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Antibacterial (*Staphylococcus aureus* and *Escherichia coli*) and Antifungal (*Saccharomyces cerevisiae*) Activity Assay on Nanoemulsion Formulation of Ethanol Extract of Mangosteen Leaves (*Garcinia mangostana* L.) as Fruit Preservative

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Abstract: Fruits after harvesting will decay faster if not handled properly. Fruits can be damaged by bacterial and fungal. Mangosteen leaves contain xanthons which are antibacterial and antifungal. 50% ethanol extract of mangosteen leaves is formulated in Nanoemulsion preparations using the SNEDDS (Self-Nanoemulsifying Drug Delivery System) method. The mangosteen leaves 50% ethanol extract nanoemulsion formulation consisted of VCO (Virgin Coconut Oil) as oil, tween 80 as surfactant, and PEG 400 as cosurfactant. There are 3 formulations with variations in the concentration of mangosteen leaves ethanol extract, there are concentrations of 1%, 2%, and 3%. All formulations have a T% of more than 90%. The results of the particles measurement of nanoemulsion using PSA were in formulation 1 amounting to 16.1 nm; formula 2 is 16.7 nm; and formula 3 is 16.6 nm. The zeta potential characterization shows that formula 1 has a zeta value of -40.9 mV. The three formulations had a pH of 5. The largest inhibitory zone in the mangosteen leaf ethanol extract against S.aureus and E. coli bacteria were 11.08 mm and 5.87 mm respectively. Whereas in the S. cerevisiae antifungal test there was no inhibition zone at all concentrations. In the antibacterial and antifungal tests nanoemulsion preparations did not produce inhibitory zones in each concentration. Nanoemulsion preparations can retain the quality of strawberries when compared to the non-nanoemulsion preservative group, both in room storage and refrigerator temperature. The best preservative result is when the fruit is coated with nanoemulsion preservative and stored in refrigerator temperature.

Keywords: mangosteen leaf extract, antibacterial, antifungal, nanoemulsion, fruit preservative

1. INTRODUCTION

Indonesia has abundant natural resources including agricultural produce in the form of fruits and vegetables. Fruits that are not handled properly will decompose faster. This is because the process of respiration and transpiration in fruit that will continue after the fruit is harvested. This respiration takes place aerobically by requiring oxygen and producing CO_2 and H_2O. Increased respiration during ripe fruit causes the overhaul of polysaccharides and cell wall compilers to run fast, so that the texture of the fruit will be softer. Besides that, fruit damage can also be caused by mechanical influences, for example due to impact, scratches on the skin or microbiological damage such as decay by microbes, so that the shelf life is relatively short (Purwadi, n.d., 2007). The example

of microbial that can damage the fruits quality is bacterial and fungal. Based on that statements, antimicrobial is needed as preservative fruits.

According to Friedman et al. (2002) and Gulmez et al. (2006), the use of plant and herbal extracts as antimicrobial agents in food and soft drinks has also been reported for centuries (Jabeen and Khanum, 2017). Chemical food preservatives have the potential for toxicity, so it will increase the demand on natural food preservatives. Antimicrobial agents used as preservatives are chemical compounds that protect food from spoilage by inhibiting the growth of pathogenic microorganisms and increasing shelf life (Jabeen and Khanum, 2017).

The mangosteen plant has antioxidant compounds consisting of xanthones, tannins, phenolic acids, and anthocyanins. Among these compounds, the ones with the highest levels of antioxidants are xanthones. Xanthones are beneficial for the body, such as anti-inflammatory, anti-diabetic, anti-cancer, antibacterial, anti-fungal, anti-plasmodial, and are able to increase immunity and are also hepatoprotective (Shiddiqi et al., 2014).

One of antimicrobial agent can be found in mangosteen leaves. Some compound that can be pulled out by 50% ethanolic solvent in mangosteen extract is phenolic, flavonoid, and saponin compounds. The research that has been done by Diniatik and Suparman (2010) reported the antibacterial activity from mangosteen leaves is better than the skin of mangosteen fruit. The first phase of the study show that 50% ethanol extract of mangosteen leaves have the best potential for antioxydant, antifungal, and antibacterial activity.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Material

The part of mangosteen plant that researcher used is mangosteen leaves part. This plant were collected from Kemranjen District, Banyumas Regency, Central Java Province, Indonesia. The mangosteen leaves were indentified in environmental laboratory Faculty of Biology, Jenderal Soedirman University, Indonesia.

2.2 Methods

2.2.1. Preparation of Ethanol Extract

Mangosteen leaves were collected, dried and pulverized by mechanical grinder. The powder of mangosteen leaves 500 grams were extracted by maseration and remaceration method for 2x24 hours with comparison between the powder and ethanol is 1:5 for the first day, and 1:4 for the second day. Stirring process is needed to improve the effectiveness during extraction. Extracts of mangosteen powder with ethanol were evaporated with pressure at 40^0C until getting thick consistency.

