Volume 16 No. 2
July 2024
ISSN : 2085 – 1669
e-ISSN : 2460 – 0288
Website : jurnal.umj.ac.id/index.php/jurtek
Email : jurnalteknologi@umj.ac.id

Jurnal Teknologi
UNIVERSITAS MUHAMMADIYAH JAKARTA

Effectiveness of Quercetin in Kenikir Leaves (*Cosmos Caudatus Kunt*) in Nanoemulsion Formulas with VCO (*Virgin Coconut Oil*) and Olive Oil Phase

Siti Hajir[1, 2]*, Ratri Ariatmi Nugrahani[2], Ismiyati[3]
[1,2,3]*Master Study Program, Chemical Engineering Dept, Faculty of Engineering, Universitas Muhammadiyah Jakarta, Indonesia.*
[2]*Chemical Engineering Study Program, Faculty of Engineering, Alkhairiyah University, Indonesia.*

*Corresponding author email: hajir.sh8129@gmail.com

Jurnal Teknologi use only:
Received 14 September 2023; Revised 21 February 2024; Accepted 28 June 2024

ABSTRACT

Kenikir (*Cosmos Caudatus Kunth*) is a herbal plant and contain quercetin compounds (flavonoids) which can have an effect as an antioxidant, antibacterial, antifungal, anti-inflammatory. This study aims to determine the effect of adding quercetin extract to a nanoemulsion formula with several oil phases, namely VCO (*Virgin Coconut Oil*) and Olive Oil. Evaluation of the characteristics of the preparation for each formula included organoleptic test, pH test, particle size, emulsion type, and determination of the level of quercetin absorbed in the nanoemulsion system as well as antioxidant activity test using the DPPH (1,1-diphenyl-2-picrylhydrazil) method. The results showed that the nanoemulsion preparations with the VCO oil phase, olives had good and stable characteristics. The pH test of the nanoemulsion was in the range of 4.5-6.5 while for the emulsion type it was an Oil in Water (M/A) type and the particle size was in accordance with the criteria for nanoemulsion preparations <200 nm, namely 13-178 nm. To see the ability of the active substance to be absorbed in the formula by calculating the % EE (Entrapping Efficiency) the % EE is obtained, which is 99%, which means that the nanoemulsion formula is able to absorb the active substance well and the antioxidant activity test using the DPPH method shows in the formulation of kenikir extract with the VCO oil phase gives an IC50 value of 52.238 ppm while the olive oil phase is 305.783 so that the nanoemulsion formula with the VCO oil phase is more reactive than the olive oil phase.

Keywords: Antioxidant, Kenikir (*Cosmos Caudatus Kunth*), Nanoemulsion, Olive Oil, Quercetin, VCO (*Virgin Coconut Oil)*

Introduction

Kenikir (*Cosmos Caudatus Kunth*) is a herbal plant that is often found in Indonesia. Kenikir leaves contain quercetin compounds (flavonoids) which can have effects as antioxidants, antibacterial, antifungal, anti-inflammatory, antitumor, anticancer and so on. The potential of quercetin in the world of health is very large, so to be able to develop the potential of quercetin from natural compounds it is necessary to extract it. With extraction, quercetin can be taken from a part of the plant, so that it can be studied and used further.

The quercetin compound found in plants has very low solubility in water, causing limitations in the adsorption process and affecting its bioavailability in the body. The

DOI: https://dx.doi.org/10.24853/jurtek.16.2.195-202

ISSN : 2085 – 1669
e-ISSN : 2460 – 0288

solubility of quercetin can be increased through the formation of nanoemulsions so that it can increase its bioavailability in the body. With this nanoemulsion system it is hoped that it will help quercetin to be easily absorbed into the carrier oil, oil which can dissolve the active ingredients is an important component in nanoemulsion formulations. The oil phase used is coconut oil (*Virgin Coconut Oil*) and olive oil.

