

FORMULATION AND EVALUATION OF CITRONELLA OIL IN ROLL-ON APPLICATION SYSTEM

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ABSTRACT

Objective: The purpose of this study to obtain the best formulation of roll-on *Citronella oil* that can be used for aromatherapy.

Methods: The citronella plant (*Cymbopogon winterianus* Jowitt) was processed with Steam-water distillation produced essential oil. Citronella oil was tested for quality and formulated. The best formulation result is then tested for product quality.

Results: The result of yield of citronella oil was 0.6%. The result of citronella oil quality parameters is obtained in accordance with the literature. The result of the hedonic test, formula 2 was chosen to be the best formula. The result of an average transferred volume was 10.0 ml. The result of the total plate number is < 10 cfu/ml. The result of the identification of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was negative. The result of the stability test was stable during storage.

Conclusion: The results showed that formula 2 was the best and has the potential to be circulated in large quantities

Keywords: *Citronella oil*, Roll-on, Aromatherapy, *Cymbopogon winterianus* Jowitt

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INTRODUCTION

Citronella oil is an essential oil obtained from distillation of the leaves of Lemongrass *Cymbopogon winterianus* and *Cymbopogon nardus*. Citronella oil contains the main component in the form of citronellal and geraniol, which can give a distinctive odor such as the aroma of grass that will be liked by the public. *Cymbopogon winterianus* produced higher levels of citronellal and geraniol than *Cymbopogon nardus*. Citronella oil is one of the essential oil commodities with high demand for export by Indonesia and it still needs to be developed to increase the selling value [1]. One of the efforts to add selling value of citronella oil in Indonesia is can be made into aromatherapy products because in the 21st century, aromatherapy has received a lot of attention [2, 3].

Aromatherapy comes from the word "aroma" which means the smell of fragrance and "therapy," which means treatment with the aim of helping provide a feeling of freshness and calm [4, 5]. An aromatherapy roll-on made from Citronella oil mixed with base oil The right one will provide a mild warm sensation and the aroma of Citronella oil can help relieve headaches and relieve stress because it has a soft aroma that can reduce mental tension [6]. Based on research, the result of the distillation of citronella oil can function as a sedative in male mice [7]. Research on the formulation and stability of roll-on aromatherapy based on Citronella oil is still limited; therefore, this research will conduct formulation and test the stability of roll-on aromatherapy preparations containing Citronella oil.

MATERIALS AND METHODS

Plant material

The main ingredients used in this study were Lemongrass (citronella) leaves (*Cymbopogon winterianus* Jowitt Ex Bor) obtained from Bukit Waruwangi, Serang, Banten, Indonesia.

Chemical ingredients

Menthol, Camphora, BHT (butylated hydroxyanisole), Propyleneglycol, Lemon oil and Virgin coconut oil were purchased from Faculty of Pharmacy, Pancasila University.

Plant determination

Plant determination was carried out at the Herbarium Depokensis (DEB), Departement of Biology, Faculty of Science and Mathematics,

Universitas Indonesia, Depok, West Java (No.701/UN2.F3.11/PDP.02.00/2021). The purpose of determination is to get to the truth and clear identity of the plant researched and avoid error in the main research material collection [8].

The distillation of citronella oil

Lemongrass leaves (Citronella) distillation was carried out using the steam-water distillation method to obtain Citronella oil [9].

Measurement of citronella oil yield

The purpose of measurement of citronella yield was to get how many grams of simplicia powder to produce 1 gram of extract [10]. The yield of the extract was calculated with the formula Eq. 1:

$$\% \text{ Yield of extract} = \frac{\text{Extract weight obtained (g)}}{\text{sampel weight (g)}} \times 100\% \dots\dots (1)$$

Determination of citronella oil quality parameters

Determination of quality requirements for Citronella oil refers to SNI 06-3953-1995 [11]. The parameters of color testing was carried out organoleptically. The density test was carried out using a pycnometer. The refractive index test was carried out using a refractometer. The total geraniol and citronellal test was carried out using gas-liquid chromatography. The solubility in ethanol 80% testing was carried out using the synthesis method and foreign substances such as fatty oil.

Citronella oil roll on aromatherapy formula

The roll-on aromatherapy formula was shown in table 1.

