

Antiseptic Effect Test of GinLemClo (Ginger, Lemongrass, and Clove) Herbal Mouthwash Against Halitosis-Causing Bacteria in Diabetes Mellitus Mice (In Vivo Method).

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ABSTRACT

GinLemClo herbal mouthwash made with ginger, lemongrass, and cloves is widely known for its healthful properties. It has been empirically used widely as a treatment for bad breath in people with diabetes mellitus. Therefore, this study aims to test the effect of the antiseptic herbal mouthwash GinLemClo on halitosis-causing bacteria and to reduce halitosis levels in diabetic mice in vivo. This is a true-experimental study conducted with a pretest-posttest-only group design at the Laboratory of Pharmacy and Microbiology, Faculty of Medicine, Hasanuddin University. The number of experimental animals used was 15 male mice suffering from DM and experiencing halitosis. The samples were selected using Random Sampling, while data were analyzed with the paired sample test and one-way ANOVA. The toxicity test conducted by using the GinLemClo herbal mouthwash showed that all mice were in good health. Before treatment, the mice had several bacteria, both pathogenic and non-pathogenic, but after the treatment, only normal flora was found. The treatment also suppressed the growth of halitosis-causing bacteria in DM mice ($p < 0.000$) and reduced halitosis levels with $f\text{-count} (18,300) > f\text{ table} (3.74)$. Based on the results, GinLemClo herbal mouthwash can be used as an antiseptic to inhibit the cause, and reduce levels of halitosis

Keywords: *Antibacterial, GinLemClo, Herbal Mouthwash, Diabetes Mellitus*

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INTRODUCTION

Non-communicable diseases such as hypertension, diabetes mellitus (DM), as well as dental and oral diseases including gingivitis, periodontitis, halitosis, etc., are becoming increasingly prevalent every year and this trend poses a significant challenge for treatment and prevention efforts (1). DM is a disorder of carbohydrate metabolism caused by damage to the islets of Langerhans cells in the pancreas, culminating in a reduction or inhibition of insulin secretion. It can also be caused by a decrease in the sensitivity of insulin receptors on target cells. Indonesia ranks fourth in the world with 8.4 million people having diabetes and this value is expected to reach 21.3 million in 2030. Furthermore, DM often causes complications in various organs of the body when not handled properly and this is related to high blood sugar levels in the long term which leads to damage to blood vessels, nerves, and other internal structures (2)(3)(4). DM also causes dysfunction of various cells and tissues in the human body, including salivary gland disorders in the oral cavity (5). Other disorders that often occur are halitosis and xerostomia, Stomatitis Aphthosa (sprue), burning mouth sensation, and dental caries (6)(7)(8)(9). The dental and oral health efforts carried out by the government in increasing the optimal degree of

public health include providing education about the impact of diseases such as halitosis on DM sufferers. Several people experience halitosis but are not aware of this condition. DM patients, in particular, are prone to this condition as usually indicated by the characteristic smell of acetone in their breath due to the presence of ketone waste. Various methods are used to remove halitosis including mouthwash. However, people sometimes complain about the effects such as dry mouth (10)(11) (12).

One of the ways to prevent these side effects is to produce mouthwash using herbal plants but this requires various investigation procedures. For example, animal trials should be carried out to ensure the efficacy and safety of the herbal plants. Furthermore, about 40% of studies used mice as laboratory models because they have advantages such as a relatively short life cycle, a large number of offspring per birth, easy handling, similar reproductive characteristics, as well as anatomical, physiological and genetic structures to humans. (13)(14)(15)(16)(17)(18)(19)(20)(21).

The use of herbs as alternative medicine is widely popular globally due to the increasing empirical evidence and scientific support (22). One of the herbal medicines often used to treat several diseases is ginger (*Zingiber officinalis*), which has a distinctive spicy taste derived from a ketone compound called zingerone. According to recent reports,

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the combination of ginger mixed with lemongrass is potentially effective in treating various health problems. Aside from using the stalks of the lemongrass (*Cymbopogon citratus*) plant for flavoring or cooking fragrances, the plant also has numerous benefits for the body. The content in lemongrass includes folic acid, riboflavin, vitamin A, vitamin C, potassium, iron, manganese, etc., which have various properties such as antifungal, antipyretic, analgesic, and antioxidant.

