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Research Article

Effectiveness of Citronella Extract (*Cymbopogon nardus*) Mouthwash From Microwave-Assisted Extraction Method Against *Streptococcus mutans*

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ABSTRACT

Cymbopogon nardus, or citronella has a distinctive aroma is known as a source of essential oils. Extract of citronella can be use as mouthwash to reduce bad breath and protect teeth from bacteria that cause dental caries. *C. nardus* contains antibacterial compounds such as flavonoid, essential oils, polyphenols, and saponin which are also act as antioxidant compounds. This research was carried out to formulate mouthwash using citronella extract and determine the antibacterial activity of the citronella mouthwash against *S. mutans* bacteria using disc diffusion method. The microwave extraction method (MAE) was used to obtain extract using 70 % and 96 % ethanol as a solvents. The mouthwash were made in 3 formulas i.e. formula 1, formula 2, and formula 3 each contain 25 % w/v, 30 % w/v, and 35 % w/v) respectively. The quality of mouthwash includes organoleptic, pH, viscosity and hedonic was evaluated and antibacterial activity was determined by measuring the value of minimum inhibitory concentration (MIC) and diameter of inhibition zone. The MIC value of citronella extract was achieved at concentration 20 %. All mouthwash formula met the requirements for a good mouthwash. Formula 1 was the most preferred formula by the panelists based on the hedonic test. Formula 3 had the strongest antibacterial activity with diameter inhibitory of 17.6 ± 0.52 mm. It was concluded that citronella mothwash has the potential to be developed into an antibacterial mouthwash.

Keywords: Citronella; *Cymbopogon nardus*; *MAE*; Mouthwash; *Streptococcus mutans*

INTRODUCTION

Almost half of the world's population is affected by dental caries, making it the most prevalent of all health conditions. High levels of dental caries occur in middle-income countries, where sugars consumption is high. Dental caries is a resulting from infectious of cariogenic bacteria *Streptococcus mutans* which metabolize sugars to produce acid which demineralizing dental enamel and destroy tooth structure over time. (Stenudd et al., 2001). Various *Lactobacilli* also were associated with progression of the lesion This condition involves the degradation of the enamel, dentin, and cementum of teeth (hard tissues) through lactic acid production (Rathee and Sapra, 2023). In addition to caries, *S. mutans* is responsible for cases of infective endocarditis with a subset of strains being indirectly implicated with the onset of additional extraoral pathologies (Lemos et al., 2019). Gargling with mouthwash can remove microbes and food debris between the teeth that cannot be reached by a toothbrush (Rasmah et al., 2016). Mouthwash can be used as a therapeutic and cosmetic agent to treat plaque, gingivitis, dental caries and stomatis (Justicia et al., 2017).

Cymbopogon nardus known as Citronella contain bioactive compounds such as flavonoids, essential oils, polyphenols and saponins which show antimicrobial properties (Bassolé et al., 2011). Flavonoids fight bacteria by building complex compounds with extracellular proteins, which can

damage the cell membrane and damage the bacterial cell (Reveny, 2011). Saponins were proven to have a good effect in inhibiting gram-positive bacteria (Astuti, 2013). Ethanol extract of *C. nardus* more optimally inhibits *S. mutans* bacteria at a concentration of 20 % with an average inhibitory diameter of 12.00 ± 0.23 mm (Hasibuan, 2021).

Microwave assisted extraction (MAE) method is a technique for extracting bioactive compounds from plant sample using the power of microwave energy to heat solvent in contact to the sample. The reaction to form esters and glycerol from triglycerin with bio-alcohol can be carried out effectively and efficiently with this method, this is because the phenomena of ionic conduction and dipole rotation cause an increase in heat and selective towards polar molecules (Shakinaz et al., 2010). MAE method plays an important role in extracting flavonoids as well as other secondary metabolite compounds such as anthocyanins, polyphenols and flavonoids (Routray, 2012), using different solvents can extract phenolic compounds such as flavonoids more effectively with higher quality than other conventional methods (Alara et al., 2018). The aim of this study was to determine the best mouthwash formulated from 70 % and 96 % ethanol extract of citronella using MAE as extraction method and to determine the effectiveness of the citronella mouthwash against caries caused bacteria *S. mutans*.

