

FORMULATION AND STABILITY TEST OF MOUTHWASH PREPARATIONS FROM LIME LEAF EXTRACT (*Citrus aurantifolia*) WITH THE ADDITION OF CHITOSAN

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A B S T R A C T

Background: Diseases of the oral cavity occur due to the accumulation of bacteria, including the bacteria that cause dental caries (*Streptococcus mutans*) and the cause of periodontal disease (*Porphyromonas gingivalis*). **Objective:** To determine the formulation of mouthwash preparations from lime leaf extract (*Citrus aurantifolia*) with the addition of chitosan whether it is effective and determine the effect of the addition of chitosan on the stability of mouthwash preparations and to evaluate whether the addition of chitosan can maintain and improve product stability. **Methods:** which are carried out in the manufacture of mouthwash, namely, the maceration method for the manufacture of lime leaf extract (*Citrus aurantifolia*) and stability testing using the *cycling test method*, which is an accelerated stability test method used to evaluate the physical stability of pharmaceutical preparations, one of which is mouthwash preparations by simulating extreme temperature changes. mouthwash preparations are stored at a temperature of 40°C for 24 hours and then placed at 40°C for approximately 24 hours during the storage of the two temperatures considered one cycle. **Results:** Negative control formulations showed near-good results, while formulations 1, 2, and 3, did not meet all the specified conditions. **Conclusion:** The combination of lime leaf extract (*Citrus aurantifolia*) with the addition of chitosan cannot be formulated into mouthwash preparations. Variations in concentration and combination of lime leaf extract (*Citrus aurantifolia*) can affect the organoleptic properties, pH, homogeneity and viscosity of mouthwash preparations that have been tested by the *cycling test method*. From the results of the *Cycling test* The negative control formulation showed results that were close to the standard, while formulations 1, 2, and 3, did not meet all the specified standard requirements.

INTRODUCTION

Maintaining dental and oral hygiene is an important part of personal hygiene because it can prevent the entry of germs that cause disease. Oral hygiene is done by cleaning the teeth, tongue, and oral cavity from food residues, for example by brushing your teeth at least twice a day (Simaremare and Wulandari., 2021). Diseases of the oral cavity can appear due to the accumulation of bacteria, including bacteria that cause dental caries *Streptococcus mutans* as well as bacteria that trigger periodontal disease *Porphyromonas gingivalis*. Caries itself is a local damage to the hard tissues of the teeth due to bacterial activity. This disease is included in the category of chronic diseases with the highest prevalence rate in the world, and it has a significant impact not only on dental and oral health, but also on systemic health (Ely and Bassy., 2022).

One of the main problems of the oral cavity is plaque, which can be controlled mechanically by brushing and dental floss, or chemically with mouthwash. *Chlorhexidine* From the bisguanide group, it is the most recommended mouthwash because it is effective in reducing plaque by suppressing growth *Streptococcus mutans*, the main caries-forming bacteria. However, its long-term use causes side effects, such as tooth staining, disruption of normal flora, and irritation (Syahrul *et al.*, 2023); (Mandalas *et al.*, 2022). Mouthwash with a high alcohol content is also at risk of increasing cancers of the mouth, throat, and pharynx by up to 50% (Octaviani *et al.*, 2021). In addition, one of the factors that also causes fragility in teeth is because they consume too much cariogenic foods

(foods that contain high carbohydrates) and contain sugar. There are many ways to take care of your teeth and mouth, one of the ways is to brush your teeth regularly and use dental cleaners such as mouthwash, toothpaste or toothpaste, especially toothpastes that function to prevent bacterial growth (Bachmid *et al.*, 2022).

Alternatively, herbal ingredients are considered safer. Lime leaves (*Citrus aurantifolia*) are known to contain *flavonoids*, saponins, terpenes, *Limonoid*, and essential oils that have antibacterial, anti-inflammatory, and antioxidant activity (Yanuary *et al.*, 2024); Manullang *et al.*, 2020; (Ancient *et al.*, 2022). Research shows that the extract is able to inhibit the growth of pathogenic bacteria *such as S. mutans, Staphylococcus aureus, Escherichia coli, Salmonella typhi, to Candida albicans* (Mulangsri *et al.*, 2019). The antibacterial activity is mainly derived from bioactive compounds such as tannins, *flavonoids*, polyphenols, as well as essential oils (Siregar *et al.*, 2020). To increase effectiveness, lime leaves can be combined with chitosan, which is a polysaccharide resulting from chitin deacetylation from shrimp skin waste. Chitosan is antibacterial, bioactive, and easily produced in various forms, so it is widely applied in pharmaceutical, agricultural, and industrial fields (Suherman *et al.*, 2018); (Pardi *et al.*, 2023). In addition, chitosan is also able to improve stability, solubility, controlled drug release, and has low immunogenicity, so it is often used as a drug delivery system (Zulvianti *et al.*, 2022).

