

## KASTURI ORANGE PEEL (*CITRUS MICROCARPA BUNGE*) ESSENTIAL OIL: CHEMICAL PROFILE, FORMULATION AS ROLL-ON AROMATHERAPY AND ITS EVALUATION

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### ABSTRACT

**Objective:** Kasturi orange (*Citrus microcarpa* Bunge) is widely cultivated and consumed in Indonesia. The fruit's flesh is a good source of vitamin C. Essential oil of kasturi orange peel is one of the ingredients used as a raw material for aromatherapy roll-on preparations. Roll-on aromatherapy is currently widely used by various age groups. This research aims to determine the content of kasturi oil, its formulation as a roll-on aromatherapy preparation, and its physical evaluation.

**Methods:** The essential oil of kasturi orange peel was extracted using the distillation method, and then the physicochemical properties were examined. The chemical content of the oil was analyzed using GC-MS. The roll-on aromatherapy preparation formulas were made by varying concentrations of kasturi oil, F0 (0%), F1 (4%), F2 (6%), and F3 (10%). Additional substances used were menthol, camphor, patchouli oil, and virgin coconut oil. Patchouli oil was used in this preparation to make the aroma last longer. Evaluation of roll-on aromatherapy preparations includes organoleptic tests, pH tests, specific gravity, viscosity, clarity, and stability.

**Results:** From the extraction results, the percentage yield of essential kasturi oil was 0.55% v/w, with a density of 0.85 g/ml and a refractive index of 1.469. From the chromatogram results, there were 18 compounds in kasturi oil; the largest component was D-limonene (32.59%). Physical evaluation results of all roll-on aromatherapy formulas had met the requirements.

**Conclusion:** From the chromatogram results, there were 18 compounds in kasturi oil, with the largest component were D-limonene (32.59%). Formula F3 had the best aroma intensity, approximately for 5 h, with a slightly strong aroma, and physical evaluation results of all roll-on aromatherapy formulas had met the requirements.

**Keywords:** Roll on, Aromatherapy, Kasturi oil, *Citrus microcarpa* bunge, Kasturi orange peel

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### INTRODUCTION

Essential oils, commonly called volatile oils, are substances that evaporate easily and have a distinctive aroma. They are insoluble in water and contain organic compounds and natural extracts from plant species from leaves, flowers, wood, seeds, and even pistils. At least 150 types of essential oils are traded on the world market, and 40 varieties are produced in Indonesia. Even though many kinds of essential oils are produced in Indonesia, only a few have been developed [1]. Essential oils have many uses, depending on the type of plant from which they are distilled. Generally, essential oils are used as raw materials for flavors and fragrances. The pharmaceutical industry uses it as an analgesic, anti-infective, and bacteria killer. Essential oils are used as fragrances to cover the unpleasant odors of other ingredients and also insecticides [2].

Kasturi orange is a plant from the Rutacea family that can be extracted into essential oil. Kasturi oranges are widely distributed throughout Southeast Asia, including Indonesia. This plant is commonly cultivated in North and West Sumatra. It is beneficial for the pharmaceutical and cosmetics industries because it contains high levels of secondary metabolism, flavonoids, vitamin C, and antioxidants [3]. The main ingredients contained in the essential oil of kasturi orange peel are D-limonene, (R)-(+)-citronellal, 3-isopropenyl-5,5-dimethyl-cyclopentene,  $\beta$ -terpinene, citronellol, and  $\beta$ -terpineol. D-limonene is a monoterpene found in many essential oils reported to have antimicrobial activity. D-limonene has also been used in the manufacture of many products, such as cosmetics and food. The D-limonene compound has quite low toxicity. D-limonene is not mutagenic, carcinogenic, or nephrotoxic in humans. Kasturi orange peel oil extracted with N-hexane and dichloromethane will produce aromatic compounds [4].

Aromatherapy is a form of alternative medicine that utilizes plant volatiles, also known as essential oils, which aim to improve mood, health, and cognitive function. Apart from that, aromatherapy is made

from various plant extracts, such as flowers, leaves, wood, plant roots, bark, and other parts of plants, with different manufacturing methods according to their respective uses and functions [5]. Various essential oils have been researched and found to be effective as a mild sedative that calms the central nervous system, which helps overcome insomnia, especially those caused by stress, anxiety, tension, and depression [6].