METHODS

Equipment

Tools used: beaker glass (Pyrex®), mortar, grinder (Philip®), funnel, erlenmeyer, dropper pipette, petri dish (Pyrex®), steam cup, filter paper, match, autoclave, Microwave Assisted Extraxtion (MAE) (Samsung ®), furnace (DAIHAN Scientific Furnace), sieve, bunsen, ose, digital scale, porcelain level, water bath (Lab PRO®, KERN®), wooden clamp, oven (Memmert®), furnace, vacuum dry (Lab PRO®, KERN®), pH stick, Brookfield viscometer, personal protective equipment.

Equipment

Materials used: distilled water, aluminum foil, *S. mutans* bacteria, *C. nardus*, 70 % and 96 % ethanol, glycerin, cotton, parchment paper, Whatman paper, filter paper, Muller Hinton Agar (MHA) media, menthol, NaCl 0.9 %, sodium benzoate, propylene glycol, spirtus and other supporting ingredients.

Extraction Procedure

About 30 g of citronella dry powder were soaked in 300 mL of 70 % and 96 % ethanol were put in microwave apparatus. The extraction process was carried out at 800 watts of electrical power for 6 minutes (1 minute of radiation on and 2 minutes off) to maintain the solvent temperature below 80 ºC (Quan et al., 2006). The filtrate was evaporated using a rotary evaporator at 60 ºC for 2 hours to obtain thick extract. The thick extract is then weighed and stored in a closed container for further use.

Water Content Test

About 2 g of dry powder, 70 % and 96 % ethanol extracts of citronella were dried using oven at 105 ºC for five hours and weighed successively one hour apart until the weight were constant (DepKes, 2000).

Ash Content Test

About 2 g of dry powder, 96 % ethanol extract on crucibles were placed in the muffle furnace at temperature of 550 ºC for 2 hours. The crucibles were removed from the furnace carefully and cooled in a desiccator to room temperature and weight again. The total ash content is calculated for materials that have been dried in air. Requirements for total ash content is ≤ 16.6 % (DepKes, 2008).

Phytochemical Test

The dry powder, 70 % and 96 % ethanol extracts of citronella were subjected to phytochemical tests including alkaloids, flavonoids, tannins and saponins tests qualitatively according to Hanani method (Hanani, 2015).

Alkaloid Test

The presence of alkaloid groups was detected using Dragendroff and Meyer reagents. About 2 mL of dry powder and citronella extract was warmed with 2 % H_2SO_4. Few drops of Dragendroff's reagent were added. Orange-red precipitate indicated the presence of alkaloids. About 2 mL of citronella extract was added with 1 mL of Meyer's reagent. The presence of pale-yellow precipitate indicated the presence of alkaloids.

Flavonoid Test

About 0.5 g of dry powder and citronella extract were dissolved in 5 mL of 95 % ethanol. About 2 mL of solution was taken then added with 0.1 g of Mg powder, and 10 drops of HCl. Formation of a red precipitate indicate the presence of Flavonoids group meanwhile the formation of orange-yellow color indicates the presence of chalcone and aurone groups.

Tannin Test

Each of 2 g of dry powder and citronella extract was dissolved in 30 mL ethanol. About 2 mL of solution were added with to a few drops of 10 % $FeCl_3$ were added. The presence of gallic tannin was indicated by formation blue color and the presence of catechol tannins was indicated by the presence green solution.

Saponin Test

Each of 2 g dry powder and citronella extract was boiled in 20 mL of distilled water. About 5 mL of solution was taken and added with a drop of Na_2CO_3. After vigorous shaking, it was left to rest for five minutes. Formation of stable foam indicated the presence of saponins group.

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) the lowest concentration of an antimicrobial compound that inhibit the visible growth of a microorganism after overnight incubation. The MIC of citronella extract was using solid dilution methods by spreading the suspension of *S. mutans* on sterile Muller Hinton agar (MHA) plate containing different concentration of citronella extract (10, 15, 20 %). The plate then incubated for 24 hours at 35 0C. MIC values were achieved on the agar surface which did not show the growth of *S. mutans*.

Mouthwash Formulation

The mouthwash formulation was made based previous research (Tampoliu et al., 2021), which proved stable with MIC value at 35% and diameter of inhibition zone at 14.20 mm. The formulation of citronella mouthwash is shown in Table 1.

Evaluation of Citronella Mouthwash

Organoleptic Test

Organoleptic examination includes shape, color and smell, which are observed using the five senses (Noval et al., 2020).

pH Test

The pH meter was calibrated, the citronella mouthwash is placed in a measuring cup, the pH meter is dipped into the mouthwash liquid until the mark is reached, then the pH value is read (Noval et al., 2020).

