

Activity and Stability Test of Antiseptic Preparations from The Formulation Combination of Betel Leaf (*Piper betle* L), Lime Peel (*Citrus aurantifolia*) and Bundung Plant (*Actinoscirpus grossus*)

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Abstract

Antiseptic are chemical compounds that are used to kill or inhibit the growth of microorganisms that found on the surface of the skin and mucous membranes. Betel leaf (*Piper betel*) has long been used in traditional medicine because it is believed to have effects that can kill bacteria. Lime peel (*Citrus aurantifolia*) has also been widely used to kill germs. While for *bundung* plants (*Actinoscirpus grossus*) by the community, it is used as wound medicine. Besides functioning as antibacterial, a flavonoid also functions to increase enzymatic activity and inhibit cell proliferation. The method of this research is completely randomized experimental design with concentration formulation of 20%, 30%, 40% and 50% and tested against *Staphylococcus aureus*, *Escherichia Coli* and *Streptococcus pyogenes* with disk diffusion method and test stability of formulations (organoleptic, pH, viscosity, cycling test and stability). The result of all formulations have an inhibitory power against *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes*. The result of F1 20% inhibition only against *Escherichia coli* 11 mm, F2 30% inhibition against *Staphylococcus aureus* 7 mm, *Escherichia coli* 22 mm, F3 40% inhibition against *Staphylococcus aureus* 23mm, and *Escherichia coli* 26 mm, and F4 50% inhibition against *Staphylococcus aureus* 40 mm, *Escherichia coli* 31 mm, and *Streptococcus pyogenes* was 18 mm. Stability test results showed that all formulations stable on odor, color and pH but unstable in syneresis. The conclusion of the research of all formulations stabil and has an inhibitory effect on bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes*.

Keywords: Betel leaf-lime peel-bundung plant, antiseptic, bacteria *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*.

Introduction

The use of natural ingredients as traditional medicine has recently increased. Traditional medicines are considered to have less side effect than chemical drugs. They have a more affordable price, 80% living in the developing world rely on herbal medicinal products as a primary source of healthcare and traditional medical practice which involves the use of herbs is viewed as an integral part of the culture in those communities (Ekor M, 2014). Betel leaf can be used as an antiseptic. The compounds of betel plant are saponin, flavonoid polyphenol and

essential oil. Saponin compounds work to damage the cytoplasmic membrane and kill microbial cells. Flavonoid is thought to have a mechanism of action of denaturing bacterial cells and irreparably damaging cell membranes(Aiello & Susan, 2012).

Lime contains flavonoid compounds wherein flavonoids is a group of polyphenol compounds largest that has activity as antioxidants and antibacterials. Lime has activity as antiviral and antifungal. Various activities owned by from essential oil content in lime. Essential oil is the largest component found in lime plants(Chusniah & Muhtadi, 2007). Bundung plant contains secondary metabolite compound, namely flavonoid, tannin, saponin, phenolic, steroid and terpenoid. The result showed that pure bundung plant extract had antibacterial activity through the content of flavonoid compound ^{26; 14}).

Antiseptic is a chemical compound that used to kill or inhibit the growth of microorganisms in living tissue such as the surface of the skin and mucous membrane(Levinson, 2008). Antiseptic is highly recommended when there is an epidemic disease, even like the current pandemic of the coronavirus disease (Covid-19) because it can slow the spread of disease or break the chain of disease spread(Health, 2008). *Staphylococcus aureus* and *Streptococcus Pyogenes* is a gram-positive bacteria often found on the skin and mucous membrane of humans and animals. This bacteria spread and penetrate the skin through the wound and can cause infection. *Escherichia coli* is a gram-negative bacteria, which is a normal flora bacteria found in the human colon. *Escherichia coli* is also a cause of diarrhea and urinary tract infections(Clinic, 2019). Therefore, we need prevention by always maintaining personal hygiene and washing hands regularly with soap or antiseptic.

Based on the explanation above, the researcher tried to make an antiseptic preparation formulation from a combination of three natural ingredients that empirically has an antimicrobial activity to investigate whether the three ingredients combination has antiseptic action through inhibition against bacteria that cause disease or infection.

Material And Methods

a. Materials

The tools used in this research are magnetic stirrer, vortex, rotary evaporator, water bath, analytical scale, maceration bottle, object glass, ove, autoclave, pH meter, viscosimeter, infusion pan, loop and other glassware.

Ingredients in this study are betel leaf (*Piper betle*), lime peel (*Citrus aurantifolia*), bundung plant (*Actinoscirpus Grosssus*), *Staphylococcus aureus* ATCC 29213, *Escherichia Coli* ATCC 25922, *Streptococcus Pyogenes*, distilled water, ethanol 95%, nutrient agar, SDA (*Saboroud*

Dextrose Agar), HMA (Hilton Muller Agar), NaCl 0,9%, propylparaben, methylparaben and ice cubes.