One of the components used in making aromatherapy preparations is a fixative such as Patchouli Oil (*Pogostemon cablin* Benth). Patchouli oil contains several compounds, including buenesen, benzaldehyde, patchouli alcohol, patchoulien, and ariophyllene. The patchouli alcohol content in patchouli oil can be used as a fixative agent (binding agent), which is useful for the pharmaceutical industry in manufacturing medicines, cosmetic products, and others. If the patchouli alcohol content is higher, the higher quality of the patchouli oil is produced [7]. The best concentration of patchouli oil as a fixative in the preparation of air freshener gel is with a concentration of 3% and a concentration of coffee oil with a concentration of 5% [8]. Patchouli oil also used as a base note, where the mixture of lemongrass and ylang-ylang oil can reduce stress levels in healthy women. In this research, the essential oil of kasturi orange peel was formulated into a roll-on preparation by adding patchouli oil as a fixative [9]. The preparation was then evaluated for its physical properties, including organoleptic, pH, viscosity, specific gravity, stability, and aroma intensity test [10].

### MATERIALS AND METHODS

#### Materials

Kasturi oranges were obtained from the Tunggul Hitam area, Padang City, Indonesia. The plant has been identified in the Andalas University herbarium with the species name *Citrus microcarpa* Bunge (Result of Plant Identification No: 389/K-ID/ANDA/VI/2023).

The materials used in this study were camphor (Kimia Jaya Abadi), menthol (Anhui Tonghui Perfume Co. Ltd), patchouli oil (Aromatherapy Study Centre, Baiturrahmah University), and virgin coconut oil (PT Barooka Global Indonesia).

### Extraction of essential oil kasturi orange peel

The extraction process for essential oil of kasturi orange peel was carried out using water-steam distillation. The material used in this research was fresh kasturi orange (*Citrus microcarpa* Bunge). Kasturi orange was cleaned, and the peel was removed. Kasturi orange peel was weighed 27.9 kg using an analytical balance (Kern®). Steam distillation was done to separate essential oils from kasturi orange peel. A distillation kettle was filled with water until the water surface was not far below the filter. Kasturi orange peel samples were put into a kettle with a certain weight. The boiler was closed tightly and connected to a condenser, adapter, and reservoir. The distillation kettle was heated until all the essential oil had been distilled. The essential oil in the distillate was separated from the water in a separating funnel (Duran®) [11].

### Measurement of kasturi oil yield

The separated kasturi essential oil was transferred to a vial, and the percent yield was calculated. The yield value is the mass ratio between the essential kasturi oil produced and the mass of kasturi orange peel [12]. The yield was calculated using equation (1).

$$\% \text{ Yield} = \frac{\text{Weight of distilled kasturi orange peel oil (g)}}{\text{Weight of kasturi orange peel used (g)}} \times 100\% \dots\dots (1)$$

### Density testing

The pycnometer was cleaned, dried, and then weighed. After that, the essential oil of kasturi orange peel was added and then weighed again [13]. Determination of the density of essential oil of kasturi orange peel was calculated using equation (2).

$$\text{Density} = \frac{\text{mass of kasturi oil sample in pycnometer}}{\text{volume of kasturi oil sample in pycnometer}} \dots\dots (2)$$

### Refractive index testing

Refractive index measurement was carried out using a refractometer (Abbe). This method is based on a direct measure of the angle of refraction in oil maintained at constant temperature conditions. Water flows through the refractometer so the instrument is at the temperature at which the reading was taken. Before kasturi oil was placed in the tool, it had to be at the same temperature, and the measurements were taken when the temperature was stable [14].

### Solubility testing

The solubility of kasturi orange peel essential oil in alcohol is the solubility of the oil in alcohol at a specific concentration, expressed in a ratio. Solubility in alcohol was tested by mixing 1 ml of kasturi orange peel essential oil with 70% alcohol at a certain concentration until a clear solution formed [15].

### Analysis of chemical compounds

Chemical compounds in kasturi peel essential oil was analyzed using Gas Chromatography-Mass Spectrometry (Shimadzu GC-MS-QP2010 Ultra). The sample was injected with 1 µl volume at 250 °C (split ratio 200, helium with linear velocity 26.9 cm/sec as carrier gas). The GC oven temperature program started at 60 °C held for 5 min, then to 150 °C at a rate of 10 °C·min<sup>-1</sup>, then to 250 °C at a rate of 10 °C·min<sup>-1</sup>, and was held for 3 min. The MS operational conditions were: ion source temperature 220 °C, interference temperature 270 °C, and scanning 35–500 m/z [16].