2.2.2. Preparation of Nanoemulsion

This nanoemulsion consist of nanoemulsion base like Virgin Coconut Oil (VCO) as oil phase 1 ml, Tween 80 as surfactant 5 ml, and PEG 400 as co-surfactant 4 ml. This nanoemulsion base is replicated to 3 formulation, each formula consist of different ethanolic mangosteen leaves extract 1 ml, there are 10mg/ml ; 20 mg/ml; and 30 mg/ml. Total volume for each formulation is 11 ml.

All base composition is mixed in one, then use vortex for 1 minute, sonicated for 10 minutes, use waterbath at 45^0C for 15 minutes to improve the homogenisation process. Then adding the ethanolic mangosteen leaves extract to the base, vortex it for 1 minute, sonicated for 10 minutes, then

put the formulation on waterbath at 45^{0}C for 15 minutes. That 100 µl SNEDDS added to 5 ml distilled water and mixed it well using vortex 30 seconds.

2.2.3. Transmittance Measurements

The sample was prepared by mixing SNEDDS 100 µl with distilled water 5 ml using vortex for 30 seconds. The emulsions were measured the precents transmittance at 650 nm using UV spectrophotometer to determine the level of clarity (Pratiwi, 2017).

2.2.4. pH Measurements

SNEDDS 100 µl was dissolved in 5 mL distilled water then use pH stick to determine nanoemulsion pH for food.

2.2.5. Particle Size and Zeta Potential Analysis

This analysis using a Particle Size Analyzer (PSA) to know the distribution of nanoparticles. A total of 1 mL SNEDDS was mixed with distilled water to 5 mL, then homogenized slowly. Zeta potential measurements were determined with a Zetasizer (Pratiwi, 2017).

2.3. Antibacterial Assay

2.3.1. Preparation of Medium

Nutrient Agar (NA) 23 grams dissolved with 1000 mL of aquadest, then heated to dissolved completely. Put the warm NA solution 5 mL for each tube, then sterilized in autoclave at 121°C for 15 minutes. This NA is used to make bacterial culture. Mueller Hinton Agar (MHA) 38 grams dissolved with 1000 mL aquadest then heated it. Put 12mL MHA warm solution into each tube, then sterilized in autoclave at 121°C for 15 minutes.

2.3.2. Culture of *Eschericia coli* (*E.coli*) and *Staphylococcus aureus* (*S. aureus*)

A total of one ose E. coli and S. aureus from stock were taken with sterile ose needle and put it into test tube containing aseptically solid NA and incubated at 37^{0}C for 24 hours.

2.3.3. Bacterial Suspension

A total of one ose E. coli and S. aureus from stock were taken with sterile ose needle and put it into 10 mL NaCl 0,9% sterile. Absorbanced in UV spectrophotometer with wavelength 625 nm. The allowed range of absorbantion is 0,08-0,13 that have same meaning colony level of bacterial 1-2 x 108 CFU/mL (Aristyawan et al., 2018).

2.3.4. Antibacterial Activity Assay

Each petry dish contain of 12 mL MHA media, 5 papper disc, then replicated to 3 petry dish for each bacterial. Each papper disc contain of negative control (DMSO 10%); positive control (Cyprofloxacin 3µg/1µl); and ethanolic mangosteen leaves extract with 1%, 2%, 3% concentration. Before putting the papper disc, take 100 µl bacterial suspension then put it in MHA when still liquid medium. After all, put the petry dish in incubator at 37°C for 24 hours. The diameter of the inhibitory zone in observed.

2.4. Antifungal Assay

2.4.1. Preparation of Medium

Potato Dextrose Agar (PDA) 39 grams dissolved with 1000 mL of aquadest, then heated to dissolved completely. Put the warm PDA solution 5 and 15 mL for each tube, then sterilized in autoclave at 121°C for 15 minutes. 5 mL PDA for fungal culture and 15 ml PDA for antifungal assay.

Potato Dextrose Broth (PDB) 24 grams dissolved with 1000 mL aquadest then heated it. Put 10 mL PDB warm solution into each tube , then sterilized in autoclave at 121°C for 15 minutes.

2.4.2. Culture of *Saccharomyces cerevisisae (S. cerevisiae)*

A total of one ose S. cerevisiae from stock were taken with sterile ose needle and put it into test tube containing aseptically solid 5 mL PDA and incubated at 28^0C for 48 hours.

2.4.3. Fungal Suspension

A total of one ose S. cerevisiae from culture was taken with sterile ose needle and put it into 10 mL PDB sterile. Absorbanced in UV spectrophotometer with wavelength 600 nm. The allowed range of transmittance is 20%-80%

2.4.4. Antifungal Activity Assay

Each petry dish contain of 15 mL PDA media, 5 papper disc, then replicated to 3 petry dish. Each papper disc contain of negative control (DMSO 10%); positive control (Cetoconazole 5µg/1µl); and ethanolic mangosteen leaves extract with 1%, 2%, 3% concentration. Before putting the papper disc, take 100 µl fungal suspension then put it in PDA when still liquid medium. After all, put the petry dish in incubator at 28°C for 48 hours. The diameter of the inhibitory zone in observed.

