PHYSICAL AND CHEMICAL STABILITY STUDY OF LINIMENT AS ANALGESIC BASED ON RED GINGER ESSENTIAL OIL (ZINGIBER OFFICINALE VAR. RUBRUM) DURING STORAGE

Titi Agni Hutahaen[1]*, Atika Nirmala[2], Faradila Arifin Putri[3], Umi Muslihatun Nikmah[4]
[1234]Universitas Nahdlatul Ulama Sunan Giri
Alamat : Jalan Ahmad yani No 10 Bojonegoro, 62115,Indonesia
E-mail: titi.agni@unugiri.ac.id[1]*

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Abstract

Red ginger essential oil (Zingiber officinale var. Rubrum) has potential as a natural analgesic applied in liniment preparations. However, the physical and chemical stability of this preparation during storage needs to be evaluated to ensure its effectiveness and safety. Changes in pH, viscosity, homogeneity, and degradation of active compounds can affect the quality of the liniment over time. Therefore, this study aims to evaluate the physical and chemical stability of red ginger essential oil-based liniment under various storage conditions. The research methods include physical stability testing with organoleptic analysis (color, odor, clarity), pH, viscosity, and homogeneity. Chemical stability was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to identify changes in the content of bioactive compounds. The liniment was stored under three conditions: room temperature (25°C), accelerated temperature (40°C), and cold temperature (4°C). The organoleptic test results showed a light yellow color with a characteristic red ginger odor. The pH test results for the liniment fell within the required pH range. The comfort test using the Visual Analogue Scale (VAS) yielded results interpreted as very comfortable. Physical stability tests at three different temperatures showed stable results. GCMS testing at 40°C showed degradation of zingiberene compounds by 30-35%, including βsesquiphellandrene, champene and alfa pinene. Therefore, proper storage is a critical factor in maintaining purity and pharmacological efficacy.

Keywords: ***Red Ginger, Stability ,Liniment , Analgesic , Temperature***

INTRODUCTION

Pain is one of the most common health complaints experienced by the general population. The use of synthetic analgesics often causes side effects, such as skin irritation and allergic reactions when used topically. Therefore, the search for natural-based analgesic alternatives has become increasingly important. Red ginger essential oil (Zingiber officinale var. Rubrum) is known to have potential anti- inflammatory and analgesic effects, making it a suitable ingredient for liniment formulations (Hernani and Winarti, 2011). Red ginger is a plant used as a remedy for colds, as a rubbing agent for rheumatism and headaches, and as an anti-inflammatory and analgesic (Mantiri et al., 2013). The rhizome of red ginger contains the highest levels of volatile (essential oil) and non-volatile (oleoresin) components compared to other ginger varieties (Lamtiur, 2015). Gingerol and shogaol can be used to relieve pain (Sugiarti et al., 2011). These compounds inhibit the cyclooxygenase enzyme, thereby reducing the formation or biosynthesis of prostaglandins, which leads to a decrease in pain (Mantiri et al., 2013). Liniment is an oil- or alcohol-based topical preparation used to relieve muscle and joint pain (Mutia Sari, 2019) (Jose et al., 2015). However, the stability of liniment during storage is very important to ensure its effectiveness. Physical changes such as viscosity, color, and homogeneity can affect product quality. Additionally, active compounds in essential oils, such as gingerol and shogaol, may degrade due to exposure to temperature, light, and oxidation, potentially reducing their analgesic effects (Tritanti & Pranita, 2019) This study aims to evaluate the physical and chemical stability of red ginger essential oil-based liniment during storage under various conditions, including room temperature, accelerated temperature, and cold temperature. Physical stability will be analyzed through organoleptic parameters, viscosity, pH, and homogeneity, while chemical stability will be determined using Gas Chromatography-Mass Spectrometry (GC-MS) to identify changes in bioactive compounds (Darmapatni, 2016). The results of this study are expected to provide information on optimal storage conditions to maintain the stability and efficacy of red ginger essential oil-based liniment as an analgesic. Thus, this study can support the development of more stable, safe, and effective natural-based products as an alternative to topical analgesics

METHOD

The materials used in this study were red ginger essential oil (Oleum Zingiberis officinale var rum), menthol, eucalyptus oil (Oleum eucalypti), and peanut oil (Oleum arachidis).The equipment used in the study included an analytical balance, mortar and pestle, measuring cup, beaker, object glass, dropper pipette, watch glass, pycnometer, digital pH meter, oven, viscometer, amber dropper bottle, and Shimadzu GCMS-QP2010 Ultra gas chromatography-mass spectrometer (GC-MS).