The extraction method used to obtain quercetin extract from kenikir leaves is the soxlet method for 2 hours with methanol solvent where this method produces a higher concentration of quercetin extract. The nanoemulsion formula was made using several oils to see the comparison of the oil phase in the nanemulsion formula with the addition of quercetin extract. For the application of the resulting nanoemulsion product, an antioxidant activity test was carried out using the DPPH (1,1- diphenyl -2-picrylhydrazil) method.

The purpose of this study was to determine the comparison of the addition of quercetin extract from kenikir leaves to nanoemulsion formulas with different oil phases, namely VCO and olive oil, and then carried out characteristic tests such as organoleptic testing, particle size, pH, emulsion type while to test its activity, namely to determine effect of the concentration of kenikir powder on the content using the VCO and olive oil oil phases as well as the antioxidant content contained in the nanoemulsion system using the DPPH method.

Methods

Tools and Materials

The tools used were oven, desiccator, blender, vacuum rotary evaporator, soxlet, Erlenmeyer 250 ml, funnel, vial, test tube , stir bar, digital scale, hotplate, dropper pipette, magnetic stirrer , volumetric flask, measuring cup, Whatman paper 1 , centrifugal apparatus, pH meter, UV-VIS Spectrophotometer, Particle Size Analyzer.

The materials used in this study were fresh kenikir leaves (Cosmos Caudatus Kunt), 96% methanol as a solvent for the active compound Quercetin, virgin coconut oil (VCO) oil phase, olive oil, Tween 80, Span 80, propylene glycol and oleic acid, phosphate buffer. pH 6 and standard solution of quercetin, $AlCl_3$ 10%, 4% NaOH, 5% $NaNO_2$, pure DPPH and Aquades.

Analysis Method

Extraction of Quercetin from kenikir leaves

Fresh kenikir leaves were dried in an oven at a temperature of 50 ^{o} C for 5 hours then mashed using a blender and then tested for water content. Testing the water content was carried out by the gravimetric method namely weighing 5 grams of sample and then putting it into the oven at a temperature of 105^{o}C and then cooling it in a desiccator and then weighing it until a constant weight is obtained. Moisture analysis was carried out three times to obtain data accuracy. The water content in simplicia must be below 10% [3]. Then extracted by soxhletation method for 2 hours using methanol (1:10). Then tested qualitatively and quantitatively with a standard solution of quercetin as a comparison. So that the concentration of quercetin contained in the kenikir extract is known.

Qualitative and Quantitative Tests of Quercetin on Kenikir Leaf Powder Extract

As much as 1 ml of *C. caudatus* leaf extract was put into a test tube until the volume became 10 mL with methanol solvent (mother liquor). From the mother liquor, 1 mL was taken and put into a test tube plus 4 mL of distilled water and 0.15 mL of 5% $NaNO_2$ and then allowed to stand for 6 minutes. The solution was added with 0.15 mL of 10% $AlCl_3$ and allowed to stand for 6 minutes. The solution was reacted with 2 mL of 4% NaOH and allowed to stand for 15 minutes. A change in color from pink to red indicates that the extract contains flavonoids [13].

Analysis of Quercetin Levels in Methanol with a UV-Vis Spectrophotometer

a. A standard quercetin solution with a concentration of 100 ppm can be prepared by weighing 10 mg of quercetin dissolved in a 100 mL measuring flask with methanol solvent up to the mark (the quercetin level becomes 0.1 mg/mL or 100 mg/L).
b. 100 µg/mL of quercetin mother liquor diluted with methanol pa to a

Jurnal Teknologi 16 (2) pp 195 - 202 © 2024

concentration of 0.5; 1; 2; 5; 10; 15 and 20 mg/L. After that, the absorbance was measured on a UV-Vis spectrophotometer with a wavelength of 371 nm. The standard curve is obtained from the relationship between quercetin concentration (mg/L) and absorbance.