2.4.5. Application of Nanoemulsion as Fruits Preservative

Fruits were filtered then soaked with nanoemulsion for 5 minutes and dried the fruits well. Fruits were covered by styrofoam and wrapping plastic. Then keep the fruits in room and refrigerator temperature**.**

2.4.6. Loosing fruits weight measurements

This measurements is calculated by decreasing of weight since the first day of keeping fruits until the last day during keeping period.

$$Loosing\ Weight\ = \frac{W1-W2}{W1} \times 100\%$$

W1 = the weight of first day keeping (g)

W2 = the weight of last day keeping (g)

2.4.7. Fruits Organoleptic Acceptable Assay

This assay aims to know the fruits quality after using mangosteen leaves nanoemulsion through the sense of colour, texture, and fragrance. A total of 20 people asked to determine the likely level of each fruits using hedonic scale. There are 7 hedonic scale start from 1 (very not interested), 2 (not interested), 3 (almost not interested), 4 (neutral), 5 (almost interested), 6 (interested), and 7 (very interested). The limit of rejected fruits is scale of under 3.5 (Marpaung, 2015).

3. RESULT AND DISCUSSION

3.1. Formulation of Self Nanemulsifying Drug Delivery System (SNEDDS)

Nanoemulsions are made in a drug delivery system called the Self-Nanoemulsifying Drug Delivery System (SNEDDS). SNEDDS is a mixture of oil, surfactants, cosurfactants and active substances which when mixed with water will form an oil/water (M/A) type nanoemulsion (Lina et al., 2017). In the development of a drug delivery system based on pharmaceutical technology, a provision is needed that can increase the ability of active compounds to penetrate, one of which is the self-nanoemulsifying drug delivery system (SNEDDS). According to Nazzal (2002), SNEDDS is an isotropic mixture consisting of oil, surfactants, and cosurfactants which quickly form an emulsion when it meets water (Sari et al., 2016).

Nanoemulsion is considered more stable than conventional emulsion because the small droplet size will prevent phase separation (Mulia et al., 2018). Conventional emulsions are unstable against flocculation, creaming, and a combination of oil and water. Nanoemulsion as a better option because of the nano-size of the emulsion droplets (5-100 nm) and their transparency, improved stability and bioavailability. According to Azeredo (2009), nanoemulsion allows the development of an edible nano layer as thin as 5 nm. An example is nanocoating which can be eaten and used in meat, cheese, fruit and vegetables, bread, and fast food. This coating can provide a barrier for moisture and gas exchange; acts as a colorant, antioxidant, antimicrobial, and antibrowning; and it can also increase the shelf life of food produced, even after the package is opened. In addition, edible coatings can also maintain the quality of processed fruits and vegetables minimally by minimizing moisture loss, gas exchange, respiration, oxidation, and physiological disturbances (Oprea, Alexandra Elena and Alexandru Mihai Grumezescu, 2017)

VCO is include the oil phase that has medium chain oil. Nanoemulsion needs oil phase with medium fatty acid chain. Tween 80 is non ionic surfactant that has high HLB value 15. This value can improve the decreasing of surface tension between oil and water. PEG 400 as co-surfactants can increase the surface fluidity through penetration way to surfactants film that can create a free space between the surfactants molecules (Pratiwi et al, 2018). Nanoemulsion of ethanolic mangosteen leaves extract can be shown on Figure 1.

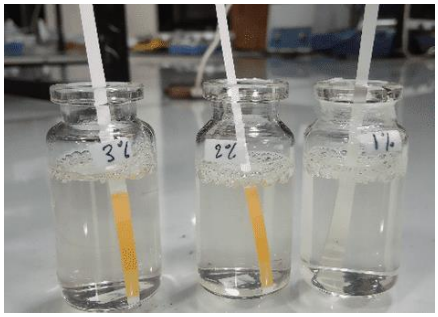


Figure 1. Ethanolic mangosteen leaves extract nanoemulsion formulation.

3.2. Transmittance Measurements

Good nanoemulsion must have a clear visual sighting with transmittance more than 90%, so that formula could be said to form a medium nanoemulsion when it was emulsified in water (Pratiwi, 2017). Based on data of transmittance, all formula have transmittance more than 90%. Decreasing of

transmittance during keeping process is caused by nanoemulsion characteristic that can not stabile for long time keeping process.

Table 1. Transmittance measurements result

	1%	2%	3%
First week	98.9 %	98.6 %	97.8 %
Second week	97.5 %	96.9 %	96.3 %
Third week	96.6 %	95.7 %	94.4 %
Fourth week	95.4 %	94.4 %	94.1 %

3.3. pH Measurements

Nanoemulsion pH testing aims to determine the safety of each formulation especially when it is used for food.