### Kasturi oil roll-on aromatherapy formulation

Each ingredient as listed in table 1, was weighed. Camphor and menthol were added into a mortar and ground until smooth. The ingredients were put in an erlenmeyer flask with a glass stopper and stirred until it dissolved. Each essential oil concentration was added to formulas F0, F1, F2, and F3. VCO was added to 100 ml of the erlenmeyer flask and stirred until homogenous. After that, the preparations were put into a calibrated 10 ml roll-on bottle.

Table 1: Roll-on aromatherapy formulas from kasturi orange peel essential oil

Composition	Concentration (%)				Use
	F0	F1	F2	F3	
Kasturi Orange Peel Essential Oil	0	4	6	10	Active substance
Menthol	40	40	40	40	Corigentia odoris
Camphora	5	5	5	5	Anti-irritant
Patchouli oil	3	3	3	3	Binding
Virgin Coconut Oil	Up to 100	Up to 100	Up to 100	Up to 100	Oil Base

### Evaluation of the physical properties of kasturi oil roll-on aromatherapy

#### Organoleptic tests

The tests were carried out by observing the physical appearance of the preparation, including shape, color, and smell [17].

#### pH measurement

The pH tests were carried out using a pH meter (Orion Star A211®). Before use, the pH meter was calibrated with pH 4, pH 7, and pH 10 buffer solution (Orion, Thermo Scientific). 50 ml of the preparation was put into a beaker glass. The pH meter was immersed and left until the pH value was read on the instrument [18].

#### Density testing

Density testing uses a pycnometer with a volume of 10 ml. The pycnometer was cleaned and dried. The empty pycnometer was weighed, then the sample was added, and the pycnometer was closed until there were no air bubbles, then weighed [10].

#### Viscosity testing

The viscosity test was carried out using a Brookfield viscometer (Atago®). 15 ml of the sample was put into a beaker glass. A3 spindle was installed and lowered until immersed in the sample. The spindle

rotation speed was set to 250 rpm. The viscosity of the sample was read on the viscometer [19].

#### Clarity testing

The aromatherapy roll-on clarity test was carried out by visually observing the preparation in a test tube on a black background. The presence of particles and sediments in the preparations were observed. The preparation is homogeneous when there are no solid particles [20].

#### Accelerated stability testing

The accelerated stability test of roll-on aromatherapy preparations was carried out using the freeze-and-thaw method. The freeze-thaw cycle for roll-on aromatherapy preparations was stored at 5 °C for the first 12 h and 35 °C for the next 12 h, carried out in 6 cycles. The aromatherapy roll-on control preparation was stored at 25 °C [21].

#### Aroma intensity

The aromatherapy roll-on was applied to the test paper, and then the remaining aroma was assessed every hour for 9 h. Each formula was given a score: very strong (score 4), strong (score 3), weak (score 2), and very weak (score 1) at every hour [22].

**RESULTS**

**Measurement of kasturi oil yield**

The yield of essential oil of kasturi orange peel obtained from the extraction results was 0.55% v/w.

**Examination of the physicochemical properties of kasturi orange peel essential oil**

The results of examining physicochemical properties of kasturi orange peel essential oil were examined, including density with the value 0.85 g/ml, refractive index 1.469 and solubility in alcohol 1:1.

**Analysis of chemical compounds in kasturi orange peel essential oil**

The results of the chromatogram of kasturi orange peel essential oil components can be seen in fig. 1. There are 18 chemical compounds in kasturi orange peel essential oil (table 2).

