Formulation Herbal Mouthwash Combination Extract of Ginger and Lemongrass as Antibacterial Causes of Halitosis in Diabetes Mellitus Patients

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Abstract

Background. Ginger and lemongrass are plants known as the king of rhizomes with a healthy stance because empirically many have used them as halitosis treatment in people with diabetes mellitus. The research objective was to formulate herbal mouthwash from extracts of natural ingredients, namely a combination of ginger and lemongrass which has the potential as antibacterial causes of bad breath (halitosis) in people with Diabetes Mellitus (DM). **Material and Methods**. The extraction of the sample will be carried out by a modified extraction method using ethanol and water solvents, then biological activity testing will be carried out in-vitro with the diffusion method so that the antibacterial activity test against several bacteria that cause halitosis will be tested. Antibacterial sample is an active compound resulting from the extraction process. Bacteria that had been inoculated into the growth medium (NB) were put into sterile soft NA media (0.7%) with a concentration of 10,000.8,000,7,500 and 6,000 ppm, respectively. **Results**. There is an inhibitory effect, on p. gingivalis but the pattern of values is uncertain. A solution of formulation A with a concentration of 75% occurs inhibition with an inhibition diameter of 15.7%. This inhibitory effect is not an activity of formulation A, because acetic acid also has the ability to inhibit bacteria. In the sample solution with a concentration of 100% chitosan (w / v), the highest inhibition occurred with an inhibition diameter of 18.7 mm / mg of the sample extract. Formulation A with a concentration of 25% (w / v), the lowest inhibition occurred. The test solution of formulation A with a concentration of 25% has shown an inhibitory effect on the growth of Streptococcus mutans. This effect is stronger at concentrations of 100%, 75%, and 50%. The antimicrobial effect actually increased with an increase in the concentration of the test solution in succession of 25%, 50%, 75%, 100%. Formulation C with a concentration of 10000ppm, 8000ppm, 7500ppm, 6000ppm showed that the antimicrobial effect actually increased with an increase in the concentration of the test solution in succession. This shows that there is a strong positive relationship between concentration and inhibition zone. **Conclusion**. All tests for both formulation A and formulation C using several concentrations showed quite good results with the antibacterial activity being directly proportional to the concentration, the greater the concentration the greater the activity.

***Keyword**: Antibacterial, Diabetes Mellitus, Halitosis, Herbal mouthwash*

Background

Assessment of health status can use several indicators that reflect the condition of mortality (death), nutritional status and morbidity (morbidity). The degree of public health in Maros Regency is illustrated through the Mortality Rate, Under-Five Mortality Rate, and Maternal Mortality Rate, Morbidity Rate (Morbidity Rate) such as several diseases including infectious diseases (pulmonary tuberculosis, dengue fever etc.), non-communicable diseases (hypertension, Diabetes Mellitus etc.), as well as oral dental diseases (gingivitis,

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periodontitis, halitosis, etc.).

Halitosis is a crippling social problem with a common complaint of up to one-third of the general population.[1] Halitosis is a lyrical term derived from the Latin word "halitus" (breath) and the Greek suffix "osis" (condition, action or pathological process). In simple words, it means "Bad Breath". It is also called as fetor ex ore or fetor oris.[2]

In most of the cases (90%), halitosis originates within the oral cavity. This is because the oral cavity harbours a large variety of microorganisms which include a large group of Gram-positive bacteria mainly *Streptococci* and a group of anerobic microorganisms such as *Porphyromonasgingivalis*, *Fusibacteriumnucleatum* and *Prevotella intermedius*. Among the latter, many are Gram-negative oral bacteria whose proteolytic activity is associated with oral malodour and periodontal disease.[3] Other bacteria associated with gingivitis and/or periodontitis (viz- *Actinobacillusactinomycetemcomitans*, *Campylobacter rectus*, *Peptostreptococcusmicros*, *Bacteroidsforsythus*, *Eubacterium* species and *Spirochetes*) are known to produce large amounts of volatile sulfur compounds (VSC) which are malodorous[4]

