Meditory
(The Journal of Medical Laboratory)
Volume 13 Issue 2 Page 205–216 December 2025
https://doi.org/10.33992/meditory.v13i2.4114

FORMULATION OF MORINGA AND EGGSHELL-BASED MOUTHWASH AS ANTIBACTERIAL AGAINST *Streptococcus mutans*

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Article History
Received, 7th July 2025
Revised, 9th July 2025
Reviewed, 29th October 2025
Posted, 25th December 2025

Editor
Jekmal Malau

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Keywords
Proteolytic enzyme, extracellular matrix, lymphovascular invasion, MMP-9

Abstract

Background: Oral health problems such as dental caries and periodontitis are common worldwide and are mainly caused by bacterial activity, particularly Streptococcus mutans. Many commercial mouthwashes contain alcohol and chemical additives, increasing the demand for effective and safer natural alternatives.

Objective: This study aimed to formulate a natural mouthwash containing Moringa oleifera leaf extract and calcium carbonate derived from eggshells, and to evaluate its antibacterial activity against Streptococcus mutans as well as its physical stability.

Methods: Moringa oleifera leaf extract was prepared using the maceration method, and eggshells were analyzed for calcium carbonate content. Mouthwash formulations were produced at concentrations of 5%, 10%, and 15%. Physical evaluations included organoleptic properties, viscosity, and pH. Antibacterial activity was assessed using the well diffusion method.

Results: Eggshell analysis showed a calcium carbonate content of 93.93%. All formulations met physical evaluation standards for oral care products. Antibacterial testing demonstrated inhibition zones of 6.15 mm and 7.21 mm for the 10% and 15% formulations, respectively, while no antibacterial activity was observed in the 5% formulation.

Conclusions: The combination of Moringa oleifera leaf extract and eggshell-derived calcium carbonate shows potential as a natural antibacterial mouthwash against Streptococcus mutans, particularly at higher concentrations, and fulfills physical evaluation criteria.

Cite this Article

Parmanantha PBMP, Gosa IPDD, Sari NLPEK. Formulation of Moringa and Eggshell-Based Mouthwash as Antibacterial Against Streptococcus mutans. Meditory J Med Lab. 2025;13(2):205-216

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INTRODUCTION

Good oral and dental hygiene are intimately related to maintaining general physical health. Dental caries, gingivitis, and periodontitis are among the conditions that can cause poor breath and other symptoms. Even while flossing and brushing twice a day are essential dental hygiene habits, they frequently fail to adequately clean disease-prone spaces between teeth. Therefore, more cleaning techniques are required to enhance oral care (1). According to the Global Burden of Disease Study 2016, periodontal disease is the eleventh most frequent health problem worldwide, and dental caries affects approximately half of the population, or 3.58 billion people. The Ministry of Health in Indonesia stated that the prevalence of periodontitis was 74.1%. Serious infections and tooth decay can be accelerated by this disease (2).

Dental caries is a common dental health issue that is caused by the bacteria Streptococcus mutans. This bacteria breaks down dental enamel by fermenting carbohydrates and generating acid (3). One way to avoid these dental problems is to use mouthwash, however a lot of commercial mouthwashes include alcohol, artificial sweeteners, and other dangerous substances. In order to reduce harmful health impacts, it is imperative that mouthwash formulations include natural chemicals. Several studies have been conducted on natural mouthwashes that can be used as a reference in formulating ingredients for more effective antibacterial properties. However, the use of moringa leaves as a base ingredient has never been studied. Secondary metabolites with antibacterial qualities found in Moringa leaves (Moringa oleifera L.) include flavonoids, terpenoids, tannins, and saponins. According to research, Moringa leaves have the ability to inhibit Streptococcus mutans, albeit the efficiency varies depending on the ethanol extract concentration (4).

Eggshells, composed of 94% calcium carbonate, can enhance dental care products by helping remove food particles and stains, supporting mineral synthesis for tooth structure, and providing antiseptic and antibacterial properties. Researchers aim to create a mouthwash that combines calcium carbonate from eggshells with Moringa oleifera leaf extract to effectively combat *Streptococcus mutans*, a key cause of tooth decay. This study presents a unique formulation that leverages the complementary benefits of these natural ingredients for antibacterial effects and remineralization, an approach that has not been previously explored.