Viscosity Test

The viscosity test is carried out by placing 100 mL of mouthwash into a tube-shaped container, then spindle 64 was submerged into citronella mouthwash. The viscometer is turned on at speed 60 rpm until the viscosity value is recorded (Noval et al., 2020).

Hedonic Test

The hedonic test was carried out on 20 student panelists aged 19-23 who were physically and mentally health. Panelists were assigned to use all citronella mouthwash formulas and rated the color, aroma and taste of the citronella mouthwash on a scale: like very much (5), like (4), quite like (3), dislike (2), and dislike very much (1).

Antibacterial Activity Test of Citronella Mouthwash

The antibacterial activity of the citronella extract mouthwash was carried out using disc diffusion method. Sterile paper discs (6 mm) were soaked in each formula of citronella extract mouthwash for 30 minutes then dry. The procedure were repeated to ensure the paper disc is completely saturated. A suspension of bacteria was prepared in NaCl solution at the turbidity equal to 0.5 Mc-Farland standard. A drop of bacterial suspension (0,2 mL) was spread thoroughly on sterile MHA surface. The prepared paper disc were put on the sterile MHA surface that had been planted with S. mutans and for 24 hours at 35 °C. Antibacterial activity was determined by measuring the diameter of the inhibition zone or the clear zone formed around the paper disc using a caliper. Formula 1 (mouthwash without citronella extract) was used as negative control and commercial antibacterial mouthwash was used as positive control used comparative commercial products.

Data Analysis

Data obtained from calculation of diameter of inhibition zone various extract concentrations was exported to SPSS for further analysis using one-way analysis of variance (ANOVA). Each data was taken with 3 replications. P value < 0.05 was considered as significant. SPSS 15.0 was employed for statistical analysis.

Table 1. Formulation of mouthwash containing citronella extracts

Material Name	Utility	Mouthwash (%)				
		F1	F2	F3	F4	F5
Citronella Stem Extract	Antibacterial	0	25	30	35	Commercial mouthwash
Glycerin	Humectant	15	15	15	15	-
Propylene Glycol	Stabilizer	10	10	10	10	-
Na saccharine	Sweetener	0.10	0.10	0.10	0.10	-
Menthol	Taste	0.25	0.25	0.25	0.25	-
Sodium benzoate	Preservative	0.20	0.20	0.20	0.20	-
Aquadest	Solvent	100	100	100	100	

Important note: *Each formula was prepared in 3 replicates. Subsequent tests including hedonic, physical quality, and antibacterial activity tests were carried out using 3 replicate of each formula.*

Table 2. Water and Ash Content of Dry Powder and Thick Extract of Citronella

Test parameter	Method	Material	Solvent Concentration (%)	Results %
Water content	gravimetry	dry powder	-	4.3851
		thick extract	70	4.3706
			96	4.4791
Ash content	gravimetry	dry powder	-	3.8179
		Thick extract	70	4.4297
			96	3.5477

RESULT AND DISCUSSION

Water and Ash Content of Citronella

The citronella dry powder (1268 g) obtained from fresh raw material (8000 g).was 15.85 %. The water and ash content of citronella dry powder and thick extract using gravimetric methods was shown in Table 2. These results meet the standard establish in Farmakope Herbal Indonesia (DepKes RI, 2017) that are no more than 10 %. The water content of plant extract affects its quality and storage time. High water content can accelerate the growth of microorganisms and material deterioration. Ash content refers to the minerals and inorganic substances left after heating to extremely high temperatures to remove moisture, volatiles, and all organic materials. Ash content can affect different characteristics of extracts including physical and chemical properties. Data on ash content ensures are no toxic minerals present in plant extracts.

Phytochemical Test Results

Phytochemical test was carried out to identify the secondary metabolite content found in dry powder and thick extract of Citronella. The presence of certain metabolite in dry sample and thick extract of citronella was characterized by change in color, the formation of precipitates, and foam forming caused by the reaction between the compounds in the sample and the reagent used. The results confirmed that dry powder and thick extract of citronella contained metabolite groups i.e alkaloids, tannins and flavonoids but no saponin was detected. These results are in line with previous research conducted by Alfiyanti et al., (2023) which confirm that there was no saponin found in dry powder and thick extract of citronella. Environmental conditions such as seasonal changes, temperature, pH mineral composition of soil are known to affect the phytochemical composition of medicinal plants. Previous studies have shown that flavonoids isolated from *C. nardus* extract many biological activities such as antibacterial (Zhao et al., 2024). The results of phytochemical test is display in Table 3.