Word halitosis is a Latin word which is derived from halitus means breathed air, and the osis means pathologic alteration and it is used to describe any unpleasant or disagreeable bad odor emanating from the mouth breath.[5] Halitosis or bad breath is a disease caused by a lack of oral hygiene. Halitosis can cause harm not only to sufferers but also other people and can affect someone's social life such as shame, avoidance of social interactions and decreased self-confidence.[6]

Research by Ravindran et al. in India in 2015 showed that the number of patients with controlled diabetes mellitus had more halitosis (26.7%) than uncontrolled diabetes mellitus patients (20%). This occurs due to bacterial decomposition and the evaporation of Volatile Sulfur Compounds (VSC).[7] Research Kumaresan et al. in India in 2017, 149 patients with type 2 diabetes mellitus showed that only 57 subjects knew and were aware of halitosis as a result of type 2 diabetes mellitus, while most subjects did not realize that halitosis was a manifestation of the oral cavity due to diabetes mellitus. This shows the lack of public knowledge about the impact of systemic diseases on oral health.[8]

Research by Bisong et al. in Cameroon in 2015 shows that disease of the oral cavity is higher in people with diabetes mellitus compared with non diabetes mellitus, where hyperglycemia is suspected to be

factors that play a role in the emergence of disorders in the patient's oral cavity diabetes mellitus. This study revealed that plaque, calculus, xerostomia and halitosis was significantly higher in people with diabetes mellitus compared to non diabetes mellitus.[9]

Everyone certainly wants healthy teeth and avoid bad breath. They think that by brushing their teeth alone, bad breath can be eliminated, even though they need continuous treatment to get maximum results. Apart from regular brushing, other efforts are also needed. Bad breath faced or experienced by a person has many causes, not only from infections in the oral cavity, it can be from other sources that can cause bad breath including throat, lung and stomach disorders, and cadaulin (Diabetes mellitus sufferers), and consumption of certain drugs.

Diabetes mellitus is a chronic disease with hyperglycemia and glucose intolerance that occurs because the pancreas cannot produce insulin adequately or because the body cannot use insulin. This disease affects many Indonesians. Oral complications that commonly occur in people with type 2 diabetes mellitus include xerostomia, periodontal disease, caries and halitosis. Halitosis describes breath that is smelly or unpleasant.[10]

Oral and dental health is very important to maintain overall health. Good oral and dental health will improve our ability to speak, speak, smile better, taste food, chew, swallow, and can even improve our facial expressions when communicating.[11]Teeth are a very important part of us, because teeth are directly related to health and

appearance. Oral health is often neglected by the public. They consider other health to be more important than oral health. In dental health problems, especially bad breath (halitosis). Many people experience a bad condition in their mouth (halitosis) but they lack awareness of the condition of their mouth. Everyone certainly wants healthy teeth and avoid bad breath. They think that by brushing their teeth alone, bad breath can be eliminated, even though they need continuous treatment to get maximum results. Apart from regular brushing, other efforts are also needed.

Word halitosis is a Latin word which is derived from halitus means breathed air, and the osis means pathologic alteration and it is used to describe any unpleasant or disagreeable bad odor emanating from the mouth breath. [12]The bad breath of diabetes mellitus is very typical like acetone. The substance comes from ketone limbs that can be secreted from the breath.

Aleman L F J et al conducted a study to determine the effectiveness and sustainability of three commercial mouthwashes against the halitosis and concluded that a decrease in VSC and organoleptic levels after use of mouthwashes for 1st and 3rd hours. Results obtained in that study indicate that mouth rinsing with essential oils, cetylpirydine chloride and triclosan represents a positive option for the treatment of halitosis.[13]

Halitosis treatment aims to improve the quality of life of people with diabetes mellitus due to prolonged disturbances in the oral cavity in the form of halitosis and other oral diseases. Herbal medicine is one of the treatment efforts and/or other methods of treatment outside of medical science and treatment science, traditional medicine needs to be nurtured, developed and supervised so that its benefits and safety can be accounted for.

