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Original Article

Formulation of Antioxidant Gel Preparations on the Cherry (*Muntingia calabura* L.) Extract from Kupang, East Nusa Tenggara Based on AQUPEC 505 HV

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Abstract

Cherry (*Muntingia calabura* L.) from Kupang, East Nusa Tenggara is a local fruit in Indonesia which has many benefits for health. One of compounds contained in Cherry is polyphenol functioning as antioxidant. The purpose of this study was to obtain information about antioxidant activity contained in Cherry in the form of extract or gel preparations with different base of aqupec 505 HV with routine comparison. **Methods:** The method used in this study was quantitative analysis. The cherry was extracted by using reflux method with 70% ethanol solvent and the quantitative testing of antioxidant activity through DPPH method with methanol solvent. For the qualitative one, Thin Layer Chromatography was used with mobile phase of butanol: acetic acid: water (4:1:5). **Results:** From the physical quality testing of gel preparations, it was obtained stable result in the room-temperature storage and the result of physical quality testing of formula 3 was totally effective to be used on the skin. From the antioxidant activity testing of Cherry extract, it showed that the value of IC_{50} is 68.50 ppm. On the formula 1 (aqupec 505 HV 0.5%), the value of IC_{50} is 189.32 ppm. On the formula 2 (aqupec 505 HV 1%), the value of IC_{50} is 186.95 ppm. On the formula 3 (aqupec 505 HV 1.5%), the value of IC_{50} is 184.75 ppm. On the formula 4 (aqupec 505 HV 2%), the value of IC_{50} is 186.60 ppm. On the formula 5 (aqupec 505 HV 1.5% regularly), the value of IC_{50} is 174.73 ppm. The results of this study showed that the gel which was made was safe to use and the most effective one was on the formula 5 which obtained IC_{50} as many as 186.60 ppm.

Keywords: *Antioxidant; Cherry extract; Gel; Aqupec 505 HV.*

Introduction

In Indonesian, cancer, heart disease, diabetes, and degenerative diseases are increasingly becoming widespread. One of them is caused by free radical. Up to now, the exposure of free radical is quite extensive in people's lives such as, pollution and unhealthy food. One of antidotes of free radicals is an antioxidant (Winarsi, 2007).

Antioxidant is inhibitors of oxidation reaction by free radical that can damage unsaturated fatty acids, wall membrane, blood vessel, DNA bases, and lipid tissue, causing disease. The method used for antioxidant testing was the DPPH radical capture method. The parameters used for the measurement of the antioxidant activity of Cherry were radical capture percent and IC_{50} which were measured using UV-Vis spectrophotometer (Salamah & Widyasari, 2015).

Cherry is considered to have high antioxidant activity due to its natural anthocyanin content. Anthocyanin is a sub class of flavonoid which is important for a plant. Cherry also contains several other polyphenol compounds such a tannin (Zhang & Lin, 2009). Antioxidant is well applied in cosmetics, one of those is in a gel form.

Gel has several advantages, namely it is non-sticky, can be applied easily, easily washed, does not leave a layer of oil on the skin, the viscosity of the gel does not change during storage (Sihombing *et al*., 2013).

Gel consists of basic ingredients and additives. One of the basic ingredients of gel is Aqupec. Aqupec is an acrylic acid polymer that can increase viscosity at small concentrations, as well as increase gel stability (Wathoni, 2012). Aqupec is an acrylic acid polymer that can increase viscosity at small concentrations, as well as increase gel stability (Carter, 1995).

Based on Herni & Rahardjo (2014) research it seen that Cherry contains active compounds including polyphenols. Polyphenol is one of the compounds that have natural antioxidant activity, kersen fruit extract has an IC_{50} value of 12.84 ppm while it has an IC_{50} value of 319.89 ppm, from the results of these studies, Kersen fruit has a very strong antioxidant activity and has more potential to be developed as antioxidant. Therefore, this study is aimed to determine the stability of the cherry extract or gel preparations with a difference base of aqupec 505 HV with routine comparison.

