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Original Article

FORMULATION OF GARGLE FROM *TRIGONA SP.* PROPOLIS EXTRACT AND ITS ACTIVITY AGAINST *STREPTOCOCCUS MUTANS*

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ABSTRACT

Objective: In this research, the formulations of a gargle with *Trigona sp.* propolis extracts as an alternative of dental caries therapy and test its activity against *Streptococcus mutans* was carried out.

Methods: The experimental laboratory have been carried out with the following stages of work, material collection and processing, extraction of *Trigona sp.* propolis by maceration with 95% ethanol, phytochemical screening, determination of minimum inhibitory concentration, formulation of the gargle, activity, contact time and stability test of the formulas.

Results: Phytochemical screening showed that *Trigona sp.* propolis extracts containing flavonoids, polyphenols, quinones, monoterpenoids and sesquiterpenoids. Minimum inhibitory concentration was 0.25% w/v. The formulation of gargle from *Trigona sp.* propolis extract were a yellow solution with mint odor and taste of mint and sweet. The antibacterial activity of gargle preparation and formulas from the market as a comparison, showed that the formula with a concentration of 1% w/v gave a significant difference to the market preparation. The formulation of gargle from *Trigona sp.* propolis extract has good inhibition against bacteria *S. mutans* with a contact time of 60 seconds and is physically stable during the 35 d of storage time.

Conclusion: The formulation of gargle from *Trigona sp.* propolis extract is an excellent preparation to be developed in the future in the treatment of dental caries.

Keywords: Dental caries, Gargle, *Trigona sp.*, Propolis, *Streptococcus mutans*

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INTRODUCTION

Dental caries is the most common disease of tooth structure caused by the activity of microorganisms due to the damage of fermented carbohydrates and tooth enamel. The etiology of caries is multifactorial because four main factors interact: microbes, carbohydrate substrates, fragile tooth surfaces (host), and time factors [1-4]. Dental caries is a disease that affects many children and adults, may affects deciduous and permanent teeth [5, 6]. The prevalence of dental and oral health problems based on the age category were about 10.4% of children aged 1-4 y, 28.9% of children aged 5-9 y and 25.2% of children aged 10-14 y, and only about 25.8%, 35.1%, and 28.3% consecutively, were treated [7].

Dental and oral health condition of children in recent decades has increased, but dental caries was still the most common oral health problem found in children worldwide. According to the WHO, the prevalence of dental caries in school-age children is 60-90%, which is generally the same as adults in most countries [8]. Caries is a global public health challenge and is continuously studied and documented in various countries. In 2020, the global prevalence of dental caries in primary and permanent teeth was estimated at 46.2 and 53.8 %, respectively, which was considered to be high [9]. A typical characteristic of children aged 6-14 is a critical age with characteristic features, the stage of mixed dentition or the early stage of the transformation of milk teeth into permanent teeth [5].

Dental caries has a high financial burden on parents of affected children [10]. In high-income countries, dental treatment is costly with 5% of the total health expenditure and 20% of out-of-pocket health expenditure [11]. The burden of oral health in the United States is annually over 20 million working days and 51 million school hours are lost alone due to oral disease and its treatment [12]. In low-and middle-income countries, the incidence of dental caries is rapidly increasing among children and adults [13].

A healthy mouth not only enables the nutrition of the physical body but also enhances social interaction and promotes self-esteem and feelings of well-being [14]. There are various ways of dental prevention method that can be done in preventing dental caries disease. One of the dental prevention methods is topical application on tooth surfaces. Since many individuals are now aware of and concerned about improving their oral health, there is sustained support for the development of new products and substances to keep the oral cavity clean and healthy [15]. Many kinds of topical preparation to use for dental caries prevention, such as gargle or mouthwash that, have gained wide acceptance because of their perceived benefits, and especially because of sustained advertising and marketing [16-18].

More recently, herbal products containing natural compounds such as mentha, guarana, eucalyptus extract, mastic, Arab acacia, and miswak, all of which also have significant antibacterial properties, have been introduced. One of these natural compounds is propolis, which has been used in many toothpastes, mouthwashes, and dentifrices as an important natural product in preventing oral diseases [18-21]. To demonstrate the health benefits of propolis, some studies have shown that its application can heal oral ulcers and gingivitis in many clients [22-24]. The formulation of gargle from *Trigona sp.* propolis extract is an excellent preparation to be developed in the future in the treatment of dental caries.

MATERIALS AND METHODS

Propolis of *Trigona sp (*North Luwu Forestry and Plantation Service), *Streptococcus mutans* ATCC 25175, ethanol (Merck, Indonesia), sorbitol (Merck, Indonesia), glycerine (Merck, Indonesia), sodium benzoat (Merck, Indonesia), peppermint (Merck, Indonesia), distilled water (Ikapharmindo Putramas, Indonesia).