A preliminary study was conducted at the Tompobulu Public Health Center in October-November 2019 regarding the effect of video method counseling, treatment in improving the quality of life of people with Diabetes Mellitus due to halitosis. The results showed that there was a relationship between counseling, treatment and quality of life for people with diabetes. In that study, provided treatment with a combination of using 0.5% chlorhexidine mouthwash with herbal mouthwash, in this case lemongrass and ginger.Based on the results of the treatment given in the form of 0.5% chlorhexidine mouthwash and it was found that some still experienced bad breath with the criteria that there was moderate mouth rinse (50%) after giving mouthwash to (20%), bad breath was very strong (10%) becomes (0%). Patients using lemongrass and ginger leaves experienced changes in bad breath criteria, namely moderate bad breath (40%) to (15%), strong breath (15%) to (5%). The research objective was to formulate a herbal mouthwash from a combination extract of ginger and lemongrass as an antibacterial cause of halitosis in DM sufferers.[14]

Material and Methods

Materials and tools

The main ingredients used are the formulations made in the Pharmaceutical Microbiology Laboratory of UMI. The bacterial cultures used in this study were Gram negative bacteria, namely P gingivalis and gram positive bacteria, namely Streptococusmutans. The agar media used were Nutrient Agar (NA), Vogel Johnson Agar (VJA), and Eosin Methylene Blue Agar (EMBA). The chemicals used were 70% alcohol, spirits, pH 4 and pH 7 buffers. The equipment used was a Kotterman-Germany brand Clean Bench (aseptic room equipped with a UV lamp), incubator cupboard, autoclave, hotplate, petri dish, analytical balance, Erlenmeyer, test tube, dropper pipette, volumetric pipette, loop needle, measuring cup, measuring flask, Bunsen lamp, and other supporting tools.

Research Treatment

The treatment applied to this test bacteria is the concentration of formulation A added. The concentration of formulation A (100 gr ginger, 100 gr lemongrass, 100 clove seeds in 100 ml water) used was 100%, 75%, 50%, 25%. For formulation C, it is 10000 ppm, 8000ppm, 7500ppm, 6000ppm with a concentration. Testing the antibacterial activity of the sample using the

agar diffusion method. [15]. The experiment was carried out with 3 replications. Observation data are presented in tabular form and then analyzed descriptively.

Preparation of Test Bacterial Culture

The test bacterial culture to be used is prepared by taking one bacterial loop from an NA agar slant, then inoculating it into 10 ml of sterile NB. Furthermore, it was vortexed to even out the bacteria in NB, then incubated at 37oC for 24 hours. After 24 hours, an inoculum is obtained which can be directly used for testing antibacterial activity.

Testing Antibacterial Activity with the Well Method

The well method (agar diffusion) is based on the ability of the tested antibacterial compound to produce the radius of the inhibition zone around the test well against the bacteria used as the tester. Testing the antibacterial activity of formulation A and formulation C was started by preparing the bacterial growth medium. Making the media begins with weighing the powder media and adding distilled water as directed on the packaging. Then, stirring while heated using a hot magnetic stirrer until the media solution is homogeneous which is marked by a clear color of the solution, then Erlenmeyer is covered with cotton and sterilized at 1210C for 15 minutes. After sterilization the media is cooled closed at room temperature until the temperature reaches 400C then 0.1% test bacteria are inoculated into each growth medium (0.1 mL of culture in NB into 100 mL of growth media) then homogenized. The growth medium used for each of the test bacteria. The media that has been inoculated with the tested bacterial culture is poured into a dish and allowed to freeze. Then five holes (wells) were made aseptically with a diameter of 7 mm and put a sample solution of 60 µL containing the added formulation A. The concentration of formulation A used was 100%, 75%, 50%, 25%. For formulation C it is 10000 ppm, 8000ppm, 7500ppm, 6000ppm. The sample solution was prepared by dissolving the sample according to the concentration in a 1% acetic acid solution. Incubation was carried out statically at 37oC for 48 hours.