Table 2. pH measurement result

	1%	2%	3%
First week	6	6	6
Second week	5	5	5
Third week	5	5	5
Fourth week	5	5	5

Based on table 2, this nanoemulsion has good pH because not include as strong acid food (pH<4,6).

3.4. Particle Size

Particle size of nanoemulsion is < 100 nm. Based on Table 3, all particle size is less than 100 nm.

Table 3. Particle size result

Concentration of ethanolic mangosteen leaves extract	**PSA (nm)**
1 %	16.1
2 %	16.7
3 %	16.6

3.5. Zeta Potential

Zeta potential values which were less than -30 mV or greater than 30 ,V indicated a stable nanoemulsion (Ujilestari, 2018). Based on the result, zeta potential in 1 % concentration extract is -40 mV. The zeta surface has potential to produce an electric repulsion between oil droplets which can inhibit droplet incorporation (Haryani et al. 2017). This zeta potential is less stable because electric repulsion not enough to refuse between each dominant droplets in nanoemulsion system and can cause droplets aglomeration (Ariviani et al., 2015).

3.6. Antibacterial Assay of Ethanolic Mangosteen Leaves Extract

According to Furukawa et al. (1996) and Chen et al. (2008), both α- and β-mangostin found mainly in mangosteen rind can inhibit some microorganisms (Palakawong et al., 2010). Based on the research of Ilnuma et al. (1996) and Sakagami et al. (2005) reported that the MIC value of α-mangostin against S. aureus was lower than that of β-mangostin (6.25 and> 100 μg / ml, respectively). From the literature and the results of this study, it can be concluded that the efficiency of microbial inhibition might result directly from α-mangostin (Palakawong et al., 2010). Xanton which is included in secondary metabolites can be isolated from the skin, whole fruit, and mangosteen leaves. Several studies have shown that xanton has biological activity as an antioxidant, anti-tumor, anti-inflammatory, allergy, antibacterial, antifungal, and antiviral (Palakawong et al, 2010). The alpha mangostin, gamma-mangostin and garsinon B compounds can inhibit the activity of Mycobacterium tuberculosis, the alpha mangostin content is also active against E. coli and S. aureus bacteria (Sujono and Nuryati, 2017). Mangosteen leaves contain two xanthone compounds, namely 1,5,8-Trihydroxy-3 methoxy-2- (3-methylbut-2 enyl) xanton and 1,6- Dihydroxy -2- (2hydroxy-3- methylbut -3enyl) - 3, 7- dimethoxy-8- (3 methylbut-2-enyl) - xanton (Obolskiy et al., 2009).

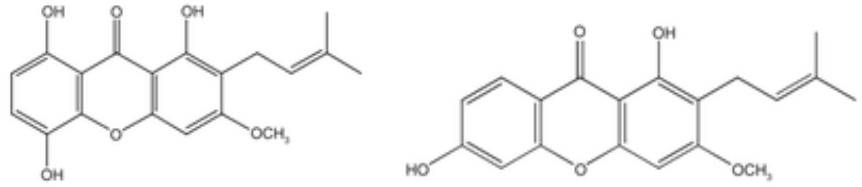


Figure 2. Structure 1,5,8-Trihydroxy- 3 methoxy-2-(3-methylbut-2 enyl) xanton and 1,6- Dihydroxy -2- (2hydroxy-3- methylbut -3enyl) – 3, 7- dimethoxy-8- (3 methylbut-2-enyl)- xanton (Obolskiy, 2009).

This antibacterial assay is used on disc diffusion method through calculate the inhibitory zone around papper disc. The result on Table 4 and 5 show that ethanolic mangosteen leaves extract has antibacterial effect to *S. aureus* and *E. coli* bacterial. Each extract concentration has different bacterial inhibitory zone. The higher concentration of the ethanol extract of mangosteen leaves, the higher the inhibition zone of *S. aureus* and *E. coli*. The difference of inhibitory zone between *E. coli* and *S. aureus* is caused by *E. coli* has more complex peptidoglycan, lipoprotein, and complex polysaccharides. The outer membrane of *E. coli* can resist both hydrophobic and hydrophilic molecules as well. Large substance molecules cannot enter the *E. coli* bacteria, whereas substances with small molecules can enter the bacteria. This difference can cause *E. coli* more resistant than *S. aureus*. Anova analyzes show that there are significancy data in *E. coli* assay. Post Hoct test has result that each extract concentration has different significancy to positive control and negative control.

Some compound that can be pulled out by 50% ethanolic solvent in mangosteen extract is phenolic, flavonoid, and saponin compounds. The research that has been done by Diniatik and Suparman (2010) reported the antibacterial activity from mangosteen leaves is better than the skin of mangosteen fruit. The first phase of the study show that 50% ethanol extract of mangosteen leaves have the best potential for antioxydant, antifungal, and antibacterial activity.