Chitosan is a poly-(2-amino-2-deoxy- β -(1-4)-D-glucokopiranose) with the molecular formula $(C_6H_{11}NO_4)_n$ obtained through full or partial deacetylation of chitin. These compounds include biopolymers with the main component D-glucosamine as well as a portion of N-acetyl-D-glucosamine bound to β -(1-4) glycosides. Chitosan can also be hydrolyzed into a form of oligomers known as chitosan oligomers (Sarni *et al.*, 2016). One of the mechanisms of action of chitosan in food preservation is its ability to interact with compounds on the surface of bacterial cells, then form a layer that inhibits cell transport. As a result, cells lack nutrients to grow and eventually die. In terms of safety, chitosan is also considered safe because it is enough to be dissolved in 1% dilute acetic acid to produce a homogeneous solution (relatively harmless). (Vitria *et al.*, 2021).

Chitosan has great prospects for use both as an active ingredient and as an excipient in the pharmaceutical field. As an active ingredient, chitosan can be formulated into pharmaceutical preparations that function to prevent or treat diseases due to its antibacterial activity. In addition, chitosan also plays an important role in the drug delivery system with its ability to control the release of active substances so as to increase the effectiveness of therapy. The use of chitosan, both as an active ingredient and an excipient, opens up vast opportunities in the development of various forms of preparations, such as tablets, hydrogels, microparticles, and nanoparticles. This advantage is supported by the non-toxic, biodegradable, bioavailable, and biocompatible properties of chitosan, so that it can help optimize drug performance according to its therapeutic goals (Akmarina & Sriwidodo, 2016).

METHOD

This research is a laboratory experimental research using maceration and *cycling test* methods. The sample used was lime leaves (*Citrus aurantifolia*), the sampling place in Betoambari District, Baubau City. In this study, tools and materials were used consisting of sieve, autoclave, stirring rod, blender, filter paper, gauze, measuring flask, mortar and stamfer, bottle, porcelain cup, funnel, erlenmeyer, measuring cup, 100 mL chemical cup, watch glass, oven, scissors, handschoon, horn spoon, volume pipette, rotary evaporator, magnetic stirrer, 1 mL spoit, pH meter, viscometer, aluminum foil, cotton, tissues, maceration tools (glass jars), test tubes and analytical scales. The ingredients used in this study are lime leaves (*Citrus aurantifolia*), 96% ethanol, aquadest, sorbitol, glycerin, chitosan, peppermint oil, aquadest, acetic acid.

Creation of simplicia

The samples are collected, wet sorted, cleaned and washed with running water, drained and then air dried. It is then dried using the sun until the moisture content decreases, then mashed using a blender until it becomes simplicia powder and sifted using a sieve, then stored in a glass jar (Blessed are you) *et al.*, 2022).

Extract manufacturing

Manufacture of lime leaf extract (*Citrus aurantifolia*) was carried out by maceration method using 96% ethanol solvent with the ratio between simplicia powder and solvent is (500 gr simplia: 1000 mL solvent). A total of 500 g of lime leaf simplicia powder (*Citrus aurantifolia*) macerated with 96% ethanol solvent covered with aluminum foil and left for 5 days while occasionally stirring well. After 5 days, decantation is carried out (applied) then filtered with filter paper so that filtrate I and residue I

are obtained. Residue I is then macerated again with 800 mL of 96% ethanol solvent, covered with aluminum foil and left for 1 day, while occasionally stirring well. After two days, the sample was decanted and filtered again with filter paper until filtrate II and residue II were obtained. Filtrate I and II are combined, then evaporated (evaporated) using a rotary evaporator, and continued with heating using an oven with a temperature of 60° C so that a thick extract of lime leaves is obtained (*Citrus aurantifolia*). Lime leaf extract (*Citrus aurantifolia*) is stored in a sealed container before being used for further testing to weigh the results of lime leaf thick extract (*Citrus aurantifolia*) (Blessed are you) *et al.*, 2022).