Several types of mouthwash available in the market are not effective in reducing high halitosis levels in the oral cavity. Additionally, their antiseptic effect in killing bacteria only lasts a short time, rendering them less effective in reducing shortness of breath in the long term (22)(23). Previous studies have also tested the formulation of herbal mouthwash using extracts of ginger, lemongrass, and cloves as an antibacterial against halitosis at the UMI Pharmacy Lab in 2020. This is due to the presence of acetic acid in the solution as a solvent. The inhibitory effect is not solely due to the activity of formulation A, as acetic acid also has the ability to inhibit bacteria. In the solution with a concentration of chitosan 100% (w/v), the highest inhibition occurred with a diameter of 18.7 mm/mg sample extract. This is presumably due to the low viscosity of formulation A which aids diffusion into the media containing *Porphyromonas gingivalis*. In contrast, the lowest inhibition occurred in formulation A with a concentration of 25%(w/v) (24)(25)(26)(27).

Masriadi, et al., examined the effect of *GinLemClo* mouthwash processed by the community and assessed the degree of halitosis after being used. The results showed that the moderate halitosis reduced from (50%) to (20%), while bad breath reduced from (10%) to (0%) after treatment. Patients who used lemongrass and ginger leaves experienced changes in moderate breath from (40%) to (15%), and bad breath from (15%) to (5%). This indicates that *GinLemClo* has a high inhibitory power against halitosis-causing bacteria (28). The study was continued with the toxicity test of the mouthwash and no death was found in the treatment group up to day 14. The test results showed that 20 ml of *GinLemClo* mouthwash was sprayed into the mice's mouths, then it was removed using a syringe after 30 seconds. This procedure was performed for 14 days with 2x daily administration. Therefore, this dose limit is considered safe and can be given to animals consecutively for 14 days. During the treatment in this study, the mice were given food and drink ad libitum.

In vivo testing using general laboratory animals is often carried out to obtain acute toxicity data. It is considered

very important due to the several advantages including the ability to obtain data related to normal physiological and biochemical conditions. The results can also be interpolated to humans or as toxicological prediction material for domestic animals and livestock. As usual medicinal compounds, herbs, or herbal medicine have detrimental effects when they are not used according to the proper dosage. Various herbal plant extracts including garlic, turmeric, ginger, katuk leaves, and many other compounds are often mixed in water without indicating the percentage compositions on the packaging. Adverse effects generally occur due to the use of non-standardized materials, the wrong dosage, the effect of combining constituent compounds, hygroscopic and volume properties, or the possibility of contamination by other microbes. These effects might also arise due to the addition of compounds in herbs.

The use of traditional medicines such as mouthwash, specifically those derived from plants, has continued to increase. These medicinal plants include Red ginger (*Zingiber officinalis Roxb*), which is commonly used by the community as a cold medicine, indigestion, analgesic, antipyretic, tonic, anti-inflammatory, etc. Various studies prove that red ginger has antioxidant properties with some of the main components such as gingerol, shogaol, and gingeron reportedly having greater activity than vitamin E [31]. Therefore, this study aims to test the antiseptic effect of *GinLemClo* mouthwash on halitosis-causing bacteria and to reduce halitosis levels in diabetic mice *in vivo*.

MATERIAL AND METHODS

This is a true-experimental study conducted with a pretest-posttest-only group design at the Pharmacy and Microbiology Laboratory, Faculty of Medicine, Hasanuddin University from September 2021 to March 2022. The number of experimental animals used was 15 healthy male mice and the operational definition of *GinLemClo* is a mouthwash that has been extracted with a combination of ginger, lemongrass, and cloves in a bottle. Meanwhile, the antiseptic effect is a state of loss/reduced growth of microorganisms that cause bad breath in the oral cavity of experimental animals.

The raw material for *GinLemClo* mouthwash consists of a combination of herbal plants, namely 100g of ginger, lemongrass, and cloves at a dose of 46.25% -100%. This dosage was designed to be sufficient for suppressing the growth of bacteria that cause halitosis. The composition of the *GinLemClo* herbal mouthwash is shown in Table 1 below

Table 1. The Composition of Herbal Mouthwash (*GinLemClo*)

Ingredient (ml)	F1	F2	F3	F4	F5
Ginger Extract	40	50	60	70	75
Lemongrass	6	4	3	7	5
Clove	0.25	0.25	0.25	0.25	0.25
Tween 80	1.80	1.80	1.80	1.80	1.80
Na-Benzoat(g)	0.10	0.10	0.10	0.10	0.10
Aquades	51.85	43.85	34.85	20.85	17.85
Total	100	100	100	100	100