Inhibition Zone Calculation

The zone of inhibition of chitosan antibacterial compounds was measured based on the radius (rp, mm) of inhibition in the form of a clear area around the test well. Measuring radius (rp, mm) is done by measuring the distance from the edge of the test well to the boundary of the zone of the inhibition zone using a caliper (accuracy of 0.05 mm) on several sides of the test well, then averaged. The value of the diameter (d, mm) of the inhibition zone as a result of direct observation is obtained using the formula d = 2 x rp.

Results

Table 1. The results of the antibacterial activity test of formulation A and formulation C with several concentrations

Consentrations	Antibacterial Activity Test						Information
	P. gingivalis			Streptococcus mutans			
	R1	R2	R3	R1	R2	R3	
100%	19	17	20	18	20	19	Formulation A 100g geger, 100g Lemongrass, 100 seeds of cloves at 100ml
75%	16	14	17	16	17	16	
50%	14	12	15	14	14	14	
25%	12	10	12	12	12	13	

Cont... **Table 1. The results of the antibacterial activity test of formulation A and formulation C with several concentrations**

10000 ppm	10	12	11	10	13	12	Formulation C ppm
8000 ppm	9	10	10	9	11	9	
75000 ppm	8	9	9	7	10	8	
6000 ppm	7	8	8	6	9	7	

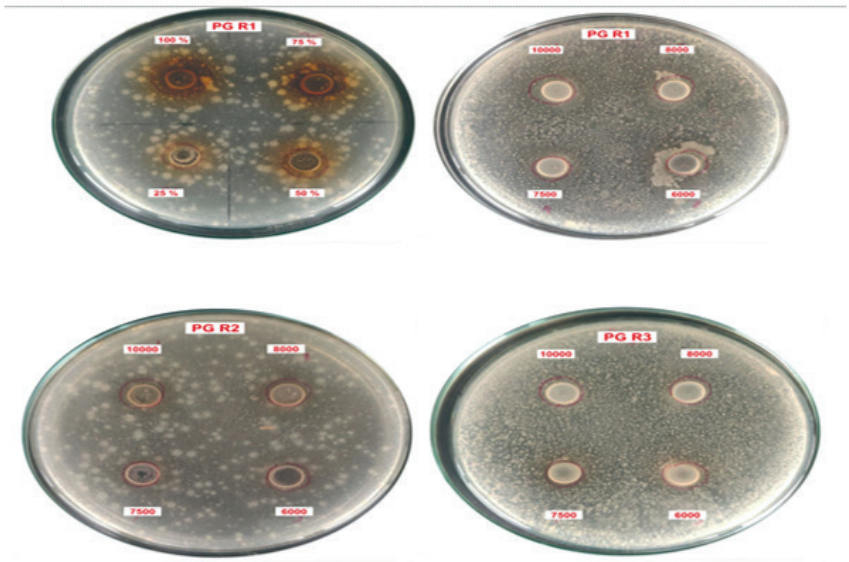

Figure 1. Antibacterial activity test results p. gingivalis (PG) with concentration (w / v) and ppm

Figure 2.The results of the antibacterial activity test for Streptococcus mutans (SAMA) with a concentration of% (w / v)