Methods

The research was carried out in Microbiology, Chemistry and Pharmaceutical Technology laboratories of Sari Mulia University. This study used a completely randomized design experimental method and used betel leaf (*Piper betle*), lime (*Citrus aurantifolia*) and bundung plant (*Actinoscirpus Grosssus*) as sample.

b. Research Procedure

1) Making lime peel extract (*Ciprus Aurantifolia*) and bundung plant extract (*Actinoscirpus grossus*) using the maceration method.

- a) Prepare the ingredients. Lime peel and bundung plants washed and dried indirectly in the sun.
- b) Each of the dried lime peel slices and bundung plants was blended to produce dry simplicia.
- c) Put each simplicia in a glass jar and add ethanol 95%, soaked for 3 days, while stirring every day
- d) The liquid obtained was separated by yield into liquid extract, with rotary evaporator for each liquid extract to become a thick extract.

2) Making betel leaf extract (*Piper betle*) using the infusion method.

- a) Prepare 40 g, 60 g, 80 g and 100 g betel leaf to make 20% w/v, 30% w/v, 40% w/v and 50% w/v betel leaf infusion
- b) Each betel leaf added 200 ml of hot distilled water in a deep infusion pan. Infusion pan with water for steam is heated to a boil, then a deep infusion pan that already contains betel leaf with hot distilled water added, and steam for 15 minutes with over low heat
- c) Steamer pan is removed from the infusion pan, cooled, then filtered, and the liquid extract of betel leaf infusion is obtained with concentration 20% w/v, 30% w/v, 40% w/v and 50% w/v.

3) Making a combination formulation of betel leaf infusion extract (*Piper betle*), lime peel ethanol extract (*Citrus aurantifolia*) and bundung plants ethanol extract (*Actinoscirpus Grosssus*)

Table 1. Combination Solution Formulations

No	Ingredients	20% w/v	30% w/v	40% w/v	50% w/v
1	Betel leaf infusion extract	100 ml	100 ml	100 ml	100 ml
2	Lime juice	8g	8g	8g	8g
3	Lime peel ethanol extract	8g	8g	8g	8g
4	Bundung plants ethanol extract	1g	1g	1g	1g
5	Methyl paraben	0,2g	0,2g	0,2g	0,2g
6	Propyl paraben	0,3g	0,3g	0,3g	0,3g
7	Positive control of hand sanitizer antiseptic	Each test	Each test	Each test	Each test
8	Negative control of distilled water	Each test	Each test	Each test	Each test

Weigh all ingredients. Dissolve the ethanol extract of the lime peel with enough betel leaf infusion extract in a porcelain dish. Ethanol extract of the bundung plant is dissolved with betel leaf infusion extract in a glass beaker assisted by a magnetic stirrer until dissolved. Also, dissolve methyl paraben and propylparaben with betel leaf infusion extract little by little. All ingredients are mixed in a beaker glass, then filtered and stored in an Erlenmeyer glass.

4) Testing on formulations with bacteria *Staphylococcus aureus*, *Escherichia Coli*, and *Streptococcus Pyogenes*.

- a) Testing the inhibition of *Staphylococcus aureus* and inhibition of *Escherichia Coli* with nutrient disc diffusion media method.
 1. Make nutrient agar media with weigh as much as 7 g, put in glass beaker add 350 ml hot water. Dissolve with magnetic stirrer 300-400 rpm until dissolved.
 2. Prepare 10 sterilized petri discs, pour the media evenly into 10 petri discs 35 ml, let cool and ready to become nutrient then add 1 ml of NaCl and 1 ml of nutrient broth.
 3. Prepare *Staphylococcus aureus* suspension and *Escherichia Coli* suspension in Bacteriology Safety Cabinet (BSC), take *Staphylococcus aureus*, *Escherichia Coli* in a tube using a sterile one. Put in an existing test tube 1 ml of sterile NaCl, homogenize it with a vortex.
 4. *Staphylococcus aureus* suspension and *Escherichia Coli* suspension are scattered in SDA media; the spread is levelled with a triangular stem.
 5. Paper discs were soaked for 15 minutes into each formulation, also in positive and negative controls. Take disc paper and place it on each of the SDA media that already have *Staphylococcus aureus* and *Escherichia Coli*.
 6. Petri discs were incubated in an incubator cabinet at 37°C and for 24 hours. Observe and measure the diameter of the inhibition zone (clear zone) with a ruler.