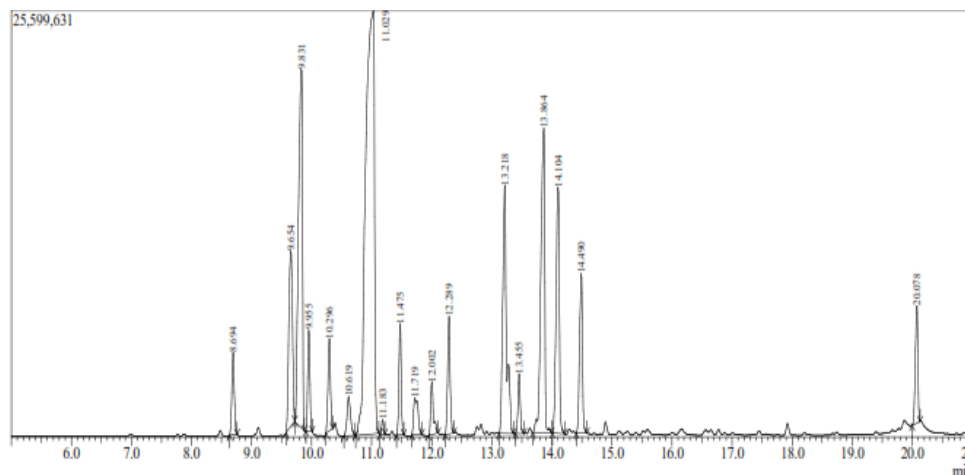


Fig. 1: GC-MS chromatogram of components kasturi orange peel essential oil

Table 2: Kasturi orange peel essential oil compounds

No	Retention time	Compound	Area %
1	8.694	α-Pinene	1.86
2	9.654	Sabinene (β-Thujene)	5.77
3	9.831	L-β-Pinene	12.52
4	9.955	β-Myrcene	1.99
5	10.296	Octanal	2.06
6	10.619	δ-Carene	1.21
7	11.029	D-Limonene	32.59
8	11.183	β-cis-Ocimene	0.23
9	11.475	γ Terpinene	2.25
10	11.719	1-Octanol	1.45
11	12.002	p-Mentha-1,8-diene	1.27
12	12.289	β-Linalool	2.69
13	13.218	(R)-(+)-Citronellal	8.49
14	13.455	Isopulegol	1.21
15	13.864	1-Terpinenol	10.49
16	14.104	α-Terpineol	7.13
17	14.490	Citronellol	4.08
18	20.078	α-Farnesene	2.71
			100.00

Table 3: Organoleptic test of kasturi orange oil roll-on aromatherapy preparation

Formula	Parameters	Observation during the day-			
		0	7	14	21
F0	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	none	none	none	none
	Color	Clear, yellow	Clear, yellow	Clear, yellow	Clear, yellow
F1	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	kasturi aroma	kasturi aroma	kasturi aroma	kasturi aroma
	Color	Clear, yellow	Clear, yellow	Clear, yellow	Clear, yellow
F2	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	kasturi aroma	kasturi aroma	kasturi aroma	kasturi aroma
	Color	Clear, yellow	Clear, yellow	Clear, yellow	Clear, yellow
F3	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	kasturi aroma	kasturi aroma	kasturi aroma	kasturi aroma
	Color	Clear, yellow	Clear, yellow	Clear, yellow	Clear, yellow
Comparator product	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Distinctive aroma	Distinctive aroma	Distinctive aroma	Distinctive aroma
	Color	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless

**Table 4: pH test results of kasturi orange oil roll-on aromatherapy**

Observation Day-	F0	F1	F2	F3	Comparator product
0	5,11±0,020	5,39±0,023	5,42±0,037	5,36±0,011	5,05±0,08
7	5,11±0,000	5,37±0,000	5,39±0,005	5,35±1,087	4,92±0,032
14	5,10±0,005	5,37±0,000	5,39±0,000	5,35±1,087	4,56±0,242
21	5,1±0,000	5,37±0,000	5,39±0,000	5,35±1,087	4,82±0,052

N=3, Data is given in mean±SD.

**pH measurement**

pH test results of roll-on aromatherapy kasturi orange oil using a pH meter on days 0, 7, 14, and 21 are shown in table 4.

**Evaluation of the physical properties of kasturi orange roll-on aromatherapy**

**Organoleptic tests**

The results of the organoleptic test of roll-on aromatherapy preparations of kasturi orange peel essential oil are shown in table 3. The results of the organoleptic evaluation of the preparations showed that roll-on aromatherapy was in the form of a liquid, yellow in color (transparent), with a weak to strong aroma of kasturi orange peel oil (F1, F2, and F3). The higher concentration of kasturi orange oil in the formula, the stronger the scent of kasturi orange oil, as shown by F3.

**Density test**

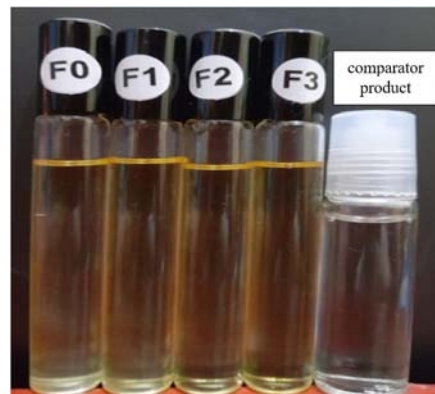
Density test results from roll-on aromatherapy kasturi orange for formulas F0, F1, F2, F3, and comparator product were respectively 0.922 g/ml; 0.901 g/ml; 0.919 g/ml; 0.912 g/ml; and 0.901 g/ml.