Minimum Inhibitory Concentration of Citronella Extract

The value of minimum inhibitory concentration was determined using solid dilution methods to find out the lowest concentration of extract that inhibit bacterial growth. The MHA plate used to determine the MIC value of 70 % ethanol extract and 96 % ethanol extract of citronella against *S. mutans* bacteria can be seen in Figure 1 and Figure 2. The concentrations of extract used for test were 70 % and 96 % ethanol extract of *C. nardus* stems used were 10, 15, and 20 %.

It can be seen in Figure 1a that at the concentration of 10 % and 15 %, the 70 % ethanol extract of citronella has no antibacterial activity against *S. mutans*. MHA plate looks clean without bacterial colonies achieved at a concentration of 20 %. It was concluded that the MIC value of 70 % ethanol extract of Citronella is 20 %.

Table 3. Phytochemical Test of Dry Powder and Thick Extract of Thick Extract

Type of Test	Dry powder	70 % Ethanol Extract	96 % Ethanol Extract
1. Alkaloid			
- Mayer	+	+	+
- Bouchardad	+	+	+
- Dragendorft	+	+	+
2. flavonoids	+	+	+
3. Saponins	-	-	-
4.Tannin	+	+	+

Note :

(+) : Indicate the presence of the test compound

(-) : Indicate the absence of the test compound

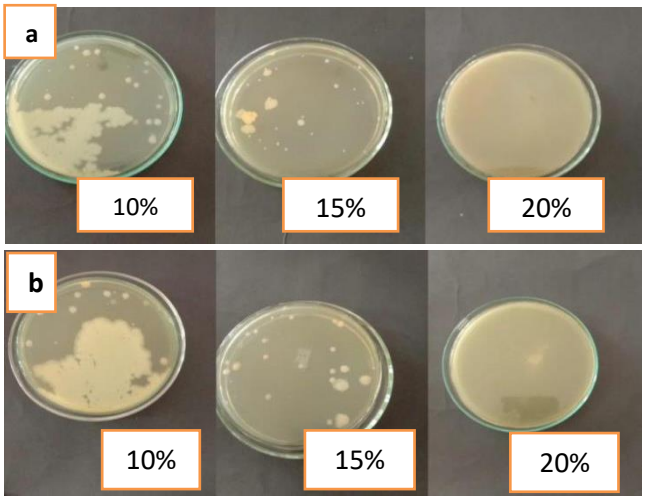

Figure 1. MIC test ethanol extract of Citronella against *S. mutans* bacteria with A. 70% ethanol and B. 96% ethanol

In Figure 1b, it can be seen that ethanol 96 % extract of citronella at a concentration from 10 % up to 20 % did not hane activity in inhibiting the growth of *S. mutans*. The MIC value of 96 % ethanol extract was achieved at concentrations above 20 %. According to Fan et al. (2020) increasing ethanol concentration can increase the dissolution and extraction rate. When the ethanol concentration is greater than 70 % the extraction rate of the target component decreases slightly, possibly because protein denaturation increases diffusion resistance at higher ethanol concentrations. It can be concluded that 70 % ethanol produces a better citronella extract than 96 % ethanol. The 70 % ethanol extract further will be used to formulate a mouthwash preparation.

Evaluation of Mouthwash Formula

Organoleptic Test

The mouthwash formulated from citronella extract was presented in Figure 3. All formula of citronella mouthwash possesses similar physical characteristics i.e brown color and strong citronella aroma. The evaluation of citronella organoleptic tests can be seen in Table 4. As a basic, formula F0 has no brown color. Formula F0 no citronella extract was added to the formula meanwhile F1, F2, and F3 contains gradually increasing concentration of citronella extract. Increasing concentration of citronella causing the color and aroma to become more intense from F1 to F3.

Evaluation of pH of Citronella Mouthwash

Evaluation of pH was carried out using a pH meter. The degree of acidity (pH) is an important parameter in mouthwash preparations. The pH value of citronella mouthwash form F1 to F3 was displayed Table 5.

The pH value of citronella mouthwash was ranged from 5 to 6. These values meet the pH of quality standard of herbal mouthwash 7 set by ministry of health ranges from 5. The rule of pH of the mouthwash should not be too acidic and should not be too alkaline. If the pH of the mouthwash is too acidic it can cause irritation to the oral cavity and if the pH of the mouthwash is too alkaline can cause canker sores (Tampoliu, 2021).