- b) Testing the inhibition of *Streptococcus Pyogenes* with Hilton Mueller media to use the disc diffusion method.
 1. Make nutrient media 9,8 g put it in a glass beaker, add 250 ml of hot water and dissolve it with a magnetic stirrer 300-400 rpm until dissolved. Pour into an Erlenmeyer glass then sterilized with autoclave 121°C for 30 minutes.
 2. Prepare 10 sterile petri disc, pour the media evenly into 10 petri disc 35 ml. Let it cool and ready to be a Hilton muller agar medium.
 3. Prepare *Streptococcus Pyogenes* suspension in BSC, take *Streptococcus Pyogenes* in a tube that already has 1 ml of sterile NaCl, homogenize it with vortex. The *Streptococcus Pyogenes* suspension is spread out in HMA medium; the spread is leveled with a triangular shaft.
 4. Paper discs were soaked for 15 minutes into each of the formulation, also under positive and negative controls.
 5. Take the disc paper, place it on the HMA media that already has *Streptococcus Pyogenes* and incubate it in an incubator cabinet at 37°C for 24 hours. Observe and measure the diameter of the inhibition zone (clear zone) with a ruler.

5) Evaluation of a combination formulation of betel leaf infusion extract (*Piper betle*), lime peel ethanol extract (*Citrus aurantifolia*) and bundung plant ethanol extract (*Actinoscirpus Grossus*).

- a) The organoleptic evaluation included color, odor and homogeneity, which were observed visually for 6 weeks.
- b) pH Evaluation

Put the formulation in beaker glass and dip the pH meter electrode. pH meter needle is allowed to move until it shows the position but then recorded.
- c) Viscosity Evaluation

Put the formulation into a 50 ml beaker glass then install the rotor and make sure that the rotor is submerged in the formulation. A viscometer stormer with rotor no. 12 rpm, 30 rpm, and 60 rpm are turned on, and it is ensured that the rotor can be rotated. Observe the needle of the viscometer that points to the number on the available viscosity scale, when the needle shows a stable direction, record the viscosity value.
- d) Cycling Test

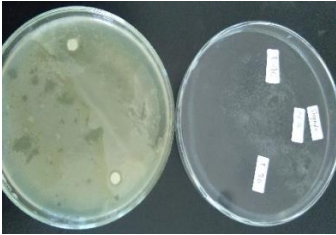
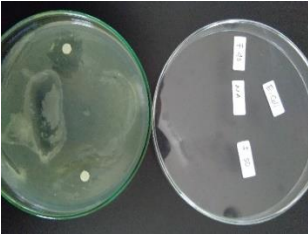
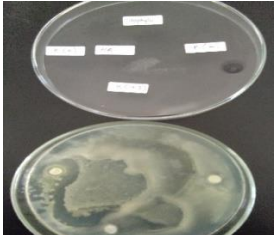
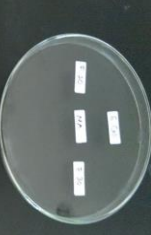
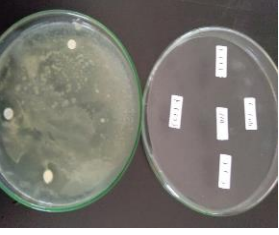
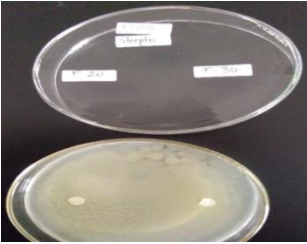
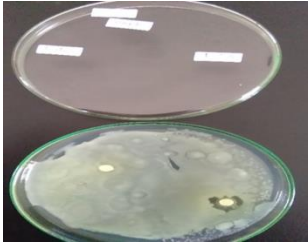
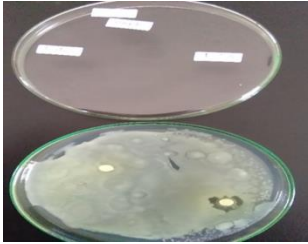
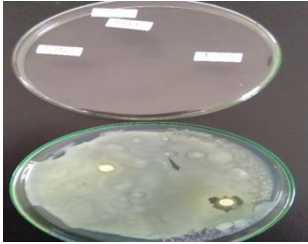
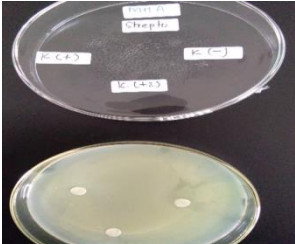
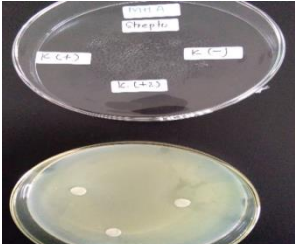
30 ml of each formulation put into a 50 ml beaker glass made in three packages for testing at a cold temperature (refrigerator), room temperature and hot temperature (oven). Once a week observed, weighed and carried out for 8 weeks.