**Viscosity test**

The viscosity test was carried out using a Brookfield viscometer. The results for formulas F0, F1, F2, F3, and the comparator product were respectively 25.15 cP; 28.56 cP; 28.99 cP; 31.55 cP; and 22.59 cP.

**Clarity test**

The clarity test results showed that the comparator product and all formulas were clear, with no particles or sediment (fig. 2).



**Fig. 2: Clarity test results of roll-on aromatherapy kasturi orange peel oil**

**Accelerated stability test**

The result of the accelerated stability test carried out using the freeze and thaw cycling method is shown in table 5.

**Table 5: Accelerated stability tests**

Formula	Parameters	Accelerated stability tests	
		Before	After
F0	Shape	Liquid	Liquid
	Smell	none	none
	Color	Clear, yellow	Clear, yellow
F1	Shape	Liquid	Liquid
	Smell	kasturi aroma	kasturi aroma
	Color	Clear, yellow	Clear, yellow
F2	Shape	Liquid	Liquid
	Smell	kasturi aroma	kasturi aroma
	Color	Clear, yellow	Clear, yellow
F3	Shape	Liquid	Liquid
	Smell	kasturi aroma	kasturi aroma
	Color	Clear, yellow	Clear, yellow
Comparator product	Shape	Liquid	Liquid
	Smell	Distinctive aroma	Distinctive aroma
	Color	Clear, colorless	Clear, colorless

**Table 6: Aroma intensity test of kasturi orange oil roll-on aromatherapy**

Formula	The aroma during the hour-								
	1	2	3	4	5	6	7	8	9
F0	none	none	none	none	none	none	none	none	none
F1	Strong	Strong	Strong	Strong	Weak	Weak	Weak	Weak	Weak
F2	Strong	Strong	Strong	Strong	Weak	Weak	Weak	Weak	Weak
F3	Strong	Strong	Strong	Strong	Strong	Weak	Weak	Weak	Weak
Comparator product	Strong	Strong	Strong	Strong	Strong	Weak	Weak	Weak	Weak

### Aroma intensity

The aroma intensity test showed that the F3 formula provides a longer aroma compared to the other formulas of kasturi orange oil roll-on aromatherapy (table 6).

## DISCUSSION

### Examination of the physicochemical properties of kasturi orange peel essential oil

The essential oils obtained were stored in tightly closed brown glass bottles and in the refrigerator to minimize changes in each essential oil's physical and chemical properties due to the storage environment. Apart from that, the length of time essential oils are stored can also change the physical and chemical properties of essential oils [23]. Kasturi orange peel oil yield was 0.7% w/w with steam distillation at a temperature of 100°C for 1 hour. According to previous research, extraction time can affect the efficiency of the extraction process and the yield obtained [24].

The kasturi orange peel oil density was 0.850 g/ml in this research. Previous research also reported a similar density value of *Citrus microcarpa* Bunge peel essential oils, which was 0.889 g/ml [25]. Density value is also an important parameter in determining the quality and purity of oil. The greater the density of the essential oil indicates the more chemical components contained in the essential oil.

The refractive index is the ratio of the sine of the angle of incidence to the angle of refraction at a specific temperature. The more long-chain compounds such as sesquiterpenes or oxygen group components are distilled, the density of the essential oil will increase, and the incoming light will be more difficult to refract. It causes the oil's refractive index to be greater. On the other hand, the presence of water causes the index value to become smaller due to the nature of water, which easily refracts incoming light. Refractive index determination is carried out to determine the presence of water in the oil content. The more water content in the essential oil, the smaller the refractive index value is [26]. The average result of determining the refractive index was 1.696. The greater the refractive index value, the greater the level of purity of an essential oil. The level of purity of an essential oil indicates the quality of the essential oil [25].

### Analysis of compounds kasturi orange peel essential oil

The main components of kasturi orange peel oil are D-limonene (32.59%), Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-,(1S)-(12.52%), 3-cyclohexen-1-ol, 4-methyl-1-(methylene) (10.49%), 6-Octenal, 3,7-dimethyl-,(R)-(8.49%). Previous researches research also shows that the composition of the compound most commonly found in kasturi orange essential oil is D-limonene. Other compounds that are also present include monoterpenes, sesquiterpenes, alkanes, and aldehydes [24, 25].