Materials and Methods

Population and Sample

The sample used in this study was the extract of Cherry (*Muntingia calabura* L.) gel with aqupec 505 HV base with a concentration of 0.5%; 1%; 1.5% and 2%.

Research variable

Classification of variables

The independent variable in this study was a formulation with aqupec 505 HV base variation and a concentration of 0.5%; 1%; 1.5% and 2% for making antioxidant gel.

The controlled variable was Cherry extract (where plants grow, plant age), mixed composition, methods and processes of making antioxidant gels along with ingredients and analytical tools.

Dependent variable was the stability of gel, physical quality of gel (organoleptic, homogeneity, viscosity, spreadability, adhesion and pH) and antioxidant activity power of Cherry.

The operational definition of the main variable

Cherry Extract was made by reflux method with 70% ethanol solvent. Cherry was obtained from the East Penfui area of Kupang Regency. Manufacture of antioxidant gels used the Aquapec 505 HV base.

Methodology

Plant Determination

Determination and identification were based on morphological features that existed in plants on the literature as evidenced in the Phytochemical Laboratory of Pharmacy, Kupang Health Polytechnic of Health Ministry.

Drying Simplicia

Cherry was sorted and washed with water so that the dirt attached to the leaves was lost, then it was roasted at 40^{o}C.

Simplisia that was already dry was then pollinated with a pollinator and sieved in mesh 40 then weighed to determine the dry percent weight to wet weight.

Determination of the moisture content of the Cherry powder

Determination of moisture content was done by weighing the Cherry powder with 2 grams of moisture content using a moisture balance tool.

Making Cherry extract

The production of 50grams of Cherry powder were refluxed with 70% ethanol solvent and refluxed for 1 hour.

Determination of the moisture content of the Cherry extract

Determination of moisture content was done by extracting Cherry weighed 2 grams of

moisture content measured using a moisture balance tool.

Determination of organoleptic extract of Cherry

Determination of organoleptic in Cherry extract by observing the color, odor, and shape of the 70% ethanol extract of Cherry.

Alcohol free test of Cherry extract

The 70% ethanol-free test of concentrated extract of Cherry aimed to ensure that the concentrated extract of Cherry was free of 70% ethanol by an esterification reaction. Acetic acid and concentrated sulfuric acid were added into the test tube containing the extract, then it was heated. If a typical ester smell of alcohol was detected, the extract still contained 70% ethanol.

Identification of chemical content of Cherry extract

Identification of Cherry chemical content could be done with two events, namely by reagents and TLC, identification of polyphenols, flavonoids,

The design of the antioxidant gel formulation of Cherry ethanol extract

The gel formulation was then made on the basis of Aqupec 505 HV with various concentrations of 0.5%; 1%; 1.5% and 2%. The design of the antioxidant gel formula can be seen in table 1.

Table 1. The draft formula of the antioxidant gel extract of Cherry

Ingredients	F_1	F_2	F_3	F_4	F_5
Aqupec 505 HV	0.5 g	1 g	1.5 g	2 g	1.5 g
TEA	2 g	2 g	2 g	2 g	2 g
Glycerin	30 g	30 g	30 g	30 g	30 g
Nipagin	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Propylene glycol	5 g	5 g	5 g	5 g	5 g
Leaf Extract	10%	10%	10%	10%	-
Cherry					
Routine	-	-	-	-	1%
Aquadest	52.3	51.8	51.3	50.8	60.3
Total	100	100	100	100	100

The process of gel production

Aqupec was used as a gel base with hot aquadest in a hot mortar then TEA (triethanolamine) was added and then crushed until homogeneous. Then Glycerin was added and propylene glycol as humectants in mortar that had been filled with aqupec and triethanolamine, crushed until homogeneous then finely crushed nipagin was added as a preservative, crushed until homogeneous. Cherry Extract as an active antioxidant was added and stirred homogeneously to form a good gel (Burke, 2007).
The physical properties test of antioxidant gel ethanol extract of Cherry Fruit.