Preparation of mouthwash preparations

The ingredients to be used are first weighed according to the dosage, lime leaf extract FI 2.5%, FII 5%, FIII 7.5% and K(-) 7.5% are put into a mortar, then glycerin 2.0% is added until it dissolves. Then 0.25% sorbitol and 0.5% chitosan were added which had been dissolved with 1% acetic acid as much as 10 mL into the mortar and crushed again until homogeneous. Add 100 mL of aquades to the mortar until it can be poured, then filtered and put in a bottle, then add the rest of the aquades up to 100 mL. Then 0.2% peppermint oil is added to the bottle and stirred well, then closed tightly (Blessed are you) *et al.*, 2022).

Stability testing

Evaluation of the stability of the preparation is carried out by the *Cycling test* Namely an accelerated stability test method used to evaluate the physical stability of pharmaceutical preparations, one of which is mouthwash preparations by simulating extreme temperature changes from cold to hot temperatures. mouthwash preparations are stored at 40oC for 24 hours and then placed at 40oC for approximately 24 hours during the storage of the two temperatures are considered one cycle. The test was carried out for 6 cycles, where each cycle observed physical changes in the mouthwash preparation including organoleptic, pH, homogeneity and viscosity (Ilyas) *et al.*, 2023)

Organoleptic testing

Organoleptic Test of Mouthwash Preparation of Lime Leaf Extract (*Citrus aurantifolia*) was carried out directly including several observation parameters: texture, color, and aroma (Blessed are you) *et al.*, 2022).

pH testing

pH measurement is carried out using a pH meter. The pH that appears on the screen and is stable is then recorded. Measurements were made on each test preparation (Rasyadi, 2018)

Viscosity testing

Viscosity test is a resistance test for a pharmaceutical preparation to flow. The higher the viscosity, the greater the resistance. The viscosity of a preparation must be high enough but still pourable (Mudhofar *et al.*, 2024). The viscosity range of mouthwash ranges from 3.64 –8 mPas. The closer the viscosity level of the formulation product is to the viscosity level of water, the easier and more convenient it is to gargle. Pure water has a viscosity of 1 mPa.s, or about 1 cP, while the market need for the viscosity of mouthwash is 7.25 mPa.s (Rachmawati) *et al.*, 2022).

Homogeneity testing

The homogeneity test was carried out by observing the preparation that had been put in a clear bottle and given a white background. A good preparation is homogeneous, not cloudy, and free from contamination and microbial growth (Sianturi, 2022)

RESULTS & DISCUSSION

This study was conducted to determine the formulation and stability of the preparation of lime leaf extract mouthwash (*Citrus aurantifolia*) with the addition of chitosan during a storage period of 12 days or 6 cycles using *the cycling test* method.

Testing of the stability of mouthwash from lime leaf extract (*Citrus aurantifolia*) with the addition of chitosan included organoleptic, homogeneity, pH, and viscosity tests. The negative control formulation showed good results, while the formulations 1, 2, 3 and K(-) although not meeting all the requirements, the results were close to the specified standard. From the results of good mouthwash formulation, it can be seen in the negative control of formulations 1, 2 and 3 which show stability close to the standard.

Table 1 Formulation of lime leaf extract mouthwash preparations (*Citrus aurantifolia*) with the addition of chitosan (Rahayu *et al.*, 2022).

Material	Function	F1 2,5%	F2 5%	F3 7,5%	K(-) 7,5%
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Lime Leaf Extract	Active ingredients	2,5%	5%	7,5%	7,5%
Papermint oil	Freshener	0,2%	0,2%	0,2%	0,2%
Sorbitol	Sweetener	0,25%	0,25%	0,25%	0,25%
Chitosan	Preservatives	0,5%	0,5%	0,5%	-
Glycerine	Hufixan	2,0%	2,0%	2,0%	2,0%
Acetic acid	Solvent	10 mL	10 mL	10 mL	-
Aquadest (ad)	Solvent	100 mL	100 mL	100 mL	100 mL

Information:

K(-) : Mouthwash formulation contains 7.5% lime leaf extract and no chitosan

F1 : The mouthwash formulation contains 2.5% lime leaf extract, with the addition of 0.5% chitosan.

F2 : The mouthwash formulation contains 5% lime leaf extract, with the addition of 0.5% chitosan.

F3 : The mouthwash formulation contains 7.5% lime leaf extract, with the addition of 0.5% chitosan.