Result

a. Microbial Inhibition Test

Results of the inhibition of formulation against microbes

Table 2. Inhibition of Microbes to Formulation Solution

Formulation	F1	F2	F3	F4	Control (+)	Control (-)
Microbes	20%	30%	40%	50%		
	0	7mm	23mm	40mm	23mm	0
<i>Staphylococcus aureus</i>						
	11mm	22mm	26mm	31mm	30mm	0
<i>Escherichia coli</i>						
	0	0	0	18mm	8mm	0
<i>Streptococcus pyogenes</i>						
	0	0	0	0	0	0

Note: Positive control: antiseptic solution and hand sanitizer
Negative control: distilled water

b. Test Stability of the Formulation

1) Organoleptic Test

Table 3. Organoleptic Test Results

Week	Formulation 1			Formulation 2			Formulation 3			Formulation 4		
	D	S	T	D	S	T	D	S	T	D	S	T
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-

Note: D = Discoloration
S = Smell
T = Transformation
(-) = Nothing Changes

2) pH Test

Table 4. pH Test Results

Formulation	pH					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	3,2	3,2	3,3	3,34	3,27	3,37
2	3,5	3,5	3,5	3,57	3,55	3,55
3	3,6	3,6	3,6	3,66	3,65	3,62
4	3,7	3,7	3,7	3,76	3,78	3,78

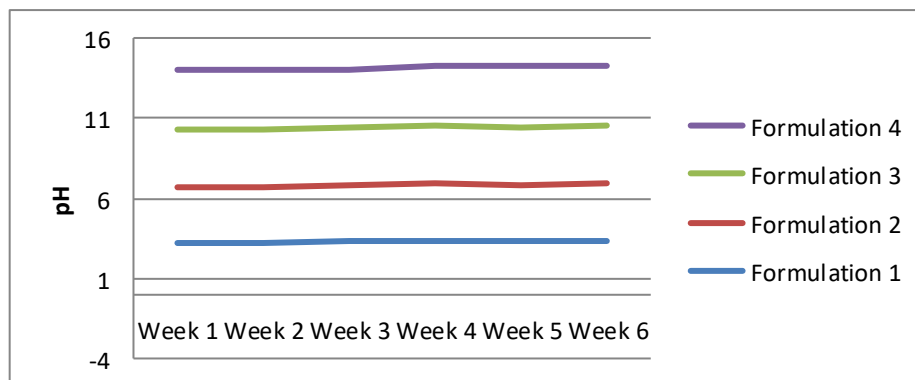


Figure 1. Graph of pH Test Results

3) Viscosity Test

Table 5. Viscosity Test Results

Formulation	rpm	Viscosity (mPa.s)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	12	189,9	183,3	233,3	166,7	189,9	176,6
	30	106,3	85,3	96	60	58,6	38,7
	60	74,7	62,6	78,7	51	55,9	60,7
2	12	223,3	179,9	223,3	166,7	130	156,7
	30	137,3	66,7	93,3	61,3	60,7	43,9
	60	80	64	74	57,9	55,9	60,7
3	12	226,7	179,9	206,7	103,3	153,3	130
	30	115,9	77,3	81,3	57,3	59,9	38,7
	60	69,3	69,3	70	55,3	56,6	64
4	12	153,3	92,6	200	63,7	146,7	130
	30	106,6	49	82,7	57,3	61,3	36
	60	74,7	61,3	70	55,9	58,6	62,7

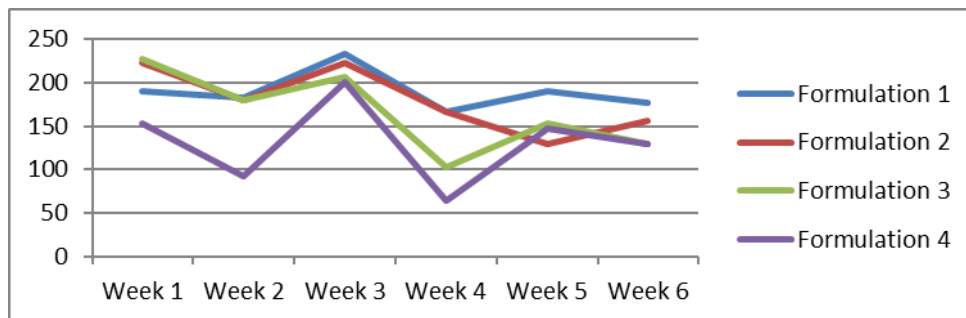


Figure 2. Graph of Viscosity Test

4) Cycling Test

Table 6. Cycling Test Results

Formulation	Test							
	0 Cycle				6 th Cycle			
	Color	Smell	Syneresis	pH	Color	Smell	Syneresis	pH
1	Light brown yellow	Fresh, slightly smelling of betel	3,19 gram	3,2	Yellow-brown	Smelling of betel	2,13 gram	3,1
2	Yellow brown	Smelling of betel	3,19 gram	3,5	Yellow brown	Smelling of betel	2,16 gram	3,4
3	Dark brown yellow	Smelling of betel	3,19 gram	3,6	Yellow brown	Smelling of betel	1,31 gram	3,6
4	Brown	Smelling of betel	3,19 gram	3,7	Yellow brown	Smelling of betel	1,35 gram	3,7