c. As much as 10 mg of kenikir leaf filtrate was dissolved in a 100 mL volumetric flask using methanol solvent up to the boundary mark (extract concentration being 0.1 mg /ml or 100 mg/L). The absorbance of the solution was measured at a wavelength of 371 nm with a UV-Vis spectrophotometer.
d. Examination of methanol-free in kenikir leaf extract produced using the esterification method with the addition of acetic acid and concentrated sulfuric acid and assisted by heating [12]. The extract was dissolved with H_2SO_4 in a test tube then added CH_3COOH and covered with cotton, then heated to boiling. Furthermore, the smell of esters was identified on cotton, if the extract did not contain methanol then there was no smell of esters.

Nanoemulsion Design

The Nanoemulsion system formula was made as much as 60 mL, each formula was replicated twice, see in **Table 1**.

Table 1. Nanoemulsion Formulation Design

No	Material	Function	F1	F2	F3	F4	F5	F6
1	Powder Extract Kenikir	Active Ingredients	-	5%	10%	15%	20%	25%
2	Tweens 80	Non-ionic surfactant	46%	46%	46%	46%	46%	46%
3	Span 80	Non Ionic Surfactants	4%	4%	4%	4%	4%	4%
4	VCO / Olive Oil	Oil Phase	5%	5%	5%	5%	5%	5%
5	Oleic Acid	Enhancers	1%	1%	1%	1%	1%	1%
6	Propylene Glycol	Kosurfactant	5%	5%	5%	5%	5%	5%
7	Phosphate buffer		Add 100%	Add 100%	Add 100%	Add 100%	Add 100%	Add 100%

Product Analysis Method

Organoleptic Test

The analytical method is used by making visual observations regarding color and shape and using the sense of smell to determine the odor of the preparation [6].

pH measurement

The resulting nanoemulsion preparation must match the pH of the skin and not cause irritation. The recommended pH for the skin is in the range of 4.5-6.6 [3]. Measurements were carried out using a pH meter, where the pH meter was first calibrated with a pH 7 buffer and then immersed in the pH value preparation and the value that appeared was recorded.

Emulsion Type

To find out the type of emulsion from nanoemulsion, a dilution test was carried out, namely by diluting the emulsion with water, if the emulsion mixes well with water, then the type of emulsion is M/A and vice versa.

Particle Size Testing

The measurement of nanoemulsion particles was used by the Zetasizer Particle Size Analyzer (Malvern) tool. This tool is able to

ISSN : 2085 – 1669
e-ISSN : 2460 – 0288

measure nanoparticle-sized particles, colloids, proteins, zeta potential and molecular weights in the range of 0.15 nm – 10 µm with a sensitivity of 3-10,000 nm [8]. Particle size is an important characteristic in nanoemulsions because drug release is affected by particle size.

The amount of quercetin that is not absorbed will be dispersed in the methanol solvent in the supernatant. The supernatant resulting from centrifugation was determined using a UV-Vis spectrophotometer [10]. This test is also known as entrapment efficiency (% EE) calculated by the formula (1):

$$\%\ EE = \frac{[(\mathrm{TD} - \mathrm{FD})}{\mathrm{TD}} x\ 100\% \qquad \text{.....(1)}$$

Note :
EE = Presentation of entrapment efficiency
TD = Amount of drug added in the system
FD = Amount that is not absorbed

The interpretation of the results of this test is (% EE) between 80-100%. The greater the entrapment efficiency, the better the resulting nanoemulsion system will be.

Nanoemulsion Antioxidant Activity Test

- Preparation of DPPH solution

A total of 2 mg of pure DPPH was weighed then dissolved with 96% methanol until the volume became 100 ml in a volumetric flask (20 ppm concentration) covered with aluminum foil and placed in a dark room.