Extraction

Propolis of Trigona sp. was cut into pieces then weighed. Extracted by maceration for 3x24 h using 95% ethanol, then evaporated to obtain a concentrated extract. The yield was obtained by equation extract weight per weight of simplicia multiplied by 100%

Phytochemical screening

The screening was carried out to determine the presence of alkaloids, polyphenolic compounds, flavonoids, quinones, tannins, saponins, steroids, triterpenoids, monoterpenoids and sesquiterpenoids.

Determination of minimum inhibitory concentration (MIC) of *Trigona sp.* propolis extract against *Streptococcus mutans*

The diffusion agar method was used for this determination. 20 µl bacterial suspension was poured into a Petri dish and 1 ml of blood and 19 ml of nutrient agar medium were added at 45 °C and then shaken until the suspension mixed well as the blood agar medium. After the medium was solid, perforated and the holes were filled with 50 µl of each concentration of *Trigona sp.* propolis extract. The plates were incubated for 18-24 h at 37 °C. The MIC value was indicated by the extract with the smallest concentration, which still gave the diameter of inhibition (clear zone) to the test bacteria medium.

The formulation of preparations were listed in table 1. *Trigona sp.* propolis extract and sodium benzoate was dissolved in distilled water each; then the solutions were mixed. Ethanol, glycerine, and sorbitol were added and mixed homogeneously. Add peppermint, put it in a bottle and add distilled water to 100 ml, then shaken homogeneously.

Gargle formulation of *Trigona sp.* propolis extract

Table 1: Gargle formulation

Materials	Formula A			Formula B		
	A_0	A_1	A_2	B_0	B_1	B_2
Trigona sp. propolis extract (g)	-	0.5	1	-	0.5	1
Sodium Benzoate (g)	-	-	-	0.1	0.1	0.1
Ethanol 70% (ml)	5	5	5	5	5	5
Sorbitol (ml)	20	20	20	20	20	20
Glycerine (ml)	10	10	10	10	10	10
Peppermint (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water add (ml)	100	100	100	100	100	100

Description: A_0: Formulation without *Trigona sp.* propolis extract and preservative, A_1:Formulation with 0.5% *Trigona sp.* propolis extract without preservative, A_2: Formulation with 1% *Trigona sp.* propolis extract without preservative, B_0:Formulation without *Trigona sp.* propolis extract, but preservative added, B_1:Formulation with 0.5% *Trigona sp.* propolis extract and preservative, B_2:Formulation with 1% *Trigona sp.* propolis extract and preservative

Antibacterial activity and comparative tests of gargle preparations of *Trigona sp.* propolis extract against *Streptococcus mutans*

The diffusion agar method was used for this testing. 20 µl bacterial suspension was poured into a Petri dish and 1 ml of blood and 19 ml of nutrient agar medium were added at 45 °C and then shaken until the suspension mixed well as the blood agar medium. After the medium was solid, perforated and the holes were filled with 50 µl of each formula solution (FA_0, FA_1, FA_2, FB_0, FB_1, FB_2). The plates were incubated for 18-24 h at 37 °C. The diameter of the inhibition formed was measured using a caliper as a parameter to determine the antibacterial activity that was tested and then compared with gargle preparations on the market.

Contact time test of *Trigona sp.* propolis extract formulation against *S. mutans*

The contact time of the preparation was carried out in sterile test tubes aseptically by inserting 100 µl bacterial suspension into 10 ml preparation. After 15 s, the mixture was taken one loop and then planted on blood agar media. This procedure was repeated for contact times of 30, 45 and 60 s. The plates were incubated at 37 °C for 18-24 h, then the results were observed. Each sector planting on blood agar media that showed the least colony growth at the shortest contact time according to the length of a person's habit of rinsing was chosen as the most effective contact time. The same treatment was also carried out on the formula without *Trigona sp.* propolis extract as a control formula and also on market preparations as a comparison.

Physical evaluation of preparations

The evaluation includes organoleptic observations, measurement of pH and viscosity of preparations during 35 d of storage.

RESULTS

Trigona sp. propolis extraction results in the form of a thick, dark brown and aromatic extract. From 1000.75 g of *Trigona sp.* propolis, 159.45 g of concentrated extract was obtained (yield was 15.93%). The phytochemical screening results that *Trigona sp.* propolis contain flavonoids, polyphenolics, quinones, monoterpenoids and sesquiterpenoids compounds, which can be seen in table 2.