Making aromatherapy roll-on citronella oil

Prepare the necessary tools and materials. Calibrated roll-on container ad 10 ml. The menthol and camphor were weighed in different glasses and mixed, then set aside to form M1 (Mass 1). BHT was weighed and dissolved with some base oil, then set aside to become M2 (Mass 2). Put M1 and M2 into the beaker glass. Propylene glycol was measured put into a beaker glass and stirred homogeneously. In Formula 3, lemon oil was measured and put into a beaker glass and stirred homogeneously. Put the mixture into the roll-on container. Added the remaining base oil ad calibration mark. Shaken ad homogeneously and equipped with a roll-on and labeled on the primary packaging. Put in secondary packaging.

Table 1: Formula design

No	Ingredients	Formula (%)		
		1	2	3
1.	Citronella oil	10	5	5
2.	Menthol	30	30	30
3.	Camphor	5	5	5
4.	BHT	0.2	0.2	0.2
5.	Propylene glycol	15	15	15
6.	Lemon oil	-	-	5
7.	Virgin coconut oil	39.8	44.8	39.8

Roll-on citronella oil quality evaluation

Product quality evaluation is carried out referring to the National Agency of Drug and Food Control of Indonesia Reg. PERKABPOM No. 32 of 2019 includes organoleptic tests, transferred volumes, and microbial contamination. Stability tests, preparations, primary irritation tests, and hedonic tests [12].

RESULTS AND DISCUSSION

Plant determination

The results of plant determinations carried out at the Faculty of Mathematics and Natural Sciences, University Indonesia, showed that the plants used were true surrender plants (*Cymbopogon winterianus* Jowitt Ex Bor) with the Poaceae family. Results the determination is based on the Book Flora of Java Vol. III [13].

Measurement of citronella oil yield

The yield of the steam distillation of *Cymbopogon winterianus* was shown in table 2.

Table 2: Result citronela oil yield

Sample weight (g)	Extract weight obtained (g)	Yield (%)
680.000	4.100	0.6

After the extraction process is complete, the result of measurement Citronella is 0.6%. The yield of extraction in this study lower than overall oil yield obtained by Singh of Citronella essential oil was 0.79% [14]. The methods of distillation process influenced citronella oil yield. The modern extractor machine showed best-distilled efficiency of oil content than the traditional alcohol boiler that shown lowest on oil content [15].

Determination of Citronella oil quality parameters

The quality parameter of citronella oil in this study was shown in table 3.

Based on the results obtained are following SNI 06-3953-1995, This means that the resulting product can be used as raw material for manufacturing roll on citronella oil as aromatherapy. Citronella oil that meet the requirement can be used on the different product as aromatherapy, insect repellent, medical, food product, and cosmetic [15]. The best quality of citronella oil was had various benefits and offered potential essential options which could be further applied to many applications

Hedonic test

Test of interest in the final results of roll-on aromatherapy preparations with an assessment using numerical parameters based on warmth and aroma. The rating scale consists of 5, namely: very like, like, neutral, don't like, and don't like it. The number of panellists consists of 30 people. The data obtained is processed using the software SPSS®25. The results of the hedonic test are presented in table 4.

Table 3: Certificate of analysis sheet Citronella oil

Parameter	Specification	Results
Appearance	Clear, sometimes slightly opalescent, mobile liquid	According to the standard
Colour	Colourless to pale yellow	According to the standard
Fragrant	Fresh, citrusy, sweet, slightly spicy	According to the standard
Density (25 °C)	0.8750-0.8930	0.8828+0.02
Refractive index (20 °C)	1.460-1.474	1.472+0.12
Optical Rotation	-7.00 °-0.00 °	-0.35+0.01 °
Citronella content	Minimum 35%	32.22+1.23%
Geraniol Content	17-30%	29.25+1.15%
Solubility	Soluble in alcohol and oil. Not soluble in water	Conform to the standard
Fat oil	Negative	Negative

Data was given in mean+SD, n=3

Table 4: Result hedonic test

Warmth				Scent			
Duncan ^{a,b}				Duncan ^{a,b}			
Sample	N	1	2	Sample	N	1	2
Formulation 1	30	3.83		Formulation 3	30	3.77	
Formulation 2	30	3.97		Formulation 2	30	3.77	
Formulation 3	30		4.33	Formulation 1	30		4.23
Sig.		.375	1.000	Sig.		1.000	1.000

Based on the hedonic test, it was known that each roll-on formula, namely, 10% citronella oil (Formula 1), 5% citronella oil (Formula 2), and a mixture of 5% citronella oil and 5% lemon (Formula 3) can be concluded that formula 2 is more dominant and much in demand by many people in terms of warmth and aroma. Therefore, formula 2 was chosen to be the best formula.