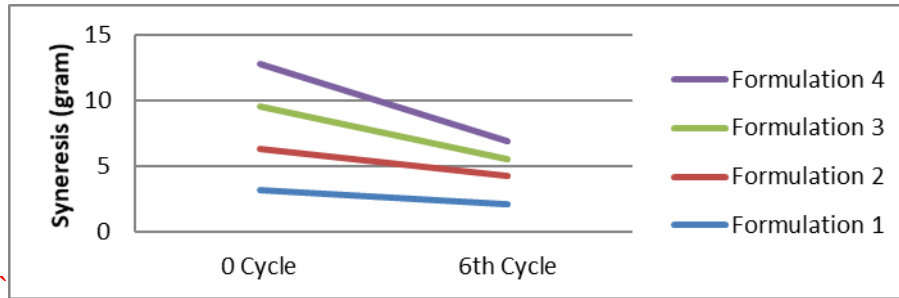


Figure 3. Graph the Results of Syneresis Cycling Test

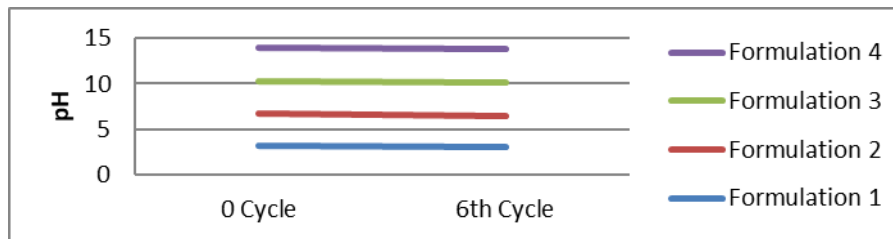


Figure 4. Graph the results of pH Cycling Test

c. Stability Test of Formulations

1) Stability Test at Low Temperature ($4 \pm 2^{\circ}\text{C}$)

Table 7. Stability Test Results at Low Temperature

Formulation	Test	Organoleptic and pH				
		Week 0	Week 2	Week 4	Week 6	Week 8
1	Color	Light brown yellow	Light brown yellow	Yellow brown	Yellow brown	Yellow brown
	Smell	Fresh, slightly smelling of betel	Smell of betel	smelling of betel	smelling of betel	smelling of betel
	Syneresis	3,19 gram	2,99 gram	2,69 gram	2,59 gram	2,29 gram
	pH	3,2	3,2	3,2	3,3	3,0
2	Color	Yellow brown	Yellow brown	Yellow brown	Yellow brown	Yellow brown
	Smell	smelling of betel	smelling of betel	smelling of betel	smelling of betel	smelling of betel
	Syneresis	3,19 gram	3,08 gram	2,88 gram	2,80 gram	2,71 gram
	pH	3,5	3,5	3,5	3,5	3,5
3	Color	Dark brown yellow	Dark brown yellow	Dark brown yellow	Dark brown yellow	Dark brown yellow
	Smell	smelling of betel	smelling of betel	smelling of betel	smelling of betel	smelling of betel
	Syneresis	3,19 gram	3,11 gram	2,97 gram	2,82 gram	2,64 gram
	pH	3,6	3,6	3,6	3,6	3,6

4	Color	Brown	Brown	Brown	Brown	Brown
	Smell	smelling of betel	smelling of betel	smelling of betel	smelling of betel	smelling of betel
	Syneresis	3,19 gram	3,03 gram	2,86 gram	2,71 gram	2,62 gram
	pH	3,7	3,7	3,7	3,8	3,8

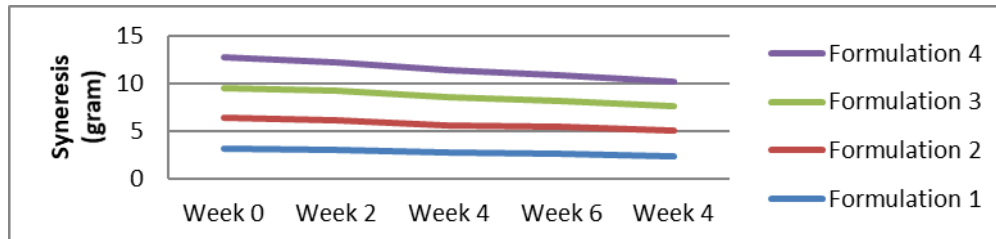


Figure 5. Graph of Syneresis Stability Test Results at Low Temperature

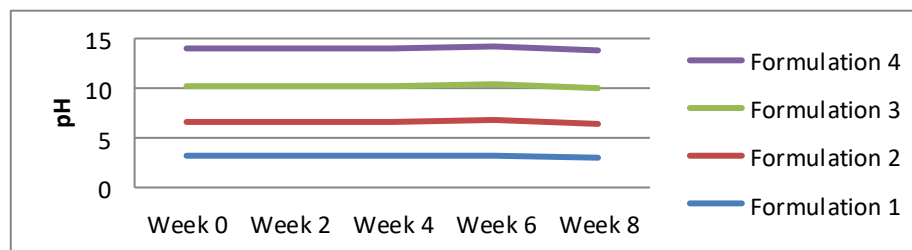


Figure 6. Graph of pH Test Results at Low Temperature

2) Stability Test at High Temperature ($40 \pm 2^\circ\text{C}$)