F: Division Composition of Herbal Mouthwash (*GinLemClo*) Groups

This study consisted of three stages, first, the toxicity test was carried out by administering *GinLemClo* mouthwash to healthy mice/without DM. About 20 ml of the mouthwash was sprayed into the mice's mouths then it was removed after 30 minutes using a syringe. This procedure was repeated 2x for 7 days with food and drink which were provided ad libitum. In the second stage, an *in-vivo* test was performed to make the mice diabetic by fasting for ± 18 hours, then the blood glucose levels and body weight were measured. The next 2 (two) hours after the wound on the tail dried up, the mice were injected with a solution of alloxan tetrahydrate 70 mg/kg body weight intravenously into the tail, subsequently, they were fed and left in the cage for two days. On the third day, blood glucose levels and body weight were measured after the mice had fasted for ± 18 hours. The blood glucose levels test results showed that the mice had been conditioned to have type 2 diabetes, while the body weight was also measured using an electronic scale. In the third stage, the preclinical test was carried out by taking saliva samples from mice before giving *GinLemClo* mouthwash to examine the growth of bacteria and measure the levels of halitosis using a Breath Checker. About 20 ml of *GinLemClo* mouthwash was sprayed into the mice's mouths, then it was removed after 30 seconds using a syringe. This procedure was performed

2x for 7 days and saliva samples were taken to examine the growth of bacteria as well as the level of halitosis.

The procedure for measuring halitosis involved using an organoleptic approach where participants were asked to cover their mouths for 30 seconds then the halitosis level was assessed by the examiner. The organoleptic assessment was based on a score of 0-5, but the average value was taken from all examinations where more than one examiner was used. The organoleptic score levels were as follows score 0: halitosis was not detected, score 1: halitosis was detected but can still be tolerated, score 2: halitosis was there and the smell has exceeded the normal limit, score 3: mild halitosis, score 4: moderate halitosis and quite disturbing, and score 5: very pronounced and disturbing halitosis. The was tested on bacteria halitosis-causing bacteria in 15 male mice. The blood glucose levels were initially measured before and after alloxan administration to induce DM and the results are shown in the table below.

mples were selected using random sampling, while the data obtained were analyzed with the *Paired Sample Test* and *One Way ANOVA* test to determine the effect of *GinLemClo* mouthwash on the inhibition of halitosis-causing bacteria.

RESULTS

This study was conducted to examine the effect of *GinLemClo* herbal mouthwash administration on

Table 2. Fasting Sugar (FS) Results / Body Weight (BW) Before and After Alloxan Administration

No	Before Giving Alloxan		After Giving Alloxan	
	FS	BW	FS	BW
1	152 mg/dl	28,85 g	317,45 mg/dl	36,8 g
2	158 mg/dl	29,51 g	352,15 mg/dl	36,8 g
3	149 mg/dl	30,75 g	325,42 mg/dl	36,8 g
4	150 mg/dl	27,12 g	330,53 mg/dl	36,8 g
5	147 mg/dl	25,39 g	340,49 mg/dl	36,8 g
6	151 mg/dl	29,98 g	312,35 mg/dl	36,8 g
7	153 mg/dl	24,87 g	316,65 mg/dl	36,8 g
8	154 mg/dl	26,71 g	319,05 mg/dl	36,8 g
9	159 mg/dl	27,15 g	320,75 mg/dl	36,8 g
10	145 mg/dl	29,15 g	333,51 mg/dl	36,8 g
11	156mg/dl	28,19 g	329,38 mg/dl	36,8 g
12	155 mg/dl	26,33 g	334,23 mg/dl	36,8 g
13	146 mg/dl	28,25 g	329,49 mg/dl	36,8 g
14	147 mg/dl	30,18 g	324,33 mg/dl	36,8 g
15	157mg/dl	29,63 g	341,25 mg/dl	36,8 g
Average	151,93 mg/dl	28,13 g	328,46 mg/dl	36,8g

Table 2 shows that all mice are already in DM condition as indicated by the increase in FS by an average of 151.93 mg/dl to 328.46 and BW from 28.13 g to 36.8 g

Table 3. Data of *GinLemClo* mouthwash *in vivo* test (Pre-Test)