MATERIALS AND METHODS

Ethanol Extraction of Moringa Leaves

Extraction is done by maceration. One of the extraction techniques that produces secondary metabolites by soaking is maceration. Easy and practical, this method is suitable for extracting compounds sensitive to heat because it does not require heating. A total of 1,500 grams of Moringa leaf powder is weighed carefully. Furthermore, 1,500 grams of Moringa leaf powder is soaked in 3.5 liters of 96% ethanol, stirred for 24 hours to be extracted by the maceration method, then filtered until the filtrate is obtained, which is then re-macerated at least three times. The separated filtrate is then concentrated using a rotary evaporator between 50 and 60 degrees Celsius. Then the extract is heated in a water bath at a temperature of 60 degrees Celsius until a thick extract is obtained.

Egg Shell Analysis

Analysis of eggshell composition method using three key techniques: EDTA titration for quantifying calcium carbonate (CaCO3) levels, proximate analysis to determine moisture, ash, and protein content, and X-ray fluorescence (XRF) for assessing elemental concentrations. First, cleaned and dried eggshells are ground into a fine powder, then subjected to EDTA titration to measure calcium levels. Proximate analysis involves drying a sample to measure moisture content, ashing it to find ash content, and using the Kjeldahl method for protein estimation. Finally, XRF analysis measures the elemental composition.

Evaluation of Mouthwash Formulation

In the first step of mouthwash production, the water-soluble elements form the first phase (water phase). Once these ingredients form Indonesia foodgrades—sodium benzoatec (koepoe-koepoe), sorbitol (Tropicana slim), tween 80, and glycerin—have been combined in a mortar, the water-insoluble constituents like peppermint oil, Eggshells, and Moringa leaf extract are introduced. The two phases are mixed and blended until smooth, distilled water is introduced, then filtered and housed in a transparent container. Using negative and positive controls with commercial items free from alcohol, (7) researched this mouthwash composition comprising a range of concentrations (5%, 10%, 15%)(7,8).

Organoleptic Test

Evaluating mouthwash preparations includes checking their shape, color, taste, and smell at room temperature. This careful inspection helps determine the quality and attractiveness of the mouthwash, making sure it has the right characteristics for effective use.

Viscosity Test

Viscosity of the mouthwash composition was measured using an Ostwald viscometer. Up to 5 mL of preparation size was seen in the sample. The viscosity value influences the viscosity of the mouthwash combination. The more pleasant and easy it is to use for gargling, the closer the mouthwash formula's viscosity is to that of water. Whereas the viscosity of pure water is roughly one mPas, or one cP, most commercial mouth rinses have a viscosity under 7.25 (9).

pH Test

The pH of the produced mouthwash has to be based on the quality requirements of herbal mouthwash, which is 4.5–10.5 according to (SNI 12 3524-1995). pH meter (Luton PH 22, Taiwan) is used to conduct this testing.

Antibacterial Test of Mouthwash Preparations

The antibacterial efficacy of the combined mouthwash made from Moringa leaves and Eggshells was evaluated against S. mutans bacteria after a 24-hour incubation period using the well method, which was carried out three times. The inhibition zone was considered very strong if it exceeded 20 mm, strong between 10-20 mm, moderate between 5-10 mm, and low below 5 mm.

Statistical Analysis

Statistical analysis using SPSS 25 software, to prove significant differences between formulations.

RESULTS AND DISCUSSION

The results of this study indicate that eggshells contain a remarkable calcium carbonate content, with an analysis revealing a high concentration of 93.93%. The use of abrasive materials needs to be reviewed because they can damage the enamel. This significant calcium carbonate (CaCO3) presence highlights the potential of eggshells as an effective scrubbing agent, particularly in toothpaste formulations. Calcium carbonate is known for its mild abrasive properties, making it an ideal ingredient for cleaning and polishing teeth while also providing essential minerals. By utilizing eggshells, a sustainable and readily available resource, the dental care industry could enhance the efficacy of oral hygiene products while promoting environmentally friendly practices. (Table 1).