Antioxidant activity test

Stock solution (Cherry extract, Cherry extract gel, routine standard) was made into 5 series of dilutions of 0.4 mM each. The mixture was incubated during the operating time and its absorbance was recorded on the length of the physical test of Kersen ethanol antioxidant gel included: organoleptic test, homogeneity test, spreadability test, viscosity test, stickiness test, pH test, gel stability test and activity test radical capture and antioxidant activity Test

Analysis Techniques

The antioxidant activity of DPPH free radical data (%) extract or gel of Cherry was calculated by Probit method from linear regression equation and determine its IC50. The scavenging activity of DPPH free radicals was calculated by the formula:

$$\text{Scavenging activity (\%)} = \frac{\text{absorbance of (blank – sample)} \times 100\%}{\text{absorbance of blank}}$$

Results

Determination results and description of Cherry

Identification of Cherry (*Muntingia calabura* L.) plant was carried out at the Phytochemical Laboratory of Pharmacy program, Kupang Health Polytechnic of Health Ministry. The key result of the plant determination was done following the method by Steenis *et al*. (2002).

Simplisia drying result

Table 2. Yields of the Cherry powder

Gross weight (gram)	Dry weight (gram)	Percentage of yield (%)
3500	950	27

Identification results of Cherry powder

Organoleptic identification results can be seen in table 3.

Table 3. Organoleptic identification results of Cherry powder

Identification types	Result
Shape	Powder
Color	Brown
Smell	Typical
Taste	No taste

The determination result of powder moisture content.

The determination result of moisture content can be seen in table 4.

Table 4. The determination result of moisture content of Cherry powder

No	Weight of Simplicia powder (gram)	Percentage of moisture content (%)
1	2.00	6.90
2	2.00	7.40
3	2.00	4.70
	Average ± SD	6.30 ± 1.44

The results of 70% ethanol extract of Cherry Production

Table 5. Yields of the Kersen Fruit extract

Powder weight (gram)	Extract weight (gram)	Percentage of yield (%)
800	121.548	15.19

Identification results of Cherry extract

Organoleptic identification results of Cherry extract.

Table 6. Organoleptic identification results of Cherry extract.

Identification Type	Result
Shape	Thick extract
Color	Brown
Smell	Typical
Test	Bitter

The results of determining the moisture content of the Kersen Fruit extract.

Table 7. The results of determining the moisture content of kersen fruit extract

No	Extract weight (gram)	Percentage of moisture content (%)
1	2.00	9.10
2	2.00	7.50
3	2.00	7.90
	Average ± SD	8.16 ± 0.83

Alcohol-free identification results of Cherry extract

The results of alcohol free identification of Cherry extract can be seen in table 8.

Table 8. Results of alcohol free identification of Kersen Fruit extract

Ingredient	References (Ansel 1989)[9]	Result
Alcohol	Typical smell of esters from alcohol	Typical smell of esters from alcohol
Extract	There is no characteristic smell of esters from alcohol	There is no characteristic smell of esters from alcohol

Results of chemical identification with reagents.

Table 9. Results of identification of chemical content in Cherry extracts by recording

No	Content Chemistry	Procedure		Result	References		Note
1.	Polyphenols	Extract + aquadest		Shapes	Shapes		+
		+ $FeCl_3$		Black	Color	Purple	
					– black		
2.	Flavonoids	Mg powder,	alcohol:	Shapes	Red,		-
		hydrochloric acid	(1:10),	Green	Orange	Or	
		amil alcohol			Yellow		

Chemical identification results by thin layer chromatography (TLC).

Table 10. Results of identifying chemical contents in extracts by TLC

Compound		Result		Note
	UV 254 nm	UV 366 nm	Spray reagents	
	colored patches	Patches	Sitoborate	
Flavonoids				+
	Dark	Fluorescence	Yellow	
Polyphenols	colored patches	Patches	Ferric Chloride	
				+
	Dark	Fluorescence	(gray black color)	

Gel physical quality test results

The physical quality tests of the gel include organoleptic observations, gel homogeneity tests, viscosity tests, dispersion tests, adhesion tests, and pH tests.

Organoleptic gel test results.

A good gel preparation has an attractive color, pleasant smell, and good consistency for comfortable use. The results obtained for organoleptic gel identification can be seen in table 11.