Table 8. Stability Test Results at High Temperature

Formulation	Test	Organoleptic and pH				
		Week 0	Week 2	Week 4	Week 6	Week 8
1	Color	Light brown yellow	Yellow brown	Yellow brown	Yellow brown	Yellow brown
	Smell	Fresh, slightly smelling of betel	Smell of betel	smelling of betel	smelling of betel	smelling of betel
	Syneresis	3,19 gram	3,02 gram	2,76 gram	2,33 gram	2,01 gram
	pH	3,2	3,2	3,2	3,3	3,3
2	Color	Yellow brown	Yellow brown	Yellow brown	Yellow brown	Yellow brown
	Smell	Smell of betel	Smell of betel	Smell of betel	Smell of betel	Smell of betel
	Syneresis	3,19 gram	2,98 gram	2,51 gram	2,38 gram	2,03 gram
3	pH	3,5	3,5	3,5	3,5	3,5
	Color	Dark brown yellow	Dark brown yellow	Dark brown yellow	Dark brown yellow	Dark brown yellow
	Smell	Smell of betel	Smell of betel	Smell of betel	Smell of betel	Smell of betel

4	Syneresis	3,19 gram	2,93 gram	2,65 gram	2,41 gram	2,26 gram
	pH	3,6	3,6	3,6	3,6	3,6
	Color	Brown	Brown	Brown	Brown	Brown
	Smell	Smell of betel	Smell of betel	Smell of betel	Smell of betel	Smell of betel
	Syneresis	3,19 gram	3,03 gram	2,87 gram	2,55 gram	2,21 gram
	pH	3,7	3,7	3,8	3,8	3,8

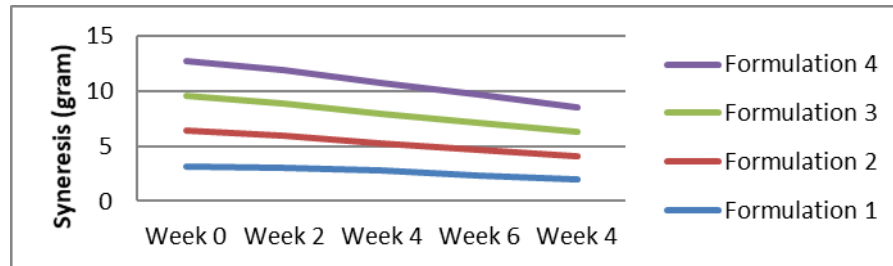


Figure 7. Graph of Syneresis Stability Test Results at High Temperature

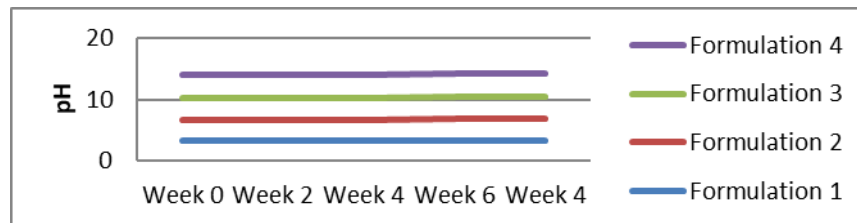


Figure 8. Graph of pH Test Results at Low Temperature

Discussion

Based on results of measurements of the diameter inhibition zone of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* bacteria in Table 2, for *Staphylococcus aureus* bacteria showed very strong inhibitory activity at F4 50% greater than positive control with an inhibitory value of 40 mm, at F3 40% also in very strong range with the same value for positive control with a value of 23 mm. whereas at F2 30% at a value of 7 mm and is the weak zone. F1 20% has no inhibition with the same value on the negative control of 0 mm. Inhibitory activity of *Escherichia coli* bacteria shows that at F4 50% have a very strong activity, greater than positive control with an inhibitory value of 31 mm, at F3 40% is also in a very strong range with same value in positive control of 23 mm. whereas at F2 30% at a value of 22 mm in the very strong zone and F1 20% has a moderate zone resistance of 11 mm. As for antimicrobial test on *Streptococcus pyogenes* bacteria showed that activity at F4 50% was very strong, greater than positive control with an inhibitory value of 18 mm, at F3 40% and F1 20% there was no inhibition equal to the negative control. For these results, it can be concluded that the greater concentration then, the greater inhibition zone formed (Noval *et al.*, 2021).