Viscosity Evaluation of Mouthwash Formula

The mouthwash viscosity test was determined using a Brookfield Viscometer with spindle number 2 at a speed of 100 rpm. This test aims to determine the viscosity of the mouthwash preparation. The viscosity of citronella mouthwash can be seen in Table 6. The viscosity level of pure water is 1 mPa.s or around ± 1 cP, while the standard viscosity of mouthwash on the market is ± 7.25 (Rowe et al., 2009). These viscosity of citronella mouthwash F1 to F3 was meet the standard of Indonesia Ministry of Health.

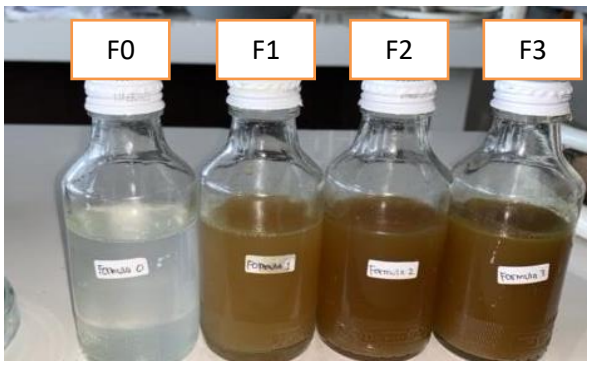

Figure 2. Mouthwash of Ethanol 70 % Extract of *C. nardus* with F0 = Basis, F1 = Mouthwash contain 25 % citronella extract, F2 = Mouthwash contain 30 % citronella extract, F3 = Mouthwash contain 35 % citronella extract

Table 4. Organoleptic Evaluation of Citronella Extract Mouthwash

Test Parameter	F0	F1	F2	F3	Comparative Preparations
Color	Clear	Light Brown	Brown	Dark Brown	Brown
Texture	Liquid	Liquid	Liquid	Liquid	Liquid
Aroma	Odorless	Mild aroma	Strong aroma	Strong aroma	Strong

Table 5. Evaluation of pH of Citronella Mouthwash

Sample	Extract Concentration (%)	pH value	Ordinance for pH	Note
Formula 0	-	6.00 ± 0.1		Qualify
Formula 1	25	5.091 ± 0.1	5-7	Qualify
Formula 2	30	5.089 ± 0.1	(BPOM, 2015)	Qualify
Formula 3	35	5.086 ± 0.1		Qualify

Table 6. The Viscosity of Citronella Extract Mouthwash

Sample	Extract Concentration (%)	Viscosity (cPs)	Ordinance	Note
Formula 0	-	0.99 ± 0.1		Qualify
Formula 1	25	1.14 ± 0.1	± 1cP	Qualify
Formula 2	30	1.16 ± 0.1	(BPOM, 2015)	Qualify
Formula 3	35	1.12 ± 0.1		Qualify

Table 7. Hedonic Evaluation of Citronella Mouthwash

Sample	Parameter		
	Color	Aroma	Flavor
Formula 1	2.85 ± 0.1^{b}	2.60 ± 0.2^{a}	2.65 ± 0.1^{c}
Formula 2	2.65 ± 0.1^{b}	3.50 ± 0.2^{b}	1.65 ± 0.1^{b}
Formula 3	1.50 ± 0.1^{a}	4.50 ± 0.2^{c}	1.20 ± 0.1^{a}

Note: *The same superscript in the same column indicates no significant difference between subject based on Duncan test at a α level of 0.05.*

Hedonic Evaluation of Citronella Mouthwash

Hedonic tests are designed to measure the degree of liking for a product. Panelists indicate their degree of liking for each sample by choosing the appropriate category. The level of panelist acceptance for each sample is marked by choosing the number of category as describe in the method. The results of the hedonic test obtained from 20 panelist can be seen in Table 7.

The results of the hedonic test show that each parameter has a significantly different rating (p 0.000 ≤

0.05). Based on color parameters, Formula 1 is most preferred by panelists with a value of 2.85 which is significantly different from formula 2 and formula 3. For the aroma parameter, formula 3 has the highest rate with a value of 4.50, although it is not significantly different from formula 2. For taste parameter, Formula 1 has the highest rate with the value of 2.65, which is significantly different from Formula 2. Formula 1 was chosen as the most preferred mouthwash by the panelists because the the most preferred formula because it has a brighter color and softer in aroma and taste.