No	Code	GRAM	CIT	UR	G	L	S	M	CAT	Information
1	A	basil gr (-)	+	+	+	-	-	+	-	<i>Proteus mirabilis</i>
2	A	basil gr (-)	+	-	+	+	+	+	-	<i>Enterobacter aerogenes</i>
3	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
4	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
5	A	basil gr (-)	+	+	+	+	+	+	-	<i>Klebsiella sp.</i>
6	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
7	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
8	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
9	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
10	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
11	A	basil gr (-)	+	+	+	+	+	+	-	<i>Klebsiella sp.</i>
12	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
13	A	basil gr (-)	+	-	-	-	-	-	-	<i>Alkaligenes faecalis</i>
14	A	basil gr (-)	+	+	+	+	+	+	-	<i>Klebsiella sp.</i>
15	A	basil gr (-)	+	+	+	+	+	+	-	<i>Klebsiella sp.</i>

Table 4. Data of *GinLemClo* mouthwash *in vivo* test (Post-Test)

No	Code	GRAM	CIT	UR	G	L	S	M	CAT	Information
1, 7, 8, 9, 11, 12, 13	A									No growth
3	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
4	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
5	A	basil gr (-)	+	+	+	+	+	+	-	<i>Klebsiella sp.</i>
6	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
10	A	basil gr (-)	-	-	+	+	+	+	-	<i>E. coli</i>
14	A	basil gr (-)	+	+	+	+	+	+	-	<i>Klebsiella sp.</i>
15	A	basil gr (-)	+	+	+	+	+	+	-	<i>Klebsiella sp.</i>

Explanation: CIT: Citrat, UR: Urea, G: Glucose, L: Lactose, S: Sucrose, M: Mannitol, CAT: Catalase

Tables 3 and 4 show that there was a change in the number of bacteria in the saliva of mice before and after administration of *GinLemClo* herbal mouthwash.

Table 4. Statistical Test Results Regarding the Effect of *GinLemClo* Herbal Mouthwash on Bacterial Growth Count

Bacterial	Mean	Paired Differences		95% Confidence Interval of the Difference		t	df	Sig (2-tailed)
		Std. Deviation	Std. Error Mean	Lower	Upper			

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Before Mouthwash	2,733	0,704	0,182	2,344	3,123	15,043	14	0.000
After Mouthwash								

Table 5. shows that *GinLemClo* herbal mouthwash significantly suppressed the growth of halitosis-causing bacteria in the oral cavity with p 0.000.

Table 6. Measurement of Halitosis Levels Pre-Posttest Administration of GinLemClo Herbal Mouthwash (*Paired Sample Test*)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pre Test - Post Test 1	.133	.352	.091	-.062	.328	1.468	14	.164
Pair 2	Pre Test - Post Test 2	.733	.458	.118	.480	.987	6.205	14	.000
Pair 3	Pre Test - Post Test 3	1.200	.414	.107	.971	1.429	11.225	14	.000
Pair 4	Pre Test - Post Test 4	1.867	.352	.091	1.672	2.062	20.546	14	.000
Pair 5	Pre Test - Post Test 5	2.200	.414	.107	1.971	2.429	20.579	14	.000
Pair 6	Pre Test - Post Test 6	2.867	.352	.091	2.672	3.062	31.553	14	.000
Pair 7	Pre Test - Post Test 7	3.733	.458	.118	3.480	3.987	31.588	14	.000

Table 6 shows that there was a significant effect before and after administration of the GinLemClo herbal mouthwash on the second to the seventh day of treatment.

Table 7. Differences in Average Halitosis Levels in the Treatment Group (*ANOVA Test*)

		Sum of Squares	df	Mean Square	F	Sig.
Post Test 1	Between Groups	2.533	2	1.267	12.667	.001
	Within Groups	1.200	12	.100		
	Total	3.733	14			
Post Test 2	Between Groups	1.733	2	.867	5.200	.024
	Within Groups	2.000	12	.167		
	Total	3.733	14			
Post Test 3	Between Groups	1.733	2	.867	8.667	.005
	Within Groups	1.200	12	.100		
	Total	2.933	14			
Post Test 4	Between Groups	2.711	2	1.356	18.300	.000
	Within Groups	.889	12	.074		
	Total	3.600	14			
Post Test 5	Between Groups	1.733	2	.867	8.667	.005
	Within Groups	1.200	12	.100		

	Total	2.933	14			
Post Test 6	Between Groups	2.711	2	1.356	18.300	.000
	Within Groups	.889	12	.074		
	Total	3.600	14			
Post Test 7	Between Groups	1.733	2	.867	5.200	.024
	Within Groups	2.000	12	.167		
	Total	3.733	14			

The OneWay Anova test was used to assess the mean difference between the posttest 1, 2, 3, 4, 5, 6 and posttest 7 groups. Based on the value of f count (18.300) > f table (3.74), there was a significant difference in the mean of all treatment groups. The highest calculated F scores were obtained in the posttest groups 4 and 6.