Another study showed that the optimized mangosteen peel extract contained alpha-mangostin compounds with strong antibacterial activity against gram-positive *Staphylococcus aureus.* The

optimized diameter (ID) inhibitor of mangisteen peel extract had greater inhibition against *Staphylococcus aureus* (ID: 18 mm) than ciprofloxacin (ID: 16 mm) (Ghasemzadeh et al, 2018).

Replication 1 **Replication 2** **Replication 3**

Figure 3. Inhibitory zone from ethanolic extract magosteen leaves in *S. aureus* bacterial

Table 4. Measurements of Inhibitory zone from ethanolic extract magosteen leaves in *S. aureus* bacterial

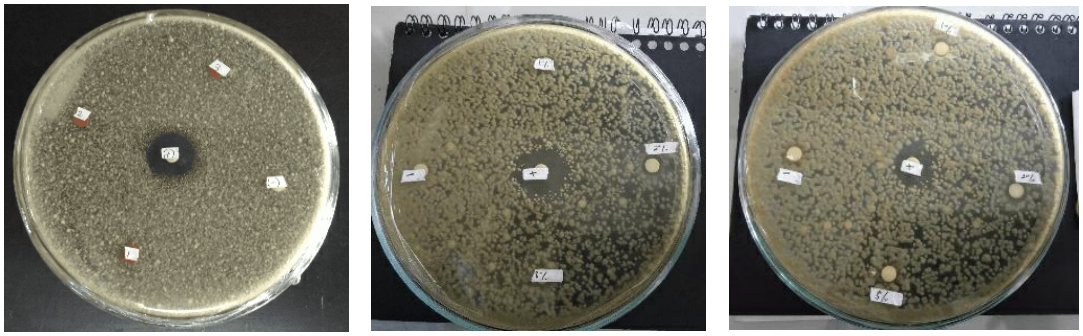
Sample	Inhibitory zone diameter (mm)			
	Replication 1	**Replication 2**	**Replication 3**	**Average ± SD**
Negative control	-	-	-	-
Positive control	38.50	51.40	39.35	43.08 ± 7.215
Consentration 1%	13.05	13.70	15.30	14.02 ± 1.158
Consentration 2%	15.00	15.40	17.68	16.03 ± 1.446
Consentration 3%	17.50	16.90	16.83	17.08 ± 0.368

Replication 1 **Replication 2** **Replication 3**

Figure 4. Inhibitory zone from ethanolic extract magosteen leaves in *E. coli* bacterial

Table 5. Measurement Inhibitory zone from ethanolic extract magosteen leaves in *E. coli* bacterial

Sample	Inhibitory zone diameter (mm)			
	Replication 1	**Replication 2**	**Replication 3**	**Average ± SD**
Positive control	-	-	-	-
Positive control	48.60	43.00	4525	45.62 ± 2.818
Concentration 1%	9.40	9.80	8.90	9.37 ± 0.451
Concentration 2%	9.85	11.60	9.38	10.28 ± 1.170
Concentration 3%	13.80	12.10	9.70	11.87 ± 2.060



Replication 1 **Replication 2** **Replication 3**

Figure 5. Inhibitory zone from ethanolic extract magosteen leaves in *S. cerevisiae* fungal

Table 6. Measurements of Inhibitory zone from ethanolic extract magosteen leaves in *S. cerevisiae* fungal

Sample	Inhibitory zone diameter (mm)			
	Replication 1	**Replication 2**	**Replication 3**	**Average ± SD**
Positive control	-	-	-	-
Positive control	24. 5	23.2	21.0	22, 9 ± 1,445
Concentration 1%	-	-	-	-
Concentration 2%	-	-	-	-
Concentration 3%	-	-	-	-

In antifungal assay on table 6, ethanol extract of mangosteen leaves do not have inhibitory zone diameter in concentration 1%, 2%, and 3%. Whereas in the Diniatik research (2012), ethanol extract of mangosteen leaves can inhibit S. cerevisiae fungi at a concentration of 1000 mg/ml at 1,0083 cm and 1250 mg/ml at 1, 071 mg/ml, but has not been able to inhibit at a concentration of 500 mg/ml and 750 mg/ml. It can be said that the ethanol extract of mangosteen leaves at a concentration of 10 mg/ml (1%), 20 mg/ml (2%), and 30 mg/ml (3%) has not been able to inhibit the growth of *S. cerevisiae* fungi because the concentration is too little.