Table 11. Organoleptic gel test results

Formula	Color		Smell		Consistency	
	Day 1	Day 21	Day 1	Day 21	Day 1	Day 21
Formula 1	Brown	Brown	Typical	Typical	A little thick	A little thick
Formula 2	Brown	Brown	Typical	Typical	Rather Thick	Rather Thick
Formula 3	Brown	Brown	Typical	Typical	Thick	Thick
Formula 4	Brown	Brown	Typical	Typical	Very Thick	Very Thick
Formula 5	Yellow	yellow	Typical	Typical	Thick	Thick

Notes :
Formula 1 : gel with *gelling agent* Aqupec 505 HV 0.5%
Formula 2 : gel with *gelling agent* Aqupec 505 HV 1%
Formula 3 : gel with *gelling agent* Aqupec 505 HV 1.5%
Formula 4 : gel with *gelling agent* Aqupec 505 HV 2%
Formula 5 : gel with gelling agent Aqupec 505 HV 1.5% with routine

A gel with very thick consistency is found in formula 4 using aqupec 505 HV is greater, and rather thick is found in formula 1 because aqupec 505 HV is used less. The greater concentration of aqupec 505 HV is used to produce a gel with a thicker consistency.

Gel homogeneity test results.

The observations of the gel homogeneity test result can be seen in table 12.

Table 12. Results of gel homogeneity test

Formula	Homogeneity	
	Day 1	Day 2
Formula 1	Homogeneous	Homogeneous
Formula 2	Homogeneous	Homogeneous
Formula 3	Homogeneous	Homogeneous
Formula 4	Homogeneous	Homogeneous
Formula 5	Homogeneous	Homogeneous

Notes :
Formula 1 : gel with *gelling agent* Aqupec 505 HV 0.5%
Formula 2 : gel with *gelling agent* Aqupec 505 HV 1%
Formula 3 : gel with *gelling agent* Aqupec 505 HV 1.5%
Formula 4 : gel with *gelling agent* Aqupec 505 HV 2%
Formula 5 : gel with gelling agent Aqupec 505 HV 1.5% with routine

Gel viscosity test results

The results of observations on the viscosity test of Kersen Fruit extract gel can be seen in table 13.

Table 13. Test results of viscosity of the Kersen Fruit extract gel

Identification	Viscosity (d Pas) ± SD				
Time	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
Day 1	176.67 ±28.968	295.35 ±11.648	335.43± ±29.968	493.53 ±56.965	363.30 ±11.758
Day 21	195.30 ±11.748	278.67 ±25.268	325.33 25.367	475.33 ±47.658	312 ±36.259

Notes :
Formula 1 : gel with *gelling agent* Aqupec 505 HV 0.5%
Formula 2 : gel with *gelling agent* Aqupec 505 HV 1%
Formula 3 : gel with *gelling agent* Aqupec 505 HV 1.5%
Formula 4 : gel with *gelling agent* Aqupec 505 HV 2%
Formula 5 : gel with gelling agent Aqupec 505 HV 1.5% with routine

Observation results show that viscosity in formula 2, formula 3, formula 4, and formula 5 decreased on the 21st day which is caused due to effect of temperature and pressure by the storage of gel. The increase in temperature will increase the distance between the atoms so that the force between the atoms will decrease, causing the viscosity of the preparation to decrease while in formula 1 viscosity increased on the 21st day due to improper storage which is due to the effects of inappropriate room temperature which causes the gel to become thicker and less stable in storage.

Gel spread test results.