- Measurement of Nanoemulsion Formula Antioxidants

Pipette the resulting nanoemulsion formula as much as 0.5 ml, 0.75 ml, 1 ml, 1.25 ml and 1.5 ml into a test tube and then add 2 mL of DPPH solution with a concentration of 20 ppm each and then measure the absorbance using a UV Vis spectrophotometer at a wavelength of 517 nm. Then read the absorbance every 5 minutes until there is no significant change in the absorbance value. Take a 30 minute dive reading. The antioxidant activity of the samples was analyzed by calculating the percentage of inhibition (inhibition of the DPPH radical according to the following equation:

Determination of Absorbed Quercetin Content in the Nanoemulsion System

1 gram of nanoemulsion extract of kenikir leaf powder added with methanol solvent up to 10 ml. the result of separation in the form of quercetin trapped in the nanoemulsion preparation will precipitate after centrifugation at 2500 rpm for 45 minutes.

$$\%\ \text{Inhibition} = \frac{(\text{Abs Blanko} - \text{Abs Sampel})}{\text{Abs Blanko}} x\ 100\%$$

Note:
Blank Abs = Absorbent at 0 minutes of adding DPPH
Sample Abs = Absorbent at 30 minutes of adding DPPH

Results and Discussions

Qualitative and Quantitative Test Results for Quercetin on Kenikir Leaf Powder Extract

- Flavanoid Qualitative Test Results

The results obtained were a color change from yellow to pink. Based on this, it can be seen that the extract contains the flavonoid quercetin [15].

-Quantitative Quercetin Test Results

The quantitative test for quercetin was carried out using a standard quercetin solution so that a standard calibration curve was obtained and the quercetin content in the kenikir leaf powder extract could be determined by plotting the absorbance value of the quercetin extract with an extract concentration of 14.26 ppm . The absorbance of quercetin was measured using the maximum wavelength of quercetin in methanol, namely 371 nm.

Preparation and Testing of Nanoemulsion Products

The nanoemulsion system of kenikir leaf extract in this study was made of 2 nanoemulsion formulas, namely with the oil phase of Virgin Coconut Oil (VCO) and phase is according to the design in **Table 1**. This nanoemulsion formula consists of an oil phase, Span 80, an aqueous phase using tween 80, propylene glycol and phosphate buffer pH 6 with the addition of oleic acid as an enhancer.

Jurnal Teknologi 16 (2) pp 195 - 202 © 2024

Figure 1. Nanoemulsion formula with VCO as the oil phase

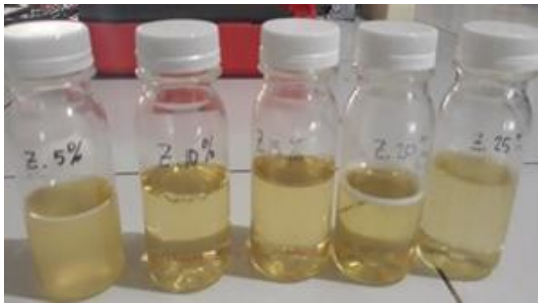

Figure 2. Nanoemulsion formula with olive as the oil phase

- Organoleptic Test

The organoleptic test was carried out by distributing questionnaires to 10 respondents at week 0 and week 6 after storage at room temperature. The tests included the color, aroma and texture of the nanoemulsion formula between the VCO and olive oil oil phases. Observation results can be seen in **Table 2 and 3**.