3.7. Antibacterial Assay of nanoemulsion of ethanolic mangosteen leaves extract

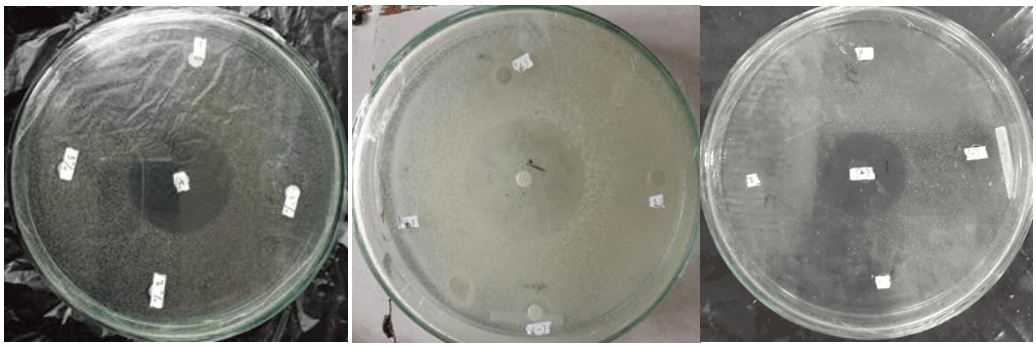
This test aims to confirm the presence of antibacterial activity or not if the ethanol extract of mangosteen leaves is made for nanoemulsion preparations. That is because the mangosteen leaf extract itself has antibacterial activity.

Replication 1 **Replication 2** **Replication 3**

Figure 6. Inhibitory zone from nanoemulsion of ethanolic magosteen leaves in *S. aureus* bacterial

Table 7. Measurements of Inhibitory zone from nanoemulsion of ethanolic magosteen leaves in *S. aureus* bacterial

Sample	Inhibitory zone diameter (mm)			
	Replication 1	**Replication 2**	**Replication 3**	**Average ± SD**
Positive control	-	-	-	-
Positive control	30.80	31.90	30.60	31.10 ± 0.572
Concentration 1%	-	-	-	-
Concentration 2%	-	-	-	-
Concentration 3%	-	-	-	-

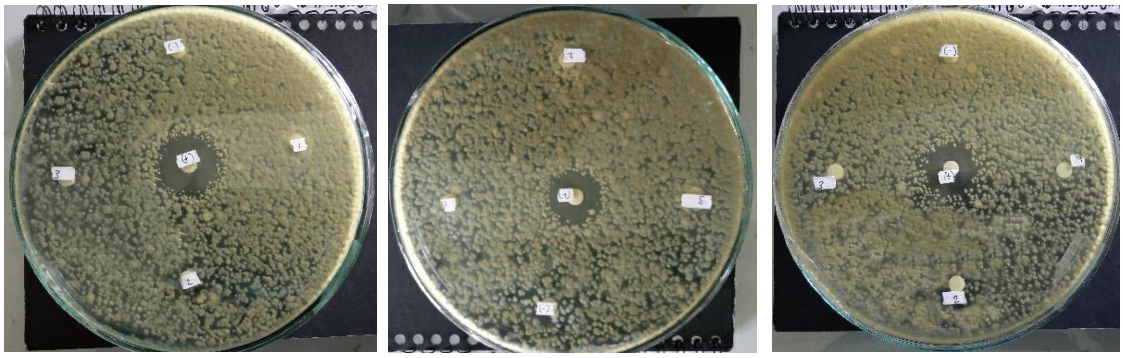


Replication 1 **Replication 2** **Replication 3**

Figure 7. Inhibitory zone from nanoemulsion of ethanolic magosteen leaves in *E. coli* bacterial

Table 8. Measurements of Inhibitory zone from nanoemulsion of ethanolic magosteen leaves in *E. coli* bacterial

Sample	Inhibitory zone diameter (mm)			
	Replication 1	**Replication 2**	**Replication 3**	**Average ± SD**
Positive control	-	-	-	-
Positive control	30.80	31.90	30.60	31.10 ± 0.572
Concentration 1%	-	-	-	-
Concentration 2%	-	-	-	-
Concentration 3%	-	-	-	-



Replication 1 **Replication 2** **Replication 3**

Figure 8. Inhibitory zone from nanoemulsion of ethanolic magosteen leaves in *S. cerevisiae* fungal

Table 9. Measurements of Inhibitory zone from nanoemulsion of ethanolic magosteen leaves in *S. cerevisiae* fungal

Sample	Inhibitory zone diameter (mm)			
	Replication 1	**Replication 2**	**Replication 3**	**Average ± SD**
Positive control	-	-	-	-
Positive control	30.80	31.90	30.60	31.10 ± 0.572
Concentration 1%	-	-	-	-
Concentration 2%	-	-	-	-
Concentration 3%	-	-	-	-

Based on table 7 and 8, naoemulsion formulation of mangosteen leaves extract with consentration 1%, 2%, 3% can not inhibit the bacterial of *S. aureus* and *E. coli*, and also *S. cerevisiae* fungal. This nanoemulsion is made to keep the fresh fruits have longer save time than before not using nanoemulsion. Although this formula does not have antibacteria and antifungal activity, this formulation can keep long save time for the fruits after harvesting.