Table 1. Egg Shell Analysis Results

No	Content	(% Weight)
1	Water	1.01
2	Protein	3.31
3	Pure Fat	0.04
4	Calcium Carbonate (CaCO3)	93.93
5	Phosphorus	0.45
No	**Content**	**(% Weight)**
6	Magnesium	0.08
7	Potassium	0.04
8	Ferro	0.03
9	Molybdenum	1

The solid maceration extract is prepared by weighing 150 grams, resulting in a yield of 10%. The extract appears dark green and has a distinct aroma reminiscent of Moringa leaves (see Table 2). 96% ethanol was selected as the solvent for isolating active compounds from the chosen plants due to its effectiveness, availability, ability to prevent microbial growth, and its capacity to extract active chemicals. Additionally, ethanol serves as a preservative by inhibiting the growth of fungi, bacteria, and mold. This solvent facilitates the dissolution of active compounds, as it can penetrate cell walls and access the interior cells where these compounds are stored (10).

Table 2. Ethanol Extract Yield Results of Moringa Leaves

No	Material	Material Weight of Simplex (g)	Weight of Extract (g)	Yield (%)
1	Moringa Leaves	1500	150	10

Phytochemical analysis was carried out using a 10% AICI3 reagent, and a 5% FeCl reagent was utilized for examining tannin compounds. Terpenoids were identified using H2SO4, while alkaloids were tested with Mayer Dragendorff's reagent, and saponins were detected with distilled water (Marbun et al., 2025). The findings from the phytochemical tests revealed that the ethanol extract of moringa leaves positively contains compounds classified as flavonoids, tannins, saponins, and terpenoids (see Table 3).

Table 3. Results of Phytochemical Screening of Moringa Leaf Maceration

No	Result

	Phytochemical Test	Literature	Observation	Conclusion
1	Flavonoid	Yellow precipitate	Dark yellow precipitate	(+)
2	Tannin	Dark greenish change	Dark greenish color formed	(+)
3	Saponin	Stable foam formed after heating and shaking	Foam formed after heating	(+)
4	Terpenoid	Purple color formed	Purple color turning brown	(+)

Conclusion: (+) positive presence of secondary metabolites; (-) negative presence of secondary metabolites.

Based on the findings from the phytochemical tests on moringa leaves presented in Table 3, it can be concluded that the maceration process using water as a solvent is capable of extracting active components contained in the moringa leaf extract. The four types of secondary metabolites from moringa leaves have the ability to combat bacteria. Flavonoids, saponins, tannins, and terpenoids have been shown to possess antioxidant and antimicrobial properties (Haki, 2009).

Formulations with 5%, 10%, and 15% concentrations create mouthwash liquids that have different colors. These color differences come from the varying levels of extract in each formula, as shown in Table 4 and Figure 1. Higher concentrations usually lead to deeper or more vibrant colors, showing how the strength of the extract affects the look of the product. This finding highlights the connection between the concentration of the formula and the colors seen in mouthwash products.

Table 4. Mouthwash Formula Combination of Moringa Leaf Extract and Egg Shell

No	**Material**	**Formula (%)**				**Function**
		K (-)	**F-1**	**F-2**	**F-3**	
1	Ethanol extract of Moringa leaves	-	5 g/mL	10 g/mL	15 g/mL	Active Substance
2	Egg shells	-	5 g/mL	10 g/mL	15 g/mL	Active Substance
3	Tween 80	5 mL	5 mL	5 mL	5 mL	Surfactant
4	Glycerin	5 mL	5 mL	5 mL	5 mL	Humectant
5	Sorbitol	3 mL	3 mL	3 mL	3 mL	Sweetener
6	Peppermint oil	0.15 mL	0.15 mL	0.15 mL	0.15 mL	Flavoring
7	Sodium Benzoate	0.15 mL	0.15 mL	0.15 mL	0.15 mL	Preservative
8	Adhesive water	100 mL	100 mL	100 mL	100 mL	Solvent
Total Volume		113.30 mL	123.3 mL	133.3 mL	143.3 mL	