2.1.Formulation Liniment Zingiber Officinale Var rubrum oil

The method of aromatherapy liniment using red ginger essential oil (Zingiber officinale var. Rubrum) uses a modified research method. The preparation process begins with grinding menthol as a corrigent and anti-irritant using a mortar and pestle. Once finely ground, eucalyptus oil is added as an anti-irritant and peanut oil as an additional ingredient. Then, red ginger essential oil is added as the active ingredient of the formula.

Table 1. Formulation of Liniment

Table head	Formulation (ml)		
	FI	**F2**	**F3**
Zingiber officinale Oil	7.5	9	12
Menthol	4	4	4
Oleum Ecalypti	10	10	10
Oleum Arachidis	Add 30 ml	Add 30 ml	Add 30 ml

2.2 Evaluation and Stability test

a. Organoleptic testing includes observation of the color, shape, and smell of the liniment that has been made with four different concentrations (Sumiyati et al, 2022).

b. Stability testing under various storage conditions: the liniment will be stored under three different conditions to evaluate the effect of the environment on stability:

1) Room temperature (25°C ± 2°C, RH 60% ± 5%) – normal storage conditions.
2) Accelerated temperature (40°C ± 2°C, RH 75% ± 5%) – to simulate faster degradation in accordance with ICH Q1A(R2) guidelines.
3) Cold temperature (4°C ± 2°C) – to assess the effect of storage at low temperatures.

c. The pH test for topical medications was conducted by immersing a digital pH meter into the liniment, then comparing the results with the pH of the skin.

d. Homogeneity testing is performed by applying 3–4 drops of the topical medication onto a glass slide, then covering it with another glass slide. This test is conducted to ensure a homogeneous composition and the absence of coarse particles in the formulation.

e. Viscosity testing is performed to determine the thickness of the topical medication using an Ostwald viscometer.

f. Chemical stability testing using Gas Chromatography-Mass Spectrometry (GC-MS) to identify changes in the content of main bioactive compounds, such as zingiberene and champene compounds.

RESULTS AND DISCUSSION

3.1 Distillation of Red Ginger Essential Oil

The method used to produce essential oils is steam distillation, which is the most commonly used method, involving the stages of evaporation, heating, and condensation. High pressure and heat force the cell sacs to open and release the aromatic substances inside. The amount of essential oil produced by this method depends on four variables: distillation time (5 hours), temperature, and the yield of essential oil is determined by comparing the weight of red ginger before distillation with the essential oil produced after distillation. The following is the yield formula for essential oil: in the distillation of red ginger at 100°C, pressure, and fresh red ginger material, so the high temperature can damage plant elements.

Rendemen = Output x 100% Input
Description:
R = yield (%)
Output = weight of essential oil produced by distillation (grams) Input = weight of the sample distilled (grams)

Thus, the yield of essential oil obtained is:

$$\text{Yield} = \frac{3\ \text{ml}}{10\ \text{kg}} \times 100\% = 30\%$$

The yield obtained from this study in the steam distillation of red ginger is 30%. This yield value is influenced by the distillation method used for the essential oil.

Fig. 1. Image 1. Distillation of Red Ginger Essential Oil *(figure caption)*

3.2 Organoleptic Test

Organoleptic testing was conducted through physical testing that included color, aroma, taste, and appearance in all formulations in liquid form and the sensation of spiciness on the skin when applied to F0, which caused an aroma similar to peanut oil. This was due to the absence of red ginger essential oil and peanut oil in the line, which acted as additives and created color. Yellow, for F1 with a red ginger essential oil concentration of 15%, it emits a characteristic aroma similar to red ginger but not very intense, with a color ranging from yellow to orange. In F2 with a red ginger concentration of 25%, it produces a unique aroma like red ginger and a bright orange color like F1. Therefore, F3 has a dark orange color with a strong characteristic aroma similar to red ginger. This is due to the main components, zingiberene and zingiberol, which give ginger its fragrant aroma. Based on a study (Gunawan, 2019), red ginger liniment is yellowish-orange in color. It is in liquid or diluted form and has a distinctive red ginger aroma. The results of the study show that the higher the concentration of essential oil, the more intense the color. Red ginger makes the color deeper to dark orange and the aroma stronger in red ginger essential oil.

Table 2. Organoleptic Test

Formulation	Color	Odor	Textur
F1	light yellow	The distinctive scent of ginger and eucalyptus	Liquid
F2	Yellow	The distinctive scent of ginger	Liquid
F3	Old yellow	The distinctive scent of menthol	Liquid

Image 2. Organoleptic *(figure caption)*

3..3 HOMOGENEITY Test

Homogeneity testing of abrasive substances was carried out on all formulas. F0, F1, F2, and F3 were homogeneous, and the formulations used glass containers filled with oil that did not contain other particles. Both were non-polar, except for a small amount of menthol, which was 4% compared to other materials. Non-polar oil compounds easily mix with other non-polar oil compounds, which are then evenly distributed and their components cannot be separated or homogenized.