3.8. The result of aplicating nanoemulsion formulations of ethanolic mangosteen leaves extract on strawberry fruits

3.8.1. Weight Loss Assay

This is one of parameter that can show the decreasing of fruits quality and levels of fruits freshness. The decreasing of weight in fruits is caused by losing water from transpiration and respiration process. During storage there is an increase in the weight loss of strawberries, both at room temperature and refrigerator temperature (Table 9). The loss of water causes strawberries have decreasing in weight. In addition, the process of respiration or the breakdown of complex compounds into simple compounds with low molecular weight also causing weight loss. Water loss during storage not only decreases weight loss, but also reduces quality and causes fruits damage so that the fruit withers and wrinkles (Marpaung, 2015).

The biggest weight loss change that occurred in strawberry storage at room temperature was strawberries with nanoemulsion concentration of 1% by 38.2%. While the smallest change in room temperature occurred in strawberries with a concentration of ethanol extract of mangosteen leaves 3% by 21.1%. Storage in the refrigerator's cold temperature, where the biggest change is in the concentration of 2% while the smallest change is in the strawberry concentration of 3%. Cold

temperatures can inhibit the ripening of fruits and vegetables through the process of inhibiting respiration and capturing the formed ethylene gas. So that storage at cold temperatures has a longer period of time than storage at room temperature.

Table 10. Inhibitor zone from nanoemulsion of ethanolic magosteen leaves in *E. coli* bacterial

Day	Weight loss (%)							
	Control		1%		2%		3%	
	Room	Refrig	Room	Refrig	Room	Refrig	Room	Refrig
0	0	0	0	0	0	0	0	0
1	2.4	2.65	3.3	1.8	3.13	1.8	4.2	2.1
3	11.2	5.23	15	7.0	15.4	6.8	17.9	5.8
5	30.2	8	38.2	8.63	29.3	12.5	21.1	10.5
7	-	5.2	-	17.3	-	18.2	-	14
9	-	14.1	-	22.7	-	23.9	-	18.3
11	-	17	-	28	-	29.3	-	21.5

3.8.2. Organoleptic Assay

Organoleptic test on the quality of strawberries during storage is determined through panelist assessment of fruit quality. Fruit quality attributes used include color, aroma, and texture. The level of panelists' preference for fruit attributes can be shown in table 11 it is known that nanoemulsion preparations can be used as preservatives in strawberries.

Table 11. The result of hedonic scale test from panelist

Parameter	Temperature	Hedonic test value							
		1%		2%		3%		Non preservative	
		A	B	A	B	A	B	A	B
Color	Room	**2.6**	**1.5**	3.5	**1.6**	3.7	**1.1**	**2.6**	**1**
	Cool	4.2	4.3	5.9	5.2	4.5	5.3	4.4	**2**
Texture	Room	4.5	**1**	4.2	**1.5**	**3.4**	**1.2**	**3.4**	**1**
	Cool	5.5	4.1	5.3	5	4.8	4.6	4.6	**2.7**
Aroma	Room	**2.9**	**1.2**	4.0	**1.1**	5.0	**1**	**3.4**	**1**
	Cool	5.0	4.5	5.4	5.2	4.8	**3.3**	3.8	**2.5**

Explanation A = Day 1

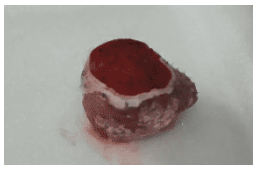
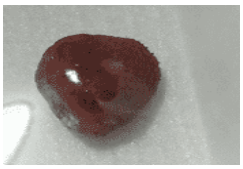
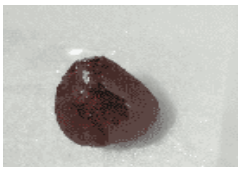
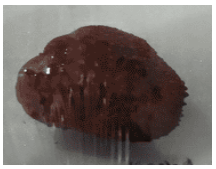
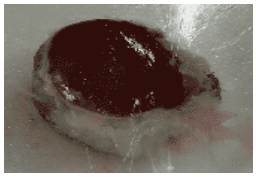
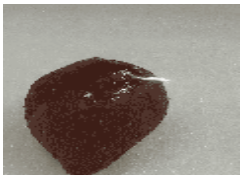
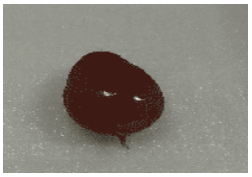
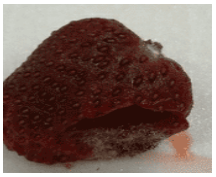
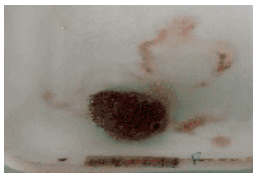
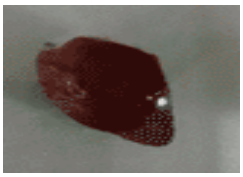
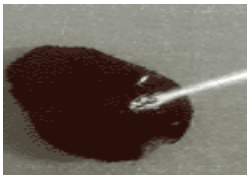
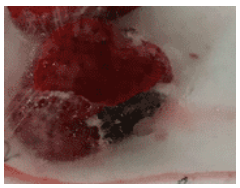
B = Day 5

On the treatment of strawberries on the first day to the fifth day, strawberries with a nanoemulsion layer of 1%, 2%, 3%, and the control group (non-preservative) could not be accepted by panelists on the fifth day with room temperature storage. This is because the fruit has experienced a decreased quality in color, aroma, and texture. These three indicators can reflect the taste in fruit. In contrast to the storage at the refrigerator temperature that can still survive its quality until day 5 (Aliya et al., 2016).