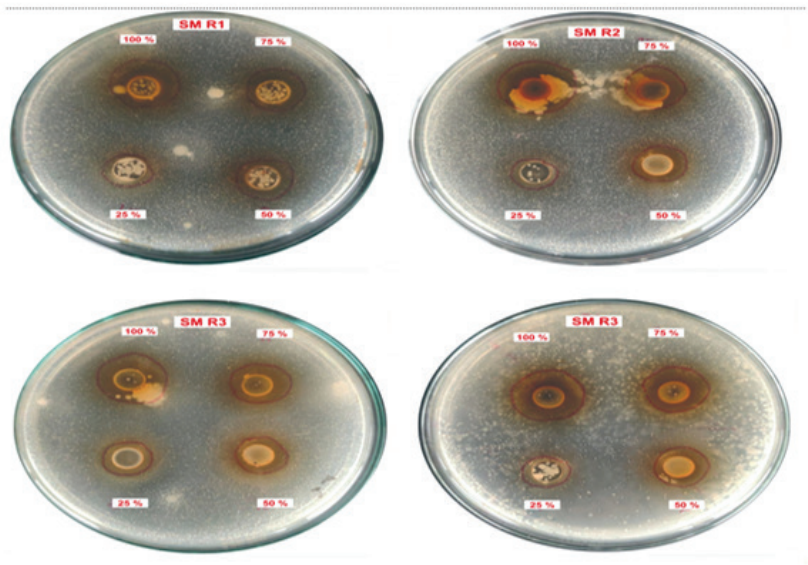

Figure 3. The results of the antibacterial activity test for Streptococcus mutans (SAMA) with a concentration of ppm

Discussion

The extract was obtained through an extraction process using 95% ethanol as a solvent. Extraction using ethanol as a solvent was chosen in order to obtain a polar active ingredient. 95% ethanol was chosen as a solvent or extractor because through a series of initial trials it was found that ethanol showed the best effect on the withdrawal of the active ingredient contained in male mayana leaves. The extract obtained is then filtered with filter paper and then evaporated under vacuum pressure, the results obtained have the physical properties of the extract, namely in the form of a very thick precipitate, which indicates that the viscosity level is very high.

Ethanol was used as the solvent because it has two groups with different polarity which are hydroxyl group that is polar and alkyl group that is non-polar, so by the existence of these groups it is expected that the compounds with different polarity levels will be extracted into the ethanol.[15]

The results of the effect test on the growth activity of Gram negative, namely P gingivalis and Gram positive bacteria, namely Streptococusmutans. In observations after 24 hours of incubation with 3 repetitions. The test results of male mayana leaf polar extract against the growth activity of Streptococusmutans and P gingivalis. has a positive value. This is indicated by the presence of a bright area around the culture hole in each of the tested bacteria. There was also inhibition against the comparative antibiotic.

In the table, it can be seen that the sample gives an inhibitory effect, on p. gingivalis but the pattern of values is uncertain. In a solution formulation A with a concentration of 75%, there was an inhibition with an inhibition diameter of 15.7%. This can occur because of the presence of acetic acid in the solution as a solvent. This inhibitory effect is not an activity of formulation A, because acetic acid also has the ability to inhibit bacteria. In the sample solution with a concentration of 100% chitosan (w/v), the highest inhibition occurred with an inhibition diameter of 18.7 mm / mg of the sample extract. This is presumably because the viscosity of formulation A is still low so that it can still diffuse into the medium so that the place where it grows p. gingivalis. In formulation A with a concentration of 25% (w/v), the lowest inhibition occurred.

The modified well method from the Kirby Bauer method was used because this method is more suitable and practical for drug testing than the diffusion method using a disc. Whereas with discs, the volume of the test solution is very limited. The volume of samples that is more than 20 µl per disc has resulted in interference of observation and measurement. The use of distilled water as a negative control is absolutely necessary to rule out the possibility of an antimicrobial effect. In the observations, it turns out that for all wells with distilled water, none of them provide an inhibition zone.