Analysis of chemical content of betel leaf extract (Ni Putu Rahayu Kusuma Pratiwi, 2016) has found 31 compounds contained in the extract, the majority of active compounds from betel leaf extract are phenolic groups which mean have antibacterial activity. Phenol which targets cell wall polypeptides will cause damage to the cell wall. Phenol works to damage the cell membrane by means of H^+ ions breaking phosphate group so that the phospholipid molecules break down into glycerol, carboxylic acid and phosphoric acid. Phenol activity as antibacterial with ability to inactivate microbial cell adhesion (molecules attached to host cells) that are presents on cell surface which will cause damage to the cell wall, so membrane will leak (interfere with permeability) and bacteria will experience groth inhibition and can even die (Noval *et al.*, 2020).

Flavonoid work by destroying the cytoplasmic membrane so that bacterial cells will be damaged and die. While flavonoid compounds play an active role as anti-inflammatory and phenolic shows antibacterial activity (Kadam *et al.*, 2012). Phytochemical analysis of lime peel extract showed good potential for free radical antioxidants through the content of flavonoids such as quercetin, hesperidin and naringerin. Naringin shows good protective activity against kidneys. Naringin is known to have anti-carcinogenesis and anti-tumorigenesis properties (Kurniadari *et al.*, 2015).

Results of chemical test showed that ethanol extract of bundung plants contained secondary metabolite compounds, namely flavonoid, tannin, saponin phenolic steroid and terpenoid. The results of these studies indicate that pure bundung plant extract has antibacterial activity through the content of flavonoid compounds (Noval *et al.*, 2019). In betel leaf infusion formulation, lime peel extract and bundung plant extract also have antibacterial or antiseptic activity even though it is only used at smallest concentration, so it is necessary to design a new formulation by increasing concentration of bundung plant extract which has antibacterial activity.

Stability Test of Formulations

1. Organoleptic Test

Organoleptic test in this study include color, odor and physical. Based on data in Table 3, it is know that color test on F1 is light brown-yellow, F2 is yellow-brown, F3 is dark brown yellow and F4 is brown. This difference is due to the concentration of extract in different solutions, giving rise to a distinctive color for each formulation. Odor test conducted showed that F1 smelled fresh with a little betel smell, F2 smelled slightly betel, F3 and F4 smelled betel. The smell of betel that dominates in F1 to F4 is due to the very distinctive and pungent smell of betel leaf extract that exceeds the smell of lime peel extract and bundung plant extract. In physical test, it was found that there was a small amount of sediment at F1 and F2, there was a deposit on F3 and a lot of sediment at F4. The precipitate is a kind of

concentrated solution which is influenced by the concentration of extract from each formula (Haryono, *et al*, 2021)

Organoleptic test results after storage for 6 weeks showed that the preparation did not change significantly from the first week of testing, is it could be said that preparation was stable during storage. This result is supported by the journal (Mardiana *et al.*, 2015) which states that there is no difference in results during storage. These results are also supported by research (Noval *et al.*, 2020) which states that gel from bundung plants extract did not change in organoleptic testing during storage (Syahrina & Noval, 2021).

2. pH tester

pH testing n preparations aims to determine the resulting pH value. From data Table 4, it can be seen that pH of preparations from 4 formulations in the first week is between 3,2-3,7 which is below the critical value of skin pH around 4,5-6,5 (Harliantika & Noval, 2021). pH can affect the absorbance of skin which mean can cause irritation. If pH value is too low, it can cause irritation, and if it is too high can cause scaly skin (Ansel, 1989; Dhawan *et al.*, 2009). pH stability test results showed an increase from F1 to F4. But still, the pH value does not meet the criteria required for the skin.

This is because one of extract used is the lime peel. Lime peel has a very acidic pH below the pH skin (Kesehatan, 2000). Another extract have a pH is also acidic, but still in the range of pH skin, such as a preservative solution from betel leaf is in pH range 4,61-4,65 (Putri & Sukma, 2019). Whereas in bundung plant extract pH exceeds range required for the skin, as reported in the study (Noval *et al.*, 2020) that bundung plant extract gel has a pH range of 7,93-8,19 but after storage for 4 weeks pH decreased to 5-6 (Novia & Noval, 2021).

Changes in pH value during storage from first week to 6th week did not show a significant change in all formulations despite increases and decreases. As in research (Ardana *et al.*, 2015) also states that after storage, there is an increase and decrease pH value of preparation. However, changes in pH value do not occur significantly. then this preparation can be said to be stable during storage (Panjaitan *et al*, 2012). Instability can damage the preparation during use, and changes in pH value can be caused by environmental factors such as temperature, poor storage (lighting and humidity), a combination of extracts that are less stable in preparation due to oxidation (Dhawan *et al.*, 2009).

3. Viscosity Test

Viscosity testing aims to determine viscosity value in a substance. According to (Ghazali, 2009) viscosity is a tendency to oppose the flow of fluids due to resistance if a material which changes shape when the material is subjected to a force (Noval *et al*, 2021). The viscosity of Newtonian flow does not change with the change in friction force between the

surface of liquid and wall. Newtonian fluids are usually chemically pure and physically homogeneous liquids. According to (Giancoli, 2001) the flow rate is different because of the difference in viscosity. Amount of viscosity is expressed by a number which indicates the viscosity of a liquid. The viscosity of each fluid is different and is expressed quantitatively by the viscosity coefficient (Garg, 2002).