When viewed from the fruit rating scale when at room temperature, there is a slight difference between the control group and the group given nanoemulsion preservatives. The rating scale of the control group (non preservative) is lower than that of the group given preservatives. Although the

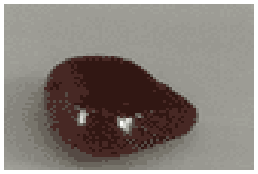
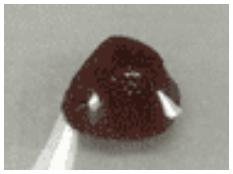
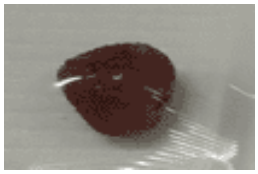
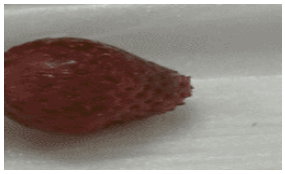
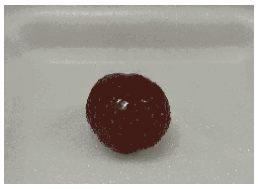
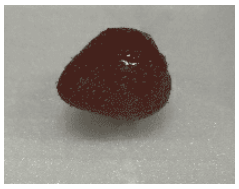
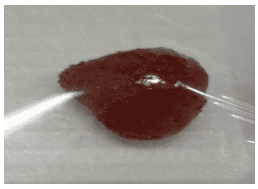
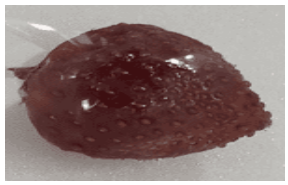
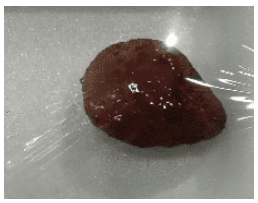
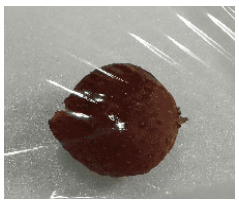
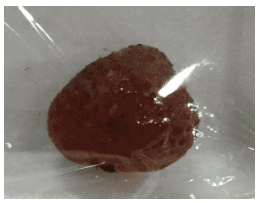
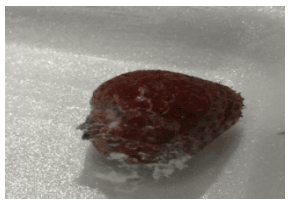
results of antibacterial and antifungal test of nanoemulsion preparations cannot provide inhibitory zones, these preparations can still help maintain fruit quality. The defense mechanism of fruit quality from nanoemulsion preparations can be through a process of minimization from loss of moisture, gas exchange, respiration, and oxidation.

Table 12. The result of nanoemulsion aplication on strawberries in room temperature storage

Nanoemulsion 1 %	**Nanoemulsion 2%**	**Nanoemulsion 3 %**	**Non nanoemulsion**
15 March 2019 			
16 March 2019 			
18 March 2019 			

Wahyudi (2018) reported that mangosteen leaves have very strong antioxidant properties with an average IC_{50} at a concentration of 1% which is 13,551 ± 0.034. Antioxidants act as donors of hydrogen atoms in free radicals to reshape fat molecules. If antioxidants are given it will slow down the auto-oxidation process. Therefore, the antioxidants contained in mangosteen leaves can increase the shelf life of strawberries at room temperature and refrigerator when compared to the non-preservative group of nanoemulsion (Table 11 and 12).

Table 13. The result of nanoemulsion aplication on strawberries in cool temperature storage

Nanoemulsion 1 %	**Nanoemulsion 2 %**	**Nanoemulsion 3 %**	**Non nanoemulsion**
15 March 2019			
			
16 March 2019			
			
18 March 2019			
			

4. CONCLUSION

Ethanol extract of mangosteen leaves have different antibacterial activity for each extract concentration. Antibacterial activity of mangosteen leaves extract for *S. aureus* is bigger than *E. coli* because of different cell membrane compound between them. In same concentration, mangosteen leaves extract can not inhibit *S. cerevisiae* as fungal that caused by the extract consentration is too small. Nanoemulsion of mangosteen leaves extract can not inhibit *S. aureus*, *E. coli*, and *S. cerevisiae* on the same extract concentration that used in extract antibacterial assay. But, nanoemulsion of ethanolic mangosteen leaves extract can keep the quality of strawberries better than non nanoemulion strawberries.

Conflicts of interest: The authors declare no conflict of interest.

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