Observations on the Streptococusmutans test bacteria (table 1), showed that distilled water as a negative control did not have an inhibitory power against the growth of Streptococcus mutans, which was indicated by the absence of an inhibition zone in the area around the well containing the distilled water. The 50 µg/50 µl ciprofloxacin comparison solution had a large zone of inhibition against the growth of Streptococcus mutans. In Table 1, the test solution of formulation A with a concentration of 25% has shown an inhibitory effect on the growth of Streptococcus mutans. This effect was stronger at concentrations of 100%, 75%, and 50% (Table 1). The antimicrobial effect actually increased with an increase in the concentration of the test solution in succession of 25%, 50%, 75%, 100%. This shows that there is a strong positive relationship between concentration and inhibition zone. This relationship can be seen in Figure 2. This means that the extract solution with the ethanol extractor has an antimicrobial effect against Streptococcus mutans.

The results of further observations using formulation C with a concentration of 10000ppm, 8000ppm, 7500ppm, 6000ppm showed that the antimicrobial effect was actually increasing with an increase in the concentration of the test solution in succession. This shows that there is a strong positive relationship between

concentration and inhibition zone. This relationship can be seen in Figure 2. This means that the extract solution of formulation C with the ethanol extractor has an antimicrobial effect on p. gingivaris and Streptococcus mutans. From the data of all the tables above, it can be shown that the order of the strength of the antimicrobial activity of the laurtan test concentrations of 10000ppm, 8000ppm, 7500ppm, 6000ppm.

Even though it has antibacterial ability, it does not mean that male mayana leaf extract is called an antibiotic substance because there is no resistance standard and an assessment of bacterial sensitivity. The ratio of the size of the light zone formed in the extract solution was smaller than that of ciprofloxacin as a positive control. The antibacterial abilities contained in formulations A and C are not only limited to the two tested bacteria used in this study but may still have antimicrobial abilities against other bacteria.The antioxidant activity of astaxanthin is stronger than other carotenoids in reducing free radical activity as a trigger the emergence of such degenerative disease cancer, heart disease, and diabetes mellitus.[16] One of the herbal medicines that have antioxidants is ginger, lemongrass and cloves.

This research is in line with research conducted by Nivetha R at.al that the Chinese used cloves more than 2000 years ago. to get rid of bad breath. The synergistic effect of clove oil along with other coriander oils, anise, coriander, and cilantro eucalyptus shows a higher level of inhibition in Gram-negative bacteria, thus proving that synergism worsens the antimicrobial activity of clove oil. Clove

The oil can be a shortterm remedy for halitosis because it is antimicrobial, but cannot be used long term because it is lacking probiotic activity.[17]Halitosis is not a disease but an inconvenience, probiotics are marketed for the treatment of oral and intestinal halitosis. Few clinical studies have proven different strains or probiotic products potent.[18]

Studies have shown an increasing number of type 2 diabetes (T2D) patients with concomitant obesity and hyperlipidemia syndromes, caused by relevant metabolic disorders. However, there are several drugs and therapies that can solve this problem completely. Research conducted by Xiaotong Yu at.all stated that herbal formula JTTZ menghasilkanpeningkatan yang aman dan signifikan pada glukosadarah, lipid darah, dan tingkatberatbadan; gejalalega; danmeningkatkanf ungsisel β untukpasien T2D denganobesitas dan hiperlipidemia. Herbal fomula JTTZ telahmenunjuk kanbahwaiaberpotensidikembangkanse bagaipengobatanalternatifuntukpasien T2D, terutamamereka yang tidakdapatmentolerir metformin atauobathipoglikemiklainnya.[19]

Conclusion

Based on the results of the antibacterial activity test, it can be said that all tests for both formulation A and formulation C using several concentrations showed quite good results with the antibacterial activity being directly proportional to the concentration, the greater the concentration the greater the activity. It should be given the opportunity to continue this research in testing and exploring herbal plants using other bacteria

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Ethical Considerations: Ethical clearance was obtained from Universitas Muslim Indonesia; with number" 579/A/KEPK- UMI/V/2020. Just before the interview, written (or thumb impression) consent was obtained from each participant in Universitas Muslim Indonesia guidelines.

Conflicts of Interest: The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

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