Inhibition zone of Mouthwash

Antibacterial activity of citronella mouthwash was determined using disc diffusion method by measuring the diameter of inhibition zone. The diameter of inhibition zone of citronella mouthwash at concentration of 25 % (F1), 30 % (F2), f 35 % (F3) was presented in Figure 4 and Table 8. The lowest concentration of citronella extract used in the mouthwash formula (25 %) was set refers to value of MIC obtained previously (20 %). Basic mouthwash without citronella extract was used as negative control and commercial antibacterial mouthwash was used as positive control.

The Figure 4 show that each mouthwash formula has antibacterial activity against *S. mutans* bacteria. This is characterized by the presence of clear zone or inhibitory zone of *C. nardus* stem extract against *S. mutans* bacteria. Increasing the concentration of extract in mouthwash preparations expands the inhibition zone formed on the MHA surface. This pattern indicates that secondary metabolite This pattern shows that the compounds contained in citronella extract such as flavonoids, tannins, phenols and alkaloids (Wijayanti and Rosyid, 2015) play an important role in the antibacterial properties of mouthwash.

The antibacterial test confirmed that all citronella mouthwash formula possess positive activity against *S. mutant*. Citronella mouthwash F1 produces an inhibition zone of 12,6 mm, which fall in the moderate category, citronella mouthwash F2 produces an inhibition zone of 15,6 mm, which fall in the moderate category and citronella mouthwash F3 produces an inhibition zone of 17,8 mm, which also fall in the moderate category. The categorization of inhibition zone was referring to Kyrbi-Bauer Disk Diffusion Susceptibility Protocol (Hudzicki, 2009), where the zone of inhibition is divide into several categories i.e <14 mm for weak category, 15-20 mm for moderate category, and >20 mm for strong category. Based on Duncan's further tests, all formulas had a different effect on the results of the inhibition test, which means that each sample had a different antibacterial activity. The highest diameter was achieved in formula 3 because this formula contains the highest levels of citronella extract. The positive control shows strong antibacterial activity because this commercial product contains a mixture of active ingredients in the form of tea tree oil, siwak (Salvadora indica) and sea salt which scientifically tested are able to reduce bacterial growth. Formula 3 has an inhibition zone value close to the positive control inhibition zone value. This shows that citronella mouthwash has the potential to be developed as an antibacterial mouthwash against *S. mutans*.

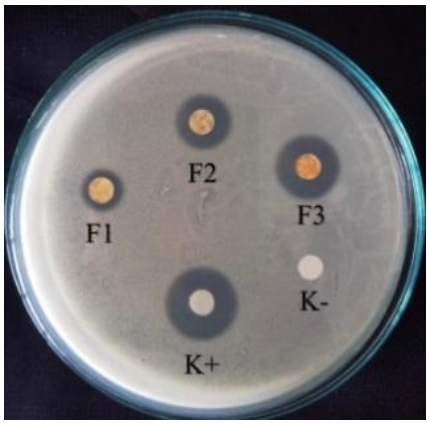

Figure 3. Inhibitory zone of Citronella Mouthwash Formula Against *S.mutans*

Table 8. Diameter of Inhibition Zone of Citronella Extract Mouthwash

Sample	Extract Concentration	Inhibition Zone (mm)	Category
Basis (F0)	K-	0	None
Formula 1	25 %	12.6 ± 0.55^{b}	Moderate
Formula 2	30 %	15.8 ± 1.37^{c}	Moderate
Formula 3	35 %	17.6 ± 0.52^{d}	Moderate
Comparison (F4)	K+	20.32 ± 1.21^{e}	Strong

Note: *Different superscripts in same columns indicate a significant difference between sample based on Duncan test at the α level of 0.05.*

CONCLUSIONS

Three mouthwash formulas have been successfully made from *Cymbopogon nardus* (citronella) extract using the MAE method. This preparation has moderate antibacterial activity against *Streptococcus mutans*. The highest activity is the F3 formula which provides an inhibition zone of 17.6 mm. Formula 3 has a MIC value of 20 % with 70 % ethanol solvent. Further research is needed to improve the quality of citronella mouthwash. In addition, it must be tested against other microorganisms.

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CONFLICT OF INTEREST

Author hereby declares that there is no conflict of interest in the writing and publication of this article.

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