Table 2. Organoleptic Test of Nanoemulsion Formula at Week 0 to 6 for VCO Oil Phase

No	**Test Component**	**Week 0 Observations**	**Week 6 Observations**
1	**Color**	Pale Yellow (Transparent)	Pale Yellow (Transparent)
2	**Odor**	Moderate Odor Tween 80	Moderate Odor Tween 80
3	**Homogeneity**	The liquid is slightly viscous and homogeneous	The liquid is slightly viscous and homogeneous

Table 3. Organoleptic Test of Nanoemulsion Formulas at Weeks 0 to 6 for the Olive Oil Phase

No	**Test Component**	**Week 0 Observations**	**Week 6 Observations**
1	**Color**	Brownish Yellow (pale)	Brownish Yellow (pale)
2	**Odor**	Moderate Odor Tween 80	Moderate Odor Tween 80
3	**Homogeneity**	The liquid is slightly viscous and homogeneous	The liquid is slightly viscous and homogeneous

Based on **Table 2 and 3** after storage until 6 weeks it can be observed that there is no change in the color, taste and texture of the resulting formulation, this means that the resulting formula is stable. Meanwhile, the criteria for a good nanoemulsion are transparent and have a viscosity that is not too high.

- pH Measurement

Based on the pH measurement results, the nanoemulsion formula still meets the standard of a preparation to be formulated for skin with a skin pH range of 4.5 – 6.5.

- Emulsion Type

After the dilution test, it can be observed that the emulsion type of this nanoemulsion formula is an O/A emulsion type because after adding water, the emulsion and water mix well.

- Particle Size Testing

The results obtained are that the preparation meets the requirements of the nanoemulsion preparation provisions, namely 10 – 200 nm [4], namely 13.01 nm for the VCO oil phase and 14.66 nm for the olive oil phase.

Table 4. Particle Size Test Results

Formulas	**Particle Size(nm)**	**PdI** (Polydispersity Index)
VCO oil	14.66	0.162
Olive oil	13.01	0.144

ISSN : 2085 – 1669
e-ISSN : 2460 – 0288

- Determination of Absorbed Quercetin Content in the Nanoemulsion System

Table 5. Results of Determination of Quercetin Compound Levels Absorbed in the Nanoemulsion System

OIL	TESTING	INITIAL CONCENTRATION(PPM)	ABSORBENT	FINAL CONCENTRATION(PPM)	%EE
VCO	I	128.34	0.035	0.640	99.9950
	II	128.34	0.032	0.661	99.9948
OLIVE	I	128.34	0.005	0.021	99.9998
	II	128.34	0.007	0.062	99.9995

Based on Table 5, it was found that %EE was 99.9 % . This means that the resulting nanoemulsion is good for the active compound to be absorbed or trapped in the nanoemulsion system. Meanwhile, between the VCO and olive oil phases, the %EE obtained in olive oil was higher than the VCO oil phase, which means that with olive oil the absorption of kenikir extract was better in the nanoemulsion formula

- Nanoemulsion Antioxidant Activity Test with DPPH method

Data on antioxidant activity test results on nanoemulsion with VCO and olive oil phases **Table 6**. The higher the concentration of the extract added, the higher the % inhibition while for the IC50 value this is in accordance with research [1]. Using the VCO oil phase has an IC50 value of 52.238 ppm in the olive oil phase a value of 305.783 ppm means that the nanoemulsion preparation with the VCO oil phase has very active antioxidant content when compared to the olive oil phase.

Table 6. Nanoemulsion Antioxidant Activity Test

Oil	Concentration (ppm)	% Inhibition	IC50 (ppm)
VCO	100	53.46	
	150	60.19	
	200	65.32	52.238
	250	69.34	
	300	73.02	
Olive	100	13.02	
	150	17.87	
	200	28.13	305.783
	250	39.24	
	300	50.28	

Conclusion

1. Based on the results of the antioxidant activity test using the DDPH method, it can be seen that the nanoemulsion formula with the VCO oil phase is very active with a value of 52,238 ppm when compared to olive oil, while based on the regression analysis it can be seen that the addition of quercetin concentration has an effect on % inhibition.
2. The resulting nanoemulsion formula met the criteria to be used as a nanoemulsion drug preparation, both in organoleptic properties, pH and in nanoemulsion particle size, namely 13.01 nm for the VCO oil phase and 14.66 nm for the olive oil phase.