DISCUSSION

Halitosis is a condition of releasing an unpleasant odor or bad breath due to the presence of bacteria and fungi in the mouth. According to several reports, it has both intraoral and extraoral sources. In some cases, the odor comes from the mouth due to insufficient plaque, periodontal disease, as well as bacterial overgrowth caused by dental caries growth or damaged restorations, dry mouth, and hairy tongue. Bad breath can also be caused by systemic disorders such as upper respiratory tract infections, liver or renal insufficiency and DM. The main cause of halitosis is Volatile Sulfur Compounds (VSC) consisting of H₂S, CH₃SH, and CH₃SCH₃.

Furthermore, DM is a metabolic disorder that constitutes a major health concern with its prevalence increasing continuously worldwide over the past few decades. It is also considered an incurable metabolic disorder affecting about 2.8% of the global population. Alloxan-induced diabetes is one of the widely used models to induce Type I DM in experimental animals(29).

Table 2 shows that all mice were already in a DM condition as indicated by an increase in average FS from 151.93 mg/dl to 328.46 and body weight from 28.13 g to 36.8 g. Before administering GinLemClo to the male mice, alloxan was given to increase blood sugar and induce DM. Amir, et al., examined the activity of durian roots and leaves ethanol extract in reducing blood glucose levels in alloxan-induced mice (30). Similarly, the administration of moringa leaf extract (*Moringa oleifera*) at a dose of 600 mg/kg for 14 days increased serum HDL cholesterol levels in alloxan-induced male Wistar rats (*Rattus norvegicus*) but the difference was not significant (p 0.05)) (31)(32)(33).

The toxicity test conducted for 7 days in the morning and evening showed that all mice were in good health conditions with great appetite, normal weight, as well as sufficient eating and drinking. According to Rahayu et al., endophytic actinobacteria from ginger can produce toxic compounds that have anticancer potential. Furthermore, flavonoids that act as cancer prevention were detected in AJ2 isolates from the phytochemical test results

(34). Djahi et al (2021) conducted a study to test the anti-diabetic effect of citronella leaves ethanol extract (*Cymbopogon citratus*) on blood glucose levels in Sprague Dawley rats induced by Alloxan. The results showed that a minimum dose of 250 mg/Kg BW reduced blood glucose levels on the second day. Similarly, moderate and high doses of 500 mg/KgBB and 650 mg/KgBB were effective in reducing blood glucose levels on days 7 to 14 of administration respectively (35).

Tables 3 and 4 show that there was a significant difference in the number of bacteria in the mice's saliva before and after treatment. This proves that GinLemClo has antibacterial properties in inhibiting the growth of halitosis-causing bacteria in mice. Furthermore, there were differences in the tongue-coating microbiota during the development of halitosis. This was indicated by the composition of the microbiota and the relative abundance of the tongue coating in the treatment and control groups which showed significant differences, even before the onset of clinical manifestations. The halitosis prediction model based on biomarkers of the tongue-coating microbiota showed shifts in microbes before the onset of the disease. Therefore, it can be used for timely diagnosis and intervention of halitosis also in children. Evaluation of tongue-coating microbiota biomarkers may assist in predicting the risk of halitosis. As a preventive measure, instructions to control tongue-coating plaque must be given to parents or guardians before the onset of halitosis (36).

Microorganisms found in the oral cavity of DM mice treated with GinLemClo mouthwash experienced a decrease in growth as indicated by the in-vitro test. According to Shibgah (2019), the application of the K-Nearest Neighbor classification method to a breath sensor system can increase the accuracy range from 66% to 80%. Although the system device has worked quite well in detection, it has not yet met the accuracy standards required in the medical world, specifically for DM patients.

Ginger was used in this study because it is traditionally believed to relieve colds, reduce or prevent influenza, rheumatism, coughs, as well as pain (analgesic) and swelling (anti-inflammatory). It also contains various substances that are needed by the body such as essential oils (0.5-5.6%) and organic acids namely malic, and oxalic. Apart from being antimicrobial, ginger also has antioxidant properties (Uhl 2000). As shown in Table 2, a composition was made to compare its effectiveness against microorganisms, while distilled water was used as a positive control because it has a normal pH (37).

GinLemClo mouthwash contains citronella leaf extract which has phenolic active compounds with antioxidant properties. Furthermore, cloves contain phenolic compounds, namely eugenol, eugenol acetate, and gallic acid, as well as flavonoids. The high eugenol content has the potential to be used in several industrial fields, including medicine, pharmaceuticals, food, and pesticides (38).