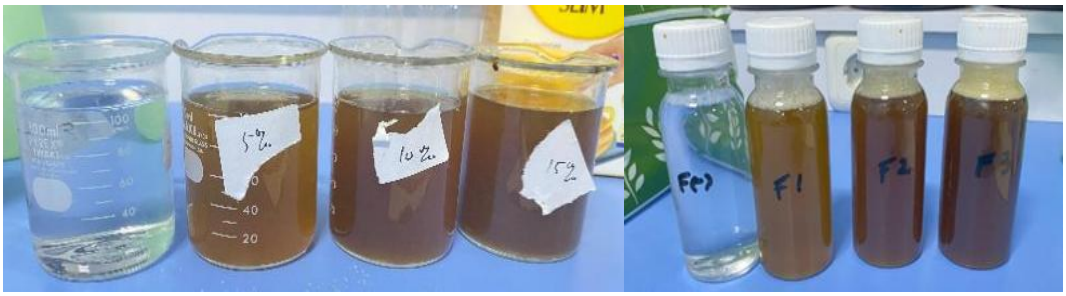

Figure 1. The mouthwash formulation consists of Moringa leaf and eggshell extract, with K (-) representing the negative control, F1 indicating the extract at a 5% concentration, F2 for the extract at a 10% concentration, and F3 for the extract at a 15% concentration.

The organoleptic test results show that the mouthwash is thin, as required, rather than thick. The active ingredients give the product its bright colors; darker preparations indicate a higher concentration of these ingredients. The minty aroma comes from peppermint oil, which adds fragrance, while sorbitol provides sweetness, and peppermint oil contributes a minty flavor. The data confirm that the materials used worked as intended and achieved the desired effects. The dark color of the product aligns with findings from Suryani's 2019 study (11). The color comes from a concentrated, dark plant extract obtained through maceration. At 10%, the color is brownish-yellow, and at 15%, it turns dark yellow, as higher concentrations produce stronger colors. The 5%, 10%, and 15% formulations each have a unique menthol and Moringa leaf scent, while glycerin adds sweetness. The Moringa leaf extract gives a slightly bitter and peppery taste, and there are no sediments in the mouthwash formulation (Table 5).

Table 5. Organoleptic Test Results of Mouthwash Combination of Moringa Leaf Extract and Egg Shell

No	Concentration	Shape	Color	Aroma	Taste
1	5%	Solution	Slightly dark brownish yellow	Typical of moringa leaves and menthol	Sweet, cold
2	10%	Solution	Dark brownish yellow	Typical of moringa leaves and menthol	Sweet, cold
3	15%	Solution	Darker brownish yellow	Typical of moringa leaves and menthol	Sweet cold

The viscosity greatly influences how thick a mouthwash solution is when gargled in the mouth. A mouthwash with a consistency similar to water will be easier and more palatable when gargling. Based on the viscosity measurement of mouthwash containing Moringa leaf extract and Eggshell, the results showed that the viscosity of the mouthwash formula with concentrations of 5%, 10%, and 15% meets the criteria. (Table 6).

Table 6. Viscosity Test Results of Mouthwash Combination of Moringa Leaf Extract and Egg Shell

No	Concentration	Time	Viscosity	According to the Ministry of Health of the Republic of Indonesia (1979)	Information
1	5%	17.93 s	0.591 mPas	<7.25	MC
2	10%	21.75 s	0.581 mPas	<7.25	MC
3	15%	23.21 s	0.513 mPas	<7.25	MC

Notes: MC = Meets Criteria

The pH test is conducted to determine the pH level of the product that is safe and meets the pH standards as an oral antibacterial. The pH level of the mouthwash product significantly affects the types of bacteria that can grow. The results of the pH test of the mixture of moringa leaf extract and egg shells can be seen in Table 6.

Table 6. Results of pH Test of Mouthwash Combination of Moringa Leaf Extract and Egg Shell

No	Concentration	pH Value	Acid/Base	pH Limits According to (SNI 12-3524-1995)	Information
1	5%	4.3	Acid	4.5-10.5	DNMC
2	10%	4.5	Acid	4.5-10.5	MC
3	15%	4.5	Acid	4.5-10.5	MC

Description: MC = Meets Criteria, DNMC = Does Not Meet Criteria

The observation result found several degrees of variation, ranging from none to moderate. The biggest inhibition zone was shown at a concentration of 15%; at 5%, the least. Antibacterial tests of moringa leaf extract against germs causing dental caries were previously conducted; however, they were done with a different approach and not in the manner of a mouthwash (Table 7).