Table 14. Test spread results of Cherry extract gel

Formula	Load (gram)	Spread diameter (cm) ± SD	
		Day 1	Day 21
Formula I	-	5.123 ± 0.712	4.268 ± 0.670
	50	5.625 ± 0.825	4.913 ± 0.475
	100	6.214 ± 0.675	5.166 ± 0.472
	150	6.576 ± 0.650	5.815 ± 0.535
	200	7.124±0.512	5.936 ± 0.563
Formula 2	-	2.813 ± 0.264	3.469 ± 0.183
	50	3.456 ± 0.163	3.976 ± 0.435
	100	3.714 ± 0.621	4.265 ± 0.563
	150	4.051 ± 0.571	4.415 ± 0.615
	200	4.324 ± 0.735	4.823 ± 0,645
Formula 3	-	2.546 ± 0.186	2.654 ± 0.345
	50	2.67 ± 0.135	2.784 ± 0.386
	100	2.953 ± 0.162	3.256 ± 0.282
	150	3.25 ± 0.175	3.424 ± 0.292
	200	3.34 ± 0.12	3.843 ± 0.417
Formula 4	-	1.61 ± 0.453	2.14±0.216
	50	1.786 ± 0.564	2.346 ± 0.217
	100	1.959 ± 0.562	2.521 ± 0.293
	150	2.235 ± 0.467	2.623 ± 0.2894
	200	2.456 ± 0.324	2.789 ± 0.267
Formula 5	-	2.353 ± 0.173	2.425 ± 0.357
	50	2.583 ± 0.298	2.656 ± 0.342
	100	3.725±0.376	2.833 ± 0.230
	150	3.243 ± 0.347	3.095 ± 0.189
	200	3.563 ± 0.254	3.315 ± 0.467

Notes :
Formula 1 : gel with *gelling agent* Aqupec 505 HV 0.5%
Formula 2 : gel with *gelling agent* Aqupec 505 HV 1%
Formula 3 : gel with *gelling agent* Aqupec 505 HV 1.5%
Formula 4 : gel with *gelling agent* Aqupec 505 HV 2%
Formula 5 : gel with gelling agent Aqupec 505 HV 1.5% and with routine

The measurement results of gel spread shows that the spread power was inversely proportional to viscosity, the greater the viscosity, the smaller the spread power and vice versa.

The data above shows that Formula 4 has a lower spread yield, while Formula 1 has the greatest spread yield because the greater the concentration of aqupec 505 HV, the smaller the spread power. The increased concentration of aqupec 505 HV causes the value of the spread power to be smaller, and the gel to be stronger.

Gel adhesion test results.

The measurement results can be seen in table 15.

Table 15. Results of gel adhesion test of Kersen Fruit extract

Test Time	Adhesion power (seconds)				
	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
Day 1	3.12 ± 0.64	6.28 ± 1.02	10.98 ± 1.26	16.39 ± 2.28	10.99 ± 0.82
Day 21	3.69 ± 0.34	8.90 ± 1.28	12.68 ± 1.36	19.48 ± 1.76	11.98 ± 1.69
Notes	:				
Formula 1	: gel with *gelling agent* Aqupec 505 HV 0.5%				
Formula 2	: gel with *gelling agent* Aqupec 505 HV 1%				
Formula 3	: gel with *gelling agent* Aqupec 505 HV 1.5%				
Formula 4	: gel with *gelling agent* Aqupec 505 HV 2%				
Formula 5	: gel with gelling agent Aqupec 505 HV 1.5%				

Formula 4 has the greatest adhesion compared to other formulas, while the smallest adhesion is in formula 1. The addition of aqupec 505 HV can increase the gel adhesion, because the aqupec 505 HV's thick nature causes molecule which is bound through dipole-dipole interactions to increasingly elongate and the molecular weight becomes larger so as to produce a gel that is getting stronger with longer adhesion.

Gel pH Test Result.

The pH test is carried out to find out the pH value of the gel to match the pH of the skin. The results of pH testing of Kersen Fruit extract gel can be seen in table 16.

Table 16. Test results for the pH of the Kersen Fruit extract gel

Test	pH Test				
Time	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
Day 1	7.75	7.20	6.15	6.68	7.15
Day 21	7.68	7.25	6.14	6.65	7.18
Notes	:				
Formula 1	: gel with *gelling agent* Aqupec 505 HV 0.5%				
Formula 2	: gel with *gelling agent* Aqupec 505 HV 1%				
Formula 3	: gel with *gelling agent* Aqupec 505 HV 1.5%				
Formula 4	: gel with *gelling agent* Aqupec 505 HV 2%				
Formula 5	: gel with gelling agent Aqupec 505 HV 1.5% with Routine				

In formula 4 and formula 5 the pH decreases. The decrease in pH is probably caused by the influence of the environment such as gases in the air which are acidic that enter the gel, but the decrease in pH that occurs in each formula is not too significant and so it can be said that pH is relatively stable based on SNI 16-4399 -1996 pH in the skin ranges from 4.5 to 8.0 (Sriningsih *et al*., 2014).