Table 2: Results of *Trigona sp.* propolis extract phytochemical screening

Secondary metabolites	Observation result
Alkaloids	-
Flavonoids	+
Polyphenolics	+
Quinones	+
Tannins	-
Saponins	-
Monoterpenoids and sesquiterpenoids	+
Steroids dan triterpenoids	-

+= detected,-= undetected

Table 3: The inhibition zone of *Trigona sp.* propolis extract against *Streptococcus mutans* (n=8)

Concentration of extract (%)	Inhibition zone diameter (cm)
10	1.38
7.5	1.32
5	1.26
2.5	1.17
1	1.11
0.5	1.06
0.25	1.02
0.125	No inhibition zone

The results of the inhibition zone from *Trigona sp* propolis extract against *Streptococcus mutans* in table 3, can be seen that the minimum inhibition concentration (MIC) of the extract was at 0.25%. This MIC is the smallest concentration of *Trigona sp.* propolis extract that can inhibit the growth of *Streptococcus mutans* bacteria. This MIC value will be used as a reference in the formulation of gargle preparations.

Table 4: The result of *Trigona sp.* propolis extract gargle formulation (n=18)

Formula	Form	Colour	Smell	Flavor	pH	Viscosity (cP)
A_0	solution	clear	mint	sweet, mint	7.44+0.00	65.8+0.00
A_1	solution	yellow clear	mint	sweet, mint	6.77+0.01	76.7+0.10
A_2	solution	yellow clear	mint	sweet, mint	6.53+0.001	82.6+0.17
B_0	solution	clear	mint	sweet, mint	7.23+0.02	65.5+0.17
B_1	solution	yellow clear	mint	sweet, mint	6.82+0.03	76.5+0.23
B_2	solution	yellow clear	mint	sweet, mint	6.66+0.03	82.4+0.35

Table 5: The result of antibacterial activity and comparative tests of *Trigona sp.* propolis extract gargle preparations against *Streptococcus mutans* (n=14)

Formula	Inhibition zone diameter (cm)		inhibition diameter (cm)
	Dish 1	Dish 2	
A_0	-	-	-
B_0	-	-	-
A_1	1.13	1.09	1.11+0.03
B_1	1.11	1.13	1.12+0.01
A_2	1.20	1.18	1.19+0.01
B_2	1.15	1.19	1.17+0.03
S	1.07	1.09	1.08+0.01

Description: S = Market preparation,-= there no inhibition

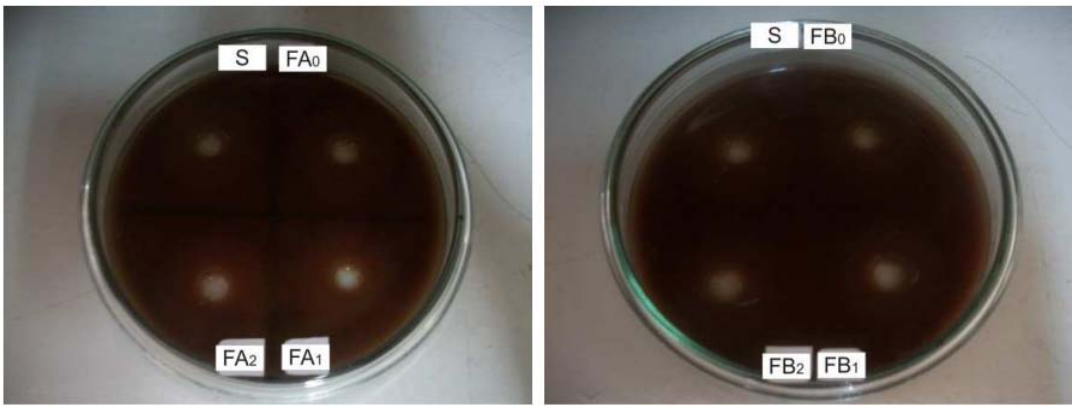

Fig. 1: The inhibition zone of antibacterial activity and comparative tests

Description

FA_0: Formulation without *Trigona sp.* propolis extract and preservative

FA_1: Formulation with 0.5% *Trigona sp.* propolis extract without preservative

FA_2: Formulation with 1% *Trigona sp.* propolis extract without preservative

FB_0: Formulation without *Trigona sp.* propolis extract, but preservative added.

FB_1: Formulation with 0.5% *Trigona sp.* propolis extract and preservative

FB_2: Formulation with 1% *Trigona sp.* propolis extract and preservative

S: Market preparation

Table 6: Result of *Trigona sp.* propolis extract formulation contact time test against *S. mutans* (n=8)

Formula	Contact time (s)			
	15	30	45	60
A_0	-	-	-	-
B_0	-	-	-	-
A_1	-	-	-	+
B_1	-	-	-	+
A_2	-	-	-	+
B_2	-	-	-	+
Control (-)	-	-	-	-
Market Prep.	-	-	-	-

Description: Control (-): The formula without extract, (+): Has antibacterial activity; organoleptic observations showed that the preparation of *Trigona sp.* propolis extract preparations did not change form, color, smell and taste during 35 d of storage. pH measurement of the *Trigona sp* propolis extract preparation can be seen in table 7.