Tablet 3.Homogeneity Test

Formula	Homogeneity
F1	Homogen
F2	Homogen
F3	Homogen

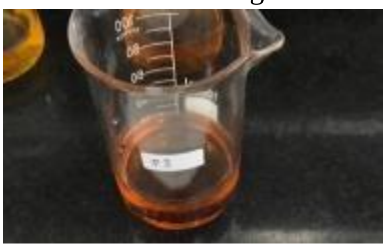

Image 3. Homogeneity *(figure caption)*

3.4 pH Test

The pH test results for all F0, F1, F2, and F3 scrub formulations showed that the pH values were within the criteria specified for human skin, which is usually between 4.5 and 6.5. Therefore, the liniment preparations were made according to the desired pH level. pH measurements using Universal pH paper showed a pH of

5 for F0 and a pH of 4 for F1, F2, and F3. Previous study findings (Gunawan, 2019) indicated that the abrasive pH produced by all formulations was outside the specified range. The normal pH range for human skin (4.5–6.5) tends to be alkaline, meaning it does not meet the criteria. However, there is a mechanism to address the "acidic layer" of the skin, which reaches neutrality after approximately 5 minutes of use and then reduces, neutralizing the "acidic layer" on the skin about one hour after use. To minimize this effect, it is recommended to clean the liner quickly and thoroughly after use

Table 4. pH Test

Formulation	**pH paper**	**pH Meter**
F1	5	5,1
F2	4	4,7
F3	4	4

3.5 Testing the sensation of comfort

One important sensory evaluation method for determining users' subjective responses to topical products. In this study, a test was conducted on a liniment product made from red ginger rhizome essential oil, involving 25 respondents. The assessment was conducted using a 100 mm Visual Analog Scale (VAS), which was evaluated for four main parameters: intensity of warmth, comfort of use, aroma, and potential for skin irritation.

VAS Assessment Results

Based on the ratings given by respondents, the following average scores were obtained:

- Intensity of warmth: 78 mm

This indicates that the product provides a strong but not stinging sensation of warmth, as expected from a liniment product. This sensation originates from the active compounds gingerol and shogaol in red ginger, which are known to stimulate heat receptors in the skin (Mekuria et al., 2022).

- Level of comfort during use: 82 mm

This score indicates that respondents felt very comfortable applying the product, without experiencing issues such as stickiness or poor absorption.

- Pleasant aroma: 85 mm

This average score indicates that the distinctive aroma of red ginger was well-received, even providing a relaxing effect for most respondents. Ginger essential oil is known to have aromatherapeutic properties that can alleviate stress and tension (Ali et al., 2008).

- Potential for skin irritation: 12 mm

This very low value indicates that the product is safe and does not cause irritation, suitable for use by various groups including students and the general public. This indicates that the liniment formulation used meets the safety standards for topical cosmetics.

Table 5. Testing the sensation of comfort

Formulation	**Result VAS (mm)**	**Interpretation**
F1	64	more comfortable
F2	88	comfortable
F3	74,5	more comfortable

3.6 Analisis Stability Test

Test results show that red ginger liniment provides a combination of comfort, pleasant aroma, and appropriate warmth, with very low irritation levels. This makes the product a potential herbal rubbing oil for daily use, especially to help warm the body, relieve aches and pains, and improve blood circulation. Stability testing of red ginger liniment under various storage temperature conditions. Stability testing of the red ginger liniment formulation was conducted by storing the product under three different environmental conditions: room temperature (±25°C), high temperature (±40°C), and low temperature (±4°C) for a specific period (e.g., 4 weeks). The purpose of this testing is to evaluate the physical, chemical, and organoleptic stability of the liniment under the influence of different storage temperatures.

Table 6. Liniment Storage Temperature Test Results

Formulation	25°C Temperature	acceleration temperature 40°C	Cold temperature 4°C
F1	Stable, no change	Stable, no change	Stable, no change
F2	Stable, no change	There are deposits and bubbles.	Stable, no change
F3	Stable, no change	There are deposits and bubbles.	Stable, no change

At high storage temperatures (±40°C), a more intense color change and slight turbidity occurred in the third week. In addition, there was a slight increase in the pungent odor of essential oils, which was thought to be due to the degradation of volatile compounds at high temperatures. Although no significant phase separation occurs, these changes indicate thermal degradation that can reduce product quality if stored for too long at high temperatures (Arya et al., 2017). At low temperatures (±4°C), the liniment experiences a slight increase in viscosity and a light sediment forms at the bottom of the bottle after the second week. The sediment can dissolve again after shaking, but it indicates a change in physical stability. This change is likely triggered by a decrease in the solubility of some components at low temperatures (Martin et al., 2002). However, no rancid odor or significant color changes were detected. Overall, red ginger liniment shows the best stability at room temperature, while storage at extreme temperatures (high or low) can affect the physical and chemical characteristics of the product. Therefore, it is recommended that the liniment be stored at a stable room temperature and protected from direct sunlight to maintain its quality and effectiveness during storage. When stored at room temperature (±25°C), the liniment showed good physical stability. There were no significant changes in color, odor, or viscosity. The liniment remained homogeneous and no phase separation occurred during the observation period. This indicates that room temperature is the optimal storage condition for maintaining product stability in the short to medium term.