Acknowledgement

The authors would like thanks to Department of Master Chemical Engineering, Engineering Faculty, Muhammadiyah University of Jakarta, for all the facilities and support provided throughout this research and also to SMK Prima Mandiri who have laboratory facilitated the completion of the research.

Funding
This research was funded by a Dikti funds with collaboration on research on Rice Brand Oil.

Author Contributions
Conception or design of the research, Data collection and data analysis and interpretation data doing with first author. Compiling journal, critical revision of the journal and final approval of the version to be published by second and third author.

Conflicts of Interest
This research has no conflict of interest.

References
[1] Anggresani, Lia. 2017. Test for Total Flavanoid Content and Antioxidant Activity of Kembang Bulan Leaf Extract (Thitonia deiversifolia (Hemsley) A. Gray). Pharmacy Study Program. STIKES Mother's Hope. Jambi
[2] Chen, et al, H. 2011. Nanonization strategies for poorly water-soluble drugs. Drug Discovery. Today 16: 354–360.
[3] Ministry of Health of the Republic of Indonesia. 2008. Indonesian Herbal Pharmacopeia, Jakarta: Ministry of Health of the Republic of Indonesia
[4] Devarajan, V. and Ravichandhandran, V. 2011. Nanoemulsions: as Modified Drug Delivery Tool. Pharmacie Globale, 2(4), 1-6
[5] Dwi, Anita. Prayogo, Lean, S. 2016. Comparison of Maceration and Soxhletation Extraction Methods on Total Phenolic Content of Kersen Leaf Ethanol Extract (Muntingia Calabura). Inside: Bachelor of Pharmacy Research. Wahid Hasyim University. Semarang
[6] Lawrence, MJ, 2000. Microemulsion-based media as a novel drug delivery system. Adv. Drug Delivery Rev. 45(1):89-121
[7] Lotulung, PDN, Minarti, and Kardono, LBS, 2005, Screening for antibacterial activity, antioxidants and toxicity to shrimp larvae Artemia salina Asteraceae plant extract, Abstract, LIPI Chemical Research Center.
[8] Malvern. 2012. A Basic Guide to Particle Characterization. Worcestershire (UK): Malvern Instruments Limited
[9] Martin, A., Swarbrick, J., Commarata, A. 1993. Physical Pharmacy 3rd Edition (Translation by Yoshita and Iis Aisyah B). Jakarta: University of Indonesia Press
[10] Pham, Thi Thuy. 2012. Colloid and Surfaces B: Biointerfaces, Liposome and Niosome Preparation Using Membrane Contractor for Scale Up . France, 94, 15-21
[11] Reynertson, KA 2007. Phytochemical analysis of bioactive constituents from edible myrtaceae fruit. University of New York, New York.
[12] School. 1988. Purity Supplementary Material for Drug Separation Method. Gadjah Mada University. Yogyakarta: University Press
[13] Talapessy, S., Suryanto, E., & Yudistira, A. (2013). Antioxidant activity test of sago waste (Metroxylon sago Rottb). Pharmaceutical Scientific Journal, 2(3), 40-44.
[14] Utami, Holy Syafitri. 2012. Formulation and InVitro Penetration Test of Nanoemulsion, Nanoemulsion Gel, and Curcumin Gel [thesis]. Depok: Faculty of Mathematics and Natural Sciences, Pharmacy Study Program
[15] Zahratunnahdhatili'ibaadatillaah. 2017. Effect of Kenikir (Cosmos Caudatus Kunt) Leaf Extract Concentration on the Characteristics and Release of Active Compounds in the Nanoemulsion System using the Virgin Coconut Oil (VCO) Oil Phase. Inside: Proceedings of Civitas Research at the State Islamic University of Maulana Malik Ibrahim.Malang
[16] Winarno. 2008. Food and Nutrition. Jakarta: Gramedia

ISSN : 2085 – 1669
e-ISSN : 2460 – 0288