Transferred volume test

Based on the results of the tests carried out, the average transferred volume was 10.0 ml from 10 repetitions. The results obtained requirements determined by the regulatory standards of the drug and food regulatory agency number 32 of 2019. The substandard of

volume is caused by several factors such as the method, labour, and machine, while the most potential factor is the volume conformity to reduce the number of defect products.

Microbial contamination testing

The result of the total plate count was shown in table 5.

Table 5: Result total plate count

Sample	Dilution					Result (cfu/ml)
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	
Roll on-1	0	0	0	0	0	<10 cfu/ml
Roll on-2	0	0	0	0	0	

In the total plate count test, 5 dilutions were carried out in duplicate on the sample. Based on the results of testing the total plate number in the roll-on Citronella oil aromatherapy preparation, there were no growing colonies (<10 cfu/ml). The Total Plate count required for external medicinal liquids according to the regulation of the drug and food regulatory agency number 32 of 2019 was 10⁷ cfu/ml. So, it

can be concluded that the tested Citronella oil roll on aromatherapy preparations met the specified requirements and was microbiologically safe for use by the public.

The identification of *Staphylococcus aureus* contamination was shown in table 6.

Table 6: Result identification of *Staphylococcus aureus*

Identification of microbial contamination	Media	
	TSB	MSA
Roll on-1	Turbid	-
Roll on-2	Turbid	-
Positive control	Turbid	fluorescence green colony
Negative control	Clear	-

Description: TSB: Tryptic Soy Broth; MSA: Mannitol Salt Agar

In the *Staphylococcus aureus* identification test, Tryptic Soy Broth (TSB) media was used as an enrichment medium and if the results were positive, then it would be carried out on Mannitol Salt Agar (MSA) media. Based on the results obtained on Tryptic Soy Broth (TSB) media is positive, this is indicated by turbidity and deposits at the bottom of the tube, so it is necessary to continue testing using selective media, namely Mannitol Salt Agar (MSA) by scratching. On Mannitol Salt Agar (MSA) media, negative results

were obtained because it did not show the formation of yellow or white colonies surrounded by a white zone. *Staphylococcus aureus* which is required for external drug fluids according to the regulation of the drug and food regulatory agency number 32 of 2019 is negative/ml.

The identification of *Pseudomonas aeruginosa* contamination was shown in table 7.

Table 6: Result identification of *Pseudomonas aeruginosa* identification of microbial contamination

	Media	
	TSB	CETA
Roll on-1	Turbid	-
Roll on-2	Turbid	-
Positive control	Turbid	Yellow colony
Negative control	Clear	-

Description: TSB: Tryptic Soy Broth; CETA: Cetrinide Agar

In testing the identification of *Pseudomonas aeruginosa*, Tryptic Soy Broth (TSB) media was used as an enrichment medium and if the results were positive, then it would be carried out on Cetrinide agar media. Based on the results obtained on Tryptic Soy Broth (TSB) media is positive, this is indicated by turbidity and deposits at the bottom of the tube, so it is necessary to continue testing using selective media, namely Cetrinide by scratching. On Cetrinide agar media, negative results were

obtained because it did not show the formation of fluorescent green colored colonies. *Pseudomonas aeruginosa* required for external medicinal fluids according to the regulation of the drug and food regulatory agency number 32 of 2019 was negative/ml.

Stability test

The stability test of citronella oil was shown in table 7 below.

Table 7: Stability test of citronella oil

Time	Description	pH	
		Room temperature (25 °C)	Temperature 40 °C
Week 0	Colorless, clear liquid, characteristic aromatic odor	6.2	-
1st week	Colorless, clear liquid, characteristic aromatic odor	6.32	6.33
2nd week	Colorless, clear liquid, characteristic aromatic odor	6.75	6.75
3 rd week	Colorless, clear liquid, characteristic aromatic odor	6.21	6.41
4 th week	Colorless, clear liquid, characteristic aromatic odor	6.44	6.40

The pH of the preparation for topical preparations must have the same pH range as the skin, namely 4.5-7.0. If the pH of the preparation is too low or too acidic, it will irritate the skin so that a

burning sensation will appear, while if it is too alkaline, it will give a dry and itchy feeling. Based on the results, it can be concluded that the pH obtained is still within the desired pH range and does not

have the potential to irritate. Besides that, there is no change in shape and aroma during 1 mo of storage. This shows that VCO is a good carrier oil capable of binding the mixed essential oils.

CONCLUSION

The results showed that formula 2 is the best and has the potential to be circulated in large quantities.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

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