The eugenol content in cloves is often used as an antibacterial, anti-inflammatory, and analgesic, while the high polyphenol composition correlates with high antioxidant activity. Furthermore, clove extracts reportedly have biological activities, such as antibacterial, antifungal, insecticidal, and antioxidant. The flowers are used traditionally as a flavoring and antimicrobial agent because they contain eugenol compounds which function as antioxidants as well as terpenoids (39)(40). Based on the statistical analysis results in Table 4, *GinLemClo* mouthwash significantly ($p < 0.000$) reduced bacterial growth in halitosis sufferers.

Table 6 shows that there was a significant effect before and after giving the *GinLemClo* herbal mouthwash from the 2nd to 7th day of administration. The OneWay Anova test was further used to assess the average difference between the posttest 1, 2, 3, 4, 5, 6 and posttest 7 groups. The f count value was $> f$ table (3.74), indicating that there was a significant difference in the mean values of all treatment groups. The highest calculated F score was obtained in the posttest groups 4 and 6 meaning that the administration of the *GinLemClo* mouthwash from the second to the seventh day can reduce halitosis levels in DM patients presumably due to its very complex content.

Ginger (*Zingiber officinale* Rosc.) is a traditional plant with many benefits and can be made in the form of extracts, powders, or gels depending on the intended use. It has active substances such as essential oils and gingerols which function as effective antibacterial and antifungal agents. Meanwhile, cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum*) are widely used as a spice for spicy dishes in European countries, the main ingredient for Indonesian typical cigarettes, as well as incense in China and Japan. Clove oil is used in aromatherapy and also to treat toothache. It is grown mainly in Indonesia (Banda Islands), Madagascar, and also thrives in Zanzibar, India, and Sri Lanka (40). Eugenol contained in cloves has pharmacological activity with analgesic, anti-inflammatory, antimicrobial, antiviral, antifungal, antiseptic, antispasmodic, antiemetic, stimulant, and local anesthetic properties. Therefore, this compound is widely used in the pharmaceutical industry. One of its derivatives namely isoeugenol can also be used as raw material for antiseptic and analgesic drugs. In the field of dentistry, the product obtained from the mixture of eugenol with zinc oxide acts as a cementing agent. Eugenol is biologically the most active part of the cement, and it blocks the transmission of nerve impulses to reduce pain caused by pulpitis (41).

Moreover, the cement has stronger antibacterial power compared to other dental cementing materials such as polycarboxylate, zinc phosphate, silicophosphate, calcium hydroxide, and composite resin. Eugenol's

antimicrobial and antiseptic properties make it a raw material for producing mouthwash, toothpaste as well as antiseptic liquid, tissue, and spray. Mouthwash containing clove eugenol can inhibit the growth of *Streptococcus mutans* and *Streptococcus viridans*, which are known to cause dental plaque.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results, *GinLemClo* herbal mouthwash is an antiseptic inhibitor of halitosis-causing bacteria in diabetic mice and it can be used twice a day. Therefore, to reduce halitosis in DM sufferers, people are advised to rinse their mouths using alternative mouthwashes such as herbal *GinLemClo*.

Declarations

Authors' contributions

This information will be published alongside your article if it is accepted for publication.

- 1) Compiling and designing experiments; (Masriadi)
- 2) Conduct experiments; (Masriadi, Sukmawati and Andi Tenri Biba Mallombasang)
- 3) Analyze and interpret data; (Tuti Alawiyah, Ardian Jaya Kusuma Amran, Mirna Febriani)
- 4) Contribution of reagents, materials, analytical tools, or data; (Hasta Handayani Idrus, Sari Aldilawati)
- 5) Writing papers (Masriadi, Sukmawati and Hasta Handayani Idrus)

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Availability of data and statement

The databases used to extract data from individual studies and the code associated with the table designs are not uploaded to public repositories. Data can be obtained by contacting the person in charge of the study.

Declaration of interests statement

The authors declare that there is no conflict of interest and informed consent (written and oral) was obtained from all the parents and participants.

Ethical policy and institutional review board statement

Ethical clearance from the Institutional Committee Universitas Muslim Indonesia (No.5934/A/KEPK-UMI/IX/2021) was obtained before the start of the study. Furthermore, national guidelines were followed in accordance with the Declaration of Helsinki

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study, data collection, analysis or interpretation, writing of the manuscript, as well as the decision to submit it for publication

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