### Evaluation of the physical properties of kasturi orange peel oil roll-on aromatherapy

#### organoleptic tests

There was no change in organoleptic test of kasturi orange peel oil roll on aromatherapy from week 0 to week 4 at F0, F1, F2, and F3. The color produced in F0 was clear yellow, F1 was clear yellow, F2 was clear yellow, and F3 was clear yellow. The smell of F0 had a typical no odor, while F1, F2, and F3 had a kasturi smell. All formulas had a liquid consistency. The color difference of the roll-on preparation occurs due to differences in the concentration of kasturi orange peel essential oil used. The higher the essential oil concentration, the more intense the yellow color in the liquid preparation.

#### pH measurement

The results of pH measurement of the preparation using a pH meter show that all roll-on aromatherapy formulas had a range pH of 5.10 to 5.42. Comparator product had a range of pH of 4.82 to 5.05. During storage, there was an increase in the roll-on aromatherapy preparation, but it was still in the range appropriate to the pH of the skin, 4-6. Topical preparations are expected to have a normal skin

pH because if it is too alkaline, it will result in scaly skin. In contrast, if it is too acidic, it can trigger skin irritation [27].

#### Density test

The density test shows that the F3 roll-on preparation has the lowest density, but the difference is insignificant from the other formulas. It may be influenced by the concentration of kasturi orange peel oil [10].

#### Viscosity test

The results showed that the viscosity of the kasturi orange peel oil roll on aromatherapy preparation ranged from 25.15-31.55 cP, and the comparator product was 22.59 cP. Viscosity tests on liquid preparations were carried out to determine the flow properties of the preparation. The viscosity of a preparation can also affect the adhesion and spreadability of the topical preparation on the skin. Suppose the preparation can spread and adhere well to the skin. In that case, the active substance of the preparation can be distributed and delivered to the skin surface constantly to provide a good and optimal therapeutic effect [28].

#### Clarity test

The clarity test aims to see the presence of undissolved particles or sediment in the aromatherapy roll-on preparation. In this study, all preparations had good clarity. There were no sediments, particles, or coarse grains that were visible when the clarity test was carried out. The preparation is said to be clear if there are no floating particles. It must be completely free from small particles seen with the eye with strong transmitted light [20].

#### Accelerated stability testing

Roll-on aromatherapy preparations that have been formulated were tested for accelerated stability, and then the results before and after were compared. After the preparations had been stored for six cycles, no organoleptic changes occurred in the four formulas and comparison preparations. If the preparation does not change before and after the stability test, the preparation is stable at any temperature [21].

#### Aroma intensity

The three formulas had almost the same aroma-lasting properties for approximately 5 h. The formula that had low aroma resistance is F0. Formulas 1 and 2 had an aroma-lasting power of approximately 4 h with the same treatment under room conditions and temperature. The formula that had high aroma resistance was F3. All formulas used 3 ml of patchouli oil, which could affect the persistence of the kasturi orange peel oil aroma because patchouli oil has good fixation or aroma binding ability. The formula is F0 as a blank and did not have the scent of kasturi orange peel oil because there were no active ingredients, so the evaporation is faster than other formulas [22].

There has been a lot of research used citrus oil as aromatherapy. The novelty of this research was that kasturi orange peel essential oil has not yet been formulated into a roll-on aromatherapy preparation. So in this study, researchers found that the aromatherapy roll on containing 10% kasturi orange peel essential oil (F3) was the best aromatherapy roll-on formula containing menthol, camphora, patchouli oil and virgin coconut oil as ingredients in formula.

## CONCLUSION

From the chromatogram results, there were 18 compounds in kasturi oil, with the largest component was D-limonene (32.59%), formula F3 had the best aroma intensity, approximately for 5 h, with a slightly strong aroma, and physical evaluation results of all roll-on aromatherapy formulas had met the requirements.

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## AUTHORS CONTRIBUTIONS

The authors contribution as follows: study conception and design: Rasyadi, Y., Ningsih, W; data collection: Mulya, WP.; analysis and

interpretation of results: Rasyadi, Y., Mulya, WP., Ningsih, W., Hanifa, D; draft manuscript preparation: Rasyadi, Y., Mulya, WP., Hanifa, D. All authors reviewed the results and approved the final version of the manuscript.

#### CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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