Table 2 Organoleptic Test Results after *Cycling Test*

	Organoleptic	Formula I	Formula II	Formula III	Control (-)
I	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Papermint oil	Papermint oil	Typical lime leaves and papermint oil	Band and mint flavor
	Color	Yellow is a bit clear	Yellow is a bit clear	Chocolate is a bit clear	Chocolate is a bit clear
Cycle 1					
II	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Papemint oil	Papermint oil	Typical lime leaves and papermit oil	Typical lime leaves and papermit oil
	Color	Yellow is a bit clear	Yellow is a bit clear	Chocolate is a bit clear	Chocolate is a bit clear
Cycle 2					
III	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Papermint oil	Papermint oil	Typical lime leaves and papermint oil	Typical lime leaves and papermint oi
	Color	Yellow is a bit clear	Yellow is a bit clear	Chocolate is a bit clear	Chocolate is a bit clear
Cycle 3					
IV	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Paprmint oil	Papermint oil	Typical lime leaves and papermint oil	Typical lime leaves and apermint oil
	Color	Yellow is a bit clear	Yellow is a bit clear	Chocolate is a bit clear	Chocolate is a bit clear
Cycle 4					
V	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Acidic and slightly smelly papermint oil	Acidic and slightly smelly papermint oil	Typical lime leaves and papemint oil	Typical lime leaves and papemint oil
	Color	Yellow is a bit clear	Yellow is a bit clear	Chocolate is a bit clear	Chocolate is a bit clear
Cycle 5					
VI	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Acidic and slight smell of papemint oil	Acidic and slight smell of papemint oil	Typical lime leaves and papemint oil	Typical lime leaves and papemint oil
	Color	Yellow is a bit clear	Yellow is a bit clear	Chocolate is a bit clear	Chocolate is a bit clear
Cycle 6					

Table 3 Homogeneity Test Results Before *Cycling Test*

Mouthwash Preparations	Homogeneity test	Standard Value of Mouthwash
FI	Homogeneous	Homogeneous

FII	Homogeneous
FIII	Homogeneous
K(-)	Homogeneous

Table 4 Homogeneity Test Results After *Cycling Test*

Formulation	Homogeneity test results in the Cycle to					
	I	II	III	IV	V	VI
F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
K(-)	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

Table 5 pH Test Results Before *Cycling Test*

Formula	Value	Information	Standard pH Value of Mouthwash
FI	3	Not compliant	5-7
FII	3	Not compliant	
FIII	4	Not compliant	
K(-)	6	Meet	

Table 6 pH Test Results After *Cycling Test*

Table 6 pH Test Results After Cycling Test								
Formulation	Average result of pH values						Information	Standard pH Value of Mouthwash
	Cycle to							
	I	II	III	IV	V	VI		
F1	4,05	2,44	2,28	2,22	2,19	2,13	Not compliant	5-7
F2	4,01	2,44	2,44	2,40	2,34	2,24	Not compliant	
F3	3,99	3,97	2,36	2,36	2,33	2,19	Not compliant	
K(-)	6,81	5,63	4,85	4,81	4,06	4,03	Meet	

Table 7 Viscosity Test Results Before *Cycling Test*

Mouthwash Preparations	Value (mPas)	Information	Viscosity Range of Mouthwash
FI	14,6	Not compliant	3.64-7.25 mPa.s
FII	10,4	Not compliant	
FIII	11,0	Not compliant	
K(-)	6,01	Meet	

Table 8 Viscosity Test Results After *Cycling Test*

Formulation	Viscosity test results in the Cycle to						Rotation/Speed (rpm)	Viscosity Range of Mouthwash
	I	II	III	IV	V	VI		
F1	15,6	13,2	10,2	6,60	4,53	4,67	No.2 Rotar with 60 RPM Speed	3.64-7.25 mPa.s
F2	11,4	10,4	9,34	6,56	6,57	7,35		
F3	18,7	10,7	7,43	6,88	7,27	3,70		
K(-)	11,0	5,90	6,88	6,98	3,67	3,54		

CONCLUSION

From the results of the research that has been conducted, it can be concluded that: The combination of lime leaf extract (*Citrus aurantifolia*) with chitosan cannot be formulated into mouthwash preparations. Variations in the concentration and combination of lime leaf extract (*Citrus aurantifolia*) with the addition of chitosan can affect the organoleptic properties, pH, homogeneity and viscosity of mouthwash preparations that have been tested by the cycling test method. From the results of the cycling test, all formulations 1,2,3 and K(-) were unstable which was shown at changes in pH and viscosity of mouthwash.

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