Table 7. Antibacterial Test Results Diameter of Bacterial Growth Inhibition Zone (mm)

No	Sample Code	Repetition	Diameter of Inhibition Zone	Diameter of Inhibition Zone (Mean)	Result
1	Negative control	1	0.00	0.00 ± 0.000 mm	None
2		2	0.00		
3		3	0.00		
4	Positive control	1	23.36	24.06 ± 1.035 mm	Very strong
5		2	25.25		
6		3	23.57		
7	F-1	1	0.00	0.00 ± 0.000 mm	None
8		2	0.00		
9		3	0.00		
10	F-2	1	6.24	6.15 ± 0.083 mm	Moderate
11		2	6.12		

12		3	6.08		
13	F-3	1	7.20	7.21 ± 0.036 mm	Moderate
14		2	7.25		
15		3	7.18		

The mouthwash formulation is made from Moringa leaf and eggshell extract. K (-) is the negative control, and K (+) uses a commercial mouthwash called Listerine Zero Alcohol. F1 represents the extract at a 5% concentration, F2 is the extract at a 10% concentration, and F3 is the extract at a 15% concentration. The results show that the 10% and 15% concentrations have antibacterial activity, but the 5% concentration does not create any inhibition zone, just like the negative control. This means that antibacterial effects are not present at lower concentrations. On the other hand, the positive control has a large inhibition zone of 24 mm, which is as expected for comparison. Since the p-value (Sig.) is less than 0.05, inhibition zone diameters differ significantly across concentrations.

The results were obtained from observing the antibacterial effectiveness of mouthwash made from Moringa leaf extract against *Streptococcus mutans* bacteria (Figure 2). In assessing the antibacterial effectiveness of the mouthwash formulations against *Streptococcus mutans*, the diameter of the inhibition zone is a critical indicator of how well the antibacterial agents function. The inhibition zone is the clear area around a sample where bacteria cannot grow, measured in millimeters (mm).

The diameter of the inhibition zone helps measure how effective the mouthwash is against *S. mutans*. This zone is the clear area around the mouthwash sample where bacteria can't grow, measured in millimeters (mm). In this study, the 10% and 15% concentrations of Moringa leaf and eggshell extract showed antibacterial activity, indicated by their clear inhibition zones. This means these concentrations can effectively stop the growth of *S. mutans*.

In contrast, the 5% concentration and the negative control (K (-)) did not create any inhibition zone, meaning their antibacterial effects are too weak at this lower concentration. The positive control, Listerine Zero Alcohol, had a large inhibition zone of 24 mm, showing that it effectively fights *Streptococcus mutans* and serves as a good comparison point. This suggests that while the natural extracts show potential at higher concentrations, they may need further improvement to match the effectiveness of commercial mouthwashes.

