, triterpenoids, and alkaloids. Meanwhile, DHW does not contain active compounds. These results are in accordance with research by Emmaitah, et al which stated that trigona honey from South Sulawesi was positive for containing flavonoid compounds, alkaloids, terpenoids, tannins while negative for saponin, and steroid compounds.[20] Another research by Tri Damayanty, et al stated that trigona honey (*Tetragonula Biroi*) is positive for the content of flavonoids, alkaloids, triterpenoids while negative for the content of saponin compounds.[21] Differences in the content of these compounds can be caused by the influence of differences in vegetation and differences in location.[22]

Flavonoid compounds are active substances found in plants and have antioxidant, anti-inflammatory, and anti-carcinogenic properties. Many studies state that flavonoid compounds have antibacterial, antifungal, and antiviral activity.[23] The research statement by Tsuchiya, et al. proved that 5-hydroxyflavanone and 5-hydroxyisoflavanone with one, two or three additional hydroxyl groups at positions 7, 2' and 4 ′ can inhibit the growth of *S.mutans*.[24] Saponin compounds are the compounds most commonly found in plants. Saponins have antibacterial, antifungal, anti-inflammatory, antiparasitic, anticancer, and antiviral properties.[25] Saponin compounds function as antibacterials by disrupting cell permeability so that intracellular compounds such as cytoplasm escape, resulting in cell death. Saponins form strong polymer bonds with porins (transmembrane proteins) on the outer membrane of bacterial cell walls, causing damage to the porins. Then, the saponin will diffuse through the cell membrane and bind to the cytoplasmic membrane, causing reduced stability and disruption of the membrane which can lead to cell death due to cytoplasmic leakage.[26]

Triterpenoid compounds are secondary metabolite compounds derived from terpenoids and have pharmacological activities that can be used, including anti-inflammatory, antibacterial, antiviral, antifungal, and anticancer.[27] The mechanism of triterpenoid antibacterial activity is to react with porins (transmembrane proteins) outside the bacterial cell wall membrane which will forming strong polymer bonds causing damage to the porins.[28]

Alkaloids are compounds that can easily be found in around 20% of plant species and play an important role in human medicine and organism defense.[29] The way alkaloid compounds work as antibacterials is by disrupting the peptidoglycan structure in bacterial cells so that the cell wall layer does not form intact and cell death occurs. It is known that the alkaloid component can inhibit the topoisomerase enzyme in bacterial cells and act as a DNA interchelator.[30]

There are three systems that play a role in honey's antibacterial properties, including osmotic pressure where honey has a solid content of around 84% fructose and glucose, with a water content of 15-21%. The strong interaction of sugar molecules with water will result in few water molecules being available for microorganisms. In this osmosis process, microorganisms will lack water and experience dehydration, causing the death of the microorganism.[31]

Apart from that, the effect of the acidity level which can be assessed from the pH value of trigona honey based on Malaysian Standards is around 2.5-3.8.[32] The acidic pH of honey will inhibit the metabolism of gram-negative bacteria, causing the bacteria to easily undergo lysis, thus inhibiting growth. bacteria. Lastly, Inhibine forms enzymes and accumulates hydrogen peroxide because it has cytotoxic properties for bacterial cells and has the ability to oxidize and form free radicals, thus facilitating damage to bacterial cells.[32, 33]

Based on research results from raw Trigona honey with a total number of bacterial colonies of 0.4 x 10^6 CFU/mL, Trigona dehumidified honey with a total number of bacterial colonies of 1.8 x 10^6 CFU/mL and hydrosol honey with a total number of colonies of 2 x 10^6 CFU/mL has quite good effectiveness compared to the positive control. It can be seen that the three honeys have antibacterial activity in inhibiting *S.mutans* bacteria and this is also supported by good results in stability tests for other physical properties and needs to be considered because the stability tests carried out did not experience changes during the storage period.

CONCLUSION

Based on the research results, it can be concluded that the process of processing honey by dehumidification produces differences in characteristics between raw trigona honey, dehumidified trigona honey and dehumidified honey water and has an effect on the antibacterial level against *Streptococcus mutans*. The most effective honey in inhibiting *Streptococcus mutans* bacteria is raw trigona honey with an average total colony of 0.4 x 10^6 CFU/mL.

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