3.7 Viscosity Liniment Test

Viscosity testing was conducted to determine the viscosity level of red ginger liniment preparations. Viscosity is one of the important parameters that affect the comfort of use, physical stability, and spreadability of topical preparations on the skin surface. The higher the viscosity, the thicker the formulation; conversely, too low viscosity can cause the product to flow quickly and not stay on the skin after application. (Sinko, 2011) Measurements were performed using an Ostwald viscometer, which operates based on the principle of the time it takes for a liquid to flow through a small capillary under the influence of gravitational force. In this test, the time required for the liniment liquid to pass through the capillary was measured and compared with standard solvent liquids such as water or ethanol. (Aulton and Taylor, 2017).

Table 7. Ostwald viscometer

Formulation	viscometer (s)
F1	11,38 S
F2	10,13 S
F3	09,24 S

To calculate the absolute viscosity of the liniment with a relative viscosity value of 11.38 from formulation F1 (the result of comparing the flow time and density to the standard solvent), we need to use the viscosity of the standard solvent in centipoise (cP) or mPa·s units. The standard solvent used is water at a test temperature of 25°C, with a viscosity of 0.890 cP.

Formula:

$$\eta \text{ liniment} = \eta \text{ solvent} \times \text{relative viscosity}$$

$$\eta\text{liniment} = 0.890 \times 11.38 = 10.13 \text{ cP (mPas)}$$

This value indicates that the liniment has a sufficiently ideal viscosity for a topical liquid formulation—not too thin, yet still easy to spread on the skin. If you wish to include this in a report, it can be stated as:

"The viscosity test result for red ginger liniment is 10.13 cP, which falls within the moderate viscosity range for liquid topical formulations (Aulton & Taylor, 2017)." In formulation F2, when 10.13 is substituted into the formula, the result is 9.02 cP, indicating that the liniment has appropriate viscosity for a liquid topical formulation (not too thin, not too thick). This is ideal for even distribution on the skin and absorption of active ingredients, as well as facilitating the rubbing process (Sinko, 2011). In formulation F3, the test result was 09.24, and when substituted into the formula, it yielded 8.23 cP. This indicates that the liniment formulation has sufficient viscosity for a liquid topical preparation. This consistency facilitates application, spreadability, and helps the formulation retain on the skin surface, enabling the active ingredients to work optimally. The results were obtained.

Table 8. Viscosity Test Results

Formulation	Viskositas (s)	result (cP)	Standart (cP)	Referensi
F1	11,38 S	10.13	5-15	Lachman et al., 1994; Sinko, 2011
F2	10,13 S	9,02	5-15	
F3	09,24 S	8.23	5-15	

3.8 Chemical stability test with GCMS

The chemical stability of red ginger essential oil was tested using Gas Chromatography–Mass Spectrometry (GC- MS) to determine changes in the composition of active compounds during storage. GC-MS is a sensitive and accurate analytical method for identifying and quantifying volatile compounds in essential oils. Essential oil samples were tested at the beginning (week 0) and after being stored under specific conditions (e.g., room temperature and high temperature for 4 weeks). The parameters analyzed included the quantity and types of major chemical compounds, such as zingiberene, chempene, zingerone, and those known to play a role in pharmacological activities such as anti- inflammatory and analgesic effects (Adams, 2007). GC-MS analysis results showed that at week 0, the main compound detected with the highest intensity was zingiberene (approximately 30–35%), followed by β-sesquiphellandrene, champene, and alpha-pinene. After 4 weeks of storage at room temperature, there was a slight decrease in the intensity of the zingiberene peak and an increase in minor degradation compounds such as geranial and citral, indicating the onset of oxidative degradation. At high temperatures (±40°C), the decrease in zingiberene and β-sesquiphellandrene content was more significant, and the appearance of new peaks indicating the formation of oxidation products was more evident. This phenomenon indicates that the active compounds in red ginger essential oil are sensitive to high temperatures, and chemical degradation can occur due to exposure to heat and light (Ali et al., 2008). GC-MS testing also showed that essential oils are more stable when stored in tightly sealed containers, protected from light, and at a constant room temperature. Thus, proper storage is an important factor in maintaining the purity and pharmacological efficacy of red ginger essential oil.