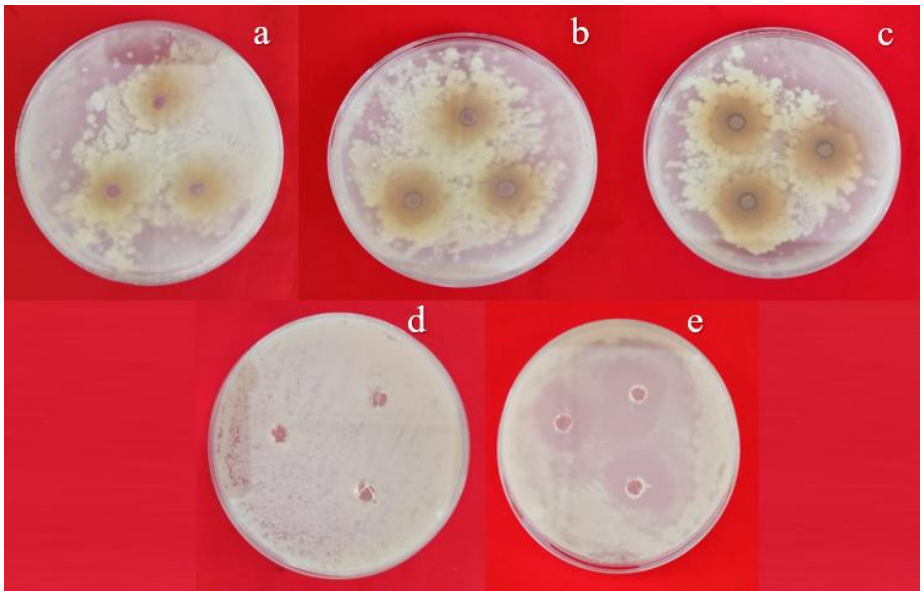

Figure 2. were obtained after an incubation period of 24 hours using the well method carried out 3 times (a) Formula 5%, (b) Formula 10%, (c) Formula 15%, (d) Negative control (Neutral solution without active ingredients , (e) Positive Control (Listerine Zero Alcohol).

The study showed that combining *Moringa oleifera L*. leaf extract and egg shells in mouthwash preparations with 5%, 10%, and 15% showed activity against bacteria. Still, no activity was detected in the formula with a concentration of 5%. This may be due to the low concentration of the extract and the possibility of contamination during the manufacture of mouthwash. Research by Tarigan (2020) suggests that moringa leaf extract can kill *Streptococcus mutans* bacteria with a notable inhibition zone. However, a remarkable inhibition zone of 15 was found. 875 mm; 11. 875 mm; and 10. 25 mm at concentrations of 80%, 40%, and 10% (13).

Extracts from *Moringa oleifera L*. leaves were tested in vitro to evaluate their antimicrobial activity on biofilms from volunteer samples against *Streptococcus mutans* compared with 70% ethanol. Both extracts showed significant antimicrobial activity and inhibited the development of biofilms that cause tooth decay, indicating that they can be used as a preventive method for dental caries (14).

All studies were reviewed, and we found that eggshell is a potential biomaterial, has a high level of biocompatibility, and can be processed using various methods (fine powder, blocks). Its properties can be enhanced using various additive techniques. There is no evidence demonstrating its ability to induce bone growth. However, there is strong evidence for its use as a supplement for calcium deficiency (15).

CLINICAL IMPLICATION

This study proposes that a combination of Moringa oleifera leaf extract and eggshell powder could provide a safer, natural alternative to alcohol-based mouthwashes. This formulation may be especially advantageous for sensitive groups, such as children and the elderly. With additional development and testing, this herbal mouthwash could enhance preventive dental care and lower the occurrence of caries related to *Streptococcus mutans*.

LIMITATIONS

This study has a number of issues. Notably, the formulation's shelf life was not assessed through long-term stability testing before it was used by humans. The study provides an important basis for further research on developing natural antibacterial mouthwashes, notwithstanding these drawbacks.

CONCLUSIONS

The natural mouthwash, which combines calcium carbonate from eggshells and Moringa oleifera leaf extract, demonstrated stability in its physical characteristics, such as viscosity, pH, and organoleptic features. Using phytochemical analysis, bioactive secondary metabolites like terpenoids, flavonoids, tannins, and saponins were found. Antibacterial testing showed that at 10% and 15% concentrations, the mouthwash successfully inhibited Streptococcus mutans, producing inhibition zones of 6.15 mm and 7.21 mm, respectively. These results imply that this herbal mouthwash can be a more secure substitute for solutions that contain alcohol. Further investigation is required to enhance its efficacy and assess its influence on additional oral pathogens.

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

The experiments and initial manuscript draft, which also provided necessary resources, were completed by Pande Bagus Mahendra Putra Parmanantha. Ni Luh Putu Eka Kartika Sari designed and supervised the experiments and analyzed the antibacterial data. I, Putu Dhananjaya Dharsila Gosa, contributed to the formatting and proofreading of the manuscript.

ACKNOWLEDGMENTS

Special appreciation goes to Universitas Warmadewa for providing the facilities and funding support for this research. Thanks also to laboratory staff and colleagues who provided technical assistance and proofread the article before the references. List here those individuals who provided help during the research, such as providing language help, writing assistance, or proofreading the article, and others.

FUNDING

This research was supported by grants 1125/Unwar/FKIK/PD-13/IX/2024 from institutions provided by the Faculty of Medicine and Health Sciences at Warmadewa University.

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors did not use to this article.

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