Based on Table 5, the results of viscosity measurements from the first week of storage to 6th week show that there was a significant increase and decrease the value of all formulations (Novia & Noval, 2021). From viscosity value, it can be said that preparation experiences instability during storage. Whereas the value of viscosity at one time whit a given rotation speed ad 12 rpm, 30 rpm and 60 rpm shows that the greater rotation speed given then viscosity will decreases (Kurniawati *et al*, 2020).

Flow properties of all dosage formulas illustrate the decrease in viscosity value with faster rotation. Viscosity is related to flow properties because viscosity will affect the resistance of a liquid to flow (Afianti and Murrukmiyadi, 2015; Noval *et al.*, 2019). Whereas according to (A. Martin, 2008) flow properties can decrease due to size and shape of a molecule, temperature and presence of colloids can increase flow properties, and presence of electrolytes can decrease flow properties (Lachman *et al.*, 2007).

Based on the figure above, the flow properties of dosage are included in the pseudoplastic-type non-newton flow because it changes when the shear rate is increased (Lieberman, 2005). In non-newton fluids pseudoplastic type, shearing rate and shearing stress have no linear relationship, and viscosity varies depending on the amount of pressure applied (Lachman *et al.*, 2007). It is also made clear by (Aulton, 2001) that a preparation which has a gradual increase in shear rate and is immediately followed by a decrease in shear rate to zero, will produce a decreasing curve that is different from ascending curve (Martin *et al.*, 1993).

4. Cycling test

Observation of stability test cycling test to test the preparation for the possibility of crystallization or cloudiness and to test the stability of preparation (Ardana *et al.*, 2015). Cycling stability test from 0th cycle to 6th cycle in formulations 1, 2, 3 and 4 showed no significant changes in color and odor. Whereas on pH testing F1 and F2 experienced an insignificant decrease. Where in Table 6, F1 on 0th cycle is pH 3,2 and 6th cycle is pH 3,1. As for F2 on 0th cycle is pH 3,5 and 6th cycle pH 3,4. At F3 and F4 pH value remains from 0th cycle to 6th cycle. These results indicate that the cycling test does not affect the organoleptic stability of the odor, color and pH of preparation.

Syneresis testing to see weight reduction during storage in a cycling test by comparing it with the initial weight (Handayani & Nurcahyanti, 2015). From the results in table and figure

above, it can be seen that during storage with cycling test, there was a reduction in weight of all formulations. The greatest syneresis was found in F3 where the weight loss reached 1,88 gram. In the sense of extreme temperature changes on cycling test the preparation experiences instability, because of the large amount of weight loss experienced. This can be due to carrier material in preparation, unable to maintain the components in preparation (Rowe, 2009).

5. Stability Test

Observation of stability test at low temperature ($4 \pm 2^{\circ}\text{C}$) and high temperature ($40 \pm 2^{\circ}\text{C}$) in 4 formulations showed that there was no significant or stable change in color and odor, this can be seen in Table 7 and Table 8 in Figure 6 and Figure 8. This is because one of the components of preparation is betel leaf extract which is not affected by changes in temperature (Hoque et al., 2011), both low ($4 \pm 2^{\circ}\text{C}$) and high temperatures ($40 \pm 2^{\circ}\text{C}$). These results indicate that stability test at all temperatures of all formulations did not affect the stability of preparation on odor, color and pH.

Syneresis testing looked at weight reduction during storage with various temperatures by comparing with initial weight (Handayani & Nurcahyanti, 2015). Based on table and figure above, it can be seen that during storage at various temperatures, there is a reduction in weight of all formulations significantly. the greatest syneresis at low temperature is F4 with a weight loss of 0,57 grams. While the high temperature at F1 is 1,18 grams. For these results, the formulations that showed a large amount of syneresis varied from each formulation at various temperatures. But the greatest synergy occurs at high temperatures. Then it can be stated that preparation experiences instability in syneresis at all test temperatures due to a large amount of weight loss. This can be caused by the carrier material in preparation not being able to maintain the components in preparation so that the carrier will escape or leave (Noval et al, 2021).

Conclusion

The conclusion is, based on research, that formulations of betel leaf infusion extract, lime peel ethanol extract and bundung plant ethanol extract have antimicrobial activity proportional to the concentration; therefore, the higher concentration used the higher microbial inhibition. F1 20% has moderate strength inhibition against *Escherichia coli*. F2 30% and F3 40% has a very strong inhibition against *Staphylococcus aureus* and *Escherichia coli*. F4 50% has a very strong inhibitory power for all tested microbes and is greater than the positive control, the formulation is stable in color, odor and pH but no stable in term of syneresis.

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