Gel stability test results

Table 17. Organoleptic test results of Kersen Fruit extract gel stability with various concentrations of Aqupec 505 HV using the freeze thaw method

Cycle	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-

Notes :

- = No separation occurred

Formula 1 : gel with *gelling agent* Aqupec 505 HV 0.5%

Formula 2 : gel with *gelling agent* Aqupec 505 HV 1%

Formula 3 : gel with *gelling agent* Aqupec 505 HV 1.5%

Formula 4 : gel with *gelling agent* Aqupec 505 HV 2%

Formula 5 : gel with gelling agent Aqupec 505 HV 1.5% with routine

Testing antioxidant activity with the DPPH method

The results of testing antioxidant activity carried out on day 1 and day 21 can be seen in table 18.

Table 18. Results of the antioxidant activity of the Cherry extract gel

	IC_{50} (ppm)	
Sample	Day 1	Day 21
Routine	6.29	-
Cherry Extract	68.50	-
Formula 1	189.32	190.95
Formula 2	186.95	192.38
Formula 3	184.75	189.25
Formula 4	186.60	187.54
Formula 5	174.73	189.02

Notes :

- No separation occurred

Formula 1 : gel with *gelling agent* Aqupec 505 HV 0.5%

Formula 2 : gel with *gelling agent* Aqupec 505 HV 1%

Formula 3 : gel with *gelling agent* Aqupec 505 HV 1.5%

Formula 4 : gel with *gelling agent* Aqupec 505 HV 2%

Formula 5 : gel with gelling agent Aqupec 505 HV 1.5% with routine

Discussion

The results of testing the antioxidant activity of Cherry extract amounted to 67.48 ppm which means that the Cherry Extract has a strong antioxidant activity (Molyneux 2004).

Cherry has high antioxidant property due to the presence of anthocyanin content. Along with that cherry also contains many polyphenol such as tannin (Zhang & Lin, 2009). The Gel formula has several usefulness and can be easily applied on skin (Sihombing *et al*., 2013). The main component of gel is Aqupec that can

increase viscosity at small concentrations, as well as increase gel stability (Wathoni, 2012), at small concentrations, as well as increase gel stability (Carter, 1995).

Numerous clinical studies have stated that the consumption of cherries and their derivatives has a positive effect on human health. Again in vitro studies have shown that natural polyphenols-rich sweet cherry extracts can protect endothelial cells from oxidative stress. Moreover, cherry extracts were detected to be a useful anti-inflammatory synthetic drug (Beconcini *et al*., 2020).

Conclusion

First, the extract of Cherry (*Muntingia calabura* L.) leaf can be formulated into a gel with a variation of aqupec 505 HV base concentration which has stable physical quality and gel preparation stability.

Secondly, Cherry (*Muntingia calabura* L.) leaf extract gel has antioxidant activity. Formula 1 (aqupec 505 HV 0.5%) has an IC_{50} value of 187.21 ppm, formula 2 (aqupec 505 HV 1%) has an IC50 value of 184.81 ppm, formula 3 (aqupec 505 HV 1.5 %) has an IC_{50} value of 182.55 ppm, formula 4 (aqupec 505 HV 2%) has an IC_{50} value of 184.49 ppm, and formula 5 (aqupec 505 HV 1.5% with routine) has an IC_{50} value of 172 .52 ppm. The formula Cherry extract gel has weak antioxidant activity.

It has been found that the Cherry extract gel formula has weak antioxidant activity. For further study, preparations must be made other than gels such as creams and tablets. It is necessary to do further research on the antioxidant gel of Cherry extract by using methods other than DPPH to find out the potential of antioxidant against other types of radicals.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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