Table 7: pH measurement of the *Trigona sp.* propolis extract preparation (n = 126)

Formula	pH of preparation on days-						
	1	3	7	14	21	28	35
A_0	7.44+0.00	7.43+0.02	7.43+0.01	7.41+0.03	7.40+0.00	7.37+0.02	7.36+0.01
A_1	6.77+0.01	6.75+0.03	6.75+0.00	6.74+0.02	6.72+0.01	6.70+0.03	6.69+0.00
A_2	6.52+0.02	6.52+0.03	6.50+0.01	6.49+0.03	6.48+0.00	6.48+0.02	6.45+0.01
B_0	7.23+0.02	7.23+0.01	7.22+0.03	7.20+0.00	7.20+0.02	7.19+0.01	7.17+0.03
B_1	6.82+0.03	6.81+0.00	6.81+0.02	6.80+0.01	6.78+0.03	6.76+0.00	6.75+0.02
B_2	6.66+0.03	6.64+0.01	6.63+0.03	6.63+0.00	6.60+0.02	6.59+0.01	6.59+0.03

Table 8: The viscosity of the *Trigona sp.* propolis extract preparation (n = 126)

Formula	Viscosity (cP) on days-						
	1	3	7	14	21	28	35
A_0	65.8+0.00	65.7+0.17	65.6+0.03	65.6+0.35	65.4+0.00	65.2+0.17	65.2+0.10
A_1	76.7+0.10	76.5+0.35	76.5+0.00	76.4+0.17	76.2+0.10	76.0+0.35	75.9+0.00
A_2	82.6+0.17	82.6+0.35	82.4+0.10	82.3+0.35	82.2+0.00	82.2+0.17	81.9+0.10
B_0	65.5+0.17	65.5+0.10	65.3+0.35	65.1+0.00	65.1+0.17	65.0+0.10	64.9+0.35
B_1	76.2+0.23	76.1+0.00	76.1+0.17	76.0+0.10	75.8+0.35	75.6+0.00	75.5+0.17
B_2	82.4+0.35	82.2+0.10	82.1+0.35	82.1+0.00	81.8+0.17	81.7+0.10	81.7+0.35

The viscosity value of *Trigona sp.* propolis extract preparation was in the range of 64.0-83.0 cP. These results indicate that the viscosity of the preparation has met the requirements, namely having an aqueous viscosity that is almost the same as the solution.

DISCUSSION

The results of the MIC determination in table 3, it can be seen that the MIC of the *Trigona sp.* propolis extract was at 0.25%. This MIC is the smallest concentration of *Trigona sp.* propolis extract that can inhibit the growth of *S. mutans* bacteria. This MIC value will be used as a reference in the formulation of gargle preparations. The result of *Trigona sp.* propolis extract gargle formulation showed at table 4.

Statistical analysis of variance (ANAVA) showed that H_0 was rejected because the calculated F value (9.227) was greater than the F table value (5.19) with a significant level of = 5%, which mean there was a significant difference in the inhibition zone diameter (table 5). The antibacterial activity on changes in concentration of *Trigona sp.* propolis extract and comparison formula. To find out whether there was a significant difference between each formula, the Newman-Keuls test was carried out with a significance level (α) = 0.05. From the results can be seen that there were significant differences between FB_1 and S, FB_1 and FA_1, FB_1 and FA_2, FB_2 and S, FB_2 and FA_1, and FA_2 with S.

The most effective contact time of preparations for formulas A_1, B_1, A_2 and B_2 was 60 s, while for formulas A_0 and B_0 there were still bacteria. It caused the formulas A_0 and B_0 were formulas that do not contain *Trigona sp.* propolis extract. The comparison formula (market preparation) requires a contact time of more than 60 s (table 6).

The pH value of *Trigona sp.* propolis extract preparations during 35 d of storage was in the range of 6.4-7.5 (table 7), and this is in accordance with the salivary pH that in the healthy state, saliva has a pH range of 6.7–7.4 [25]. The viscosity value of *Trigona sp.* propolis extract preparation was in the range of 64.0-83.0 cP (table 8). These results indicate that the viscosity of the preparation has met the requirements, namely having an aqueous viscosity that is almost the same as the solution.

CONCLUSION

The formulation of gargle preparation products containing a certainly amount of stinglessos *Trigona sp.* propolis extract were successfully prepared. The formulation has good inhibition against the bacteria *Streptococcus mutans* with a contact time of 60 s, appropriate stability and most effective than market preparation. The formulation of gargle from *Trigona sp.* propolis extract is an excellent preparation to be developed in the future in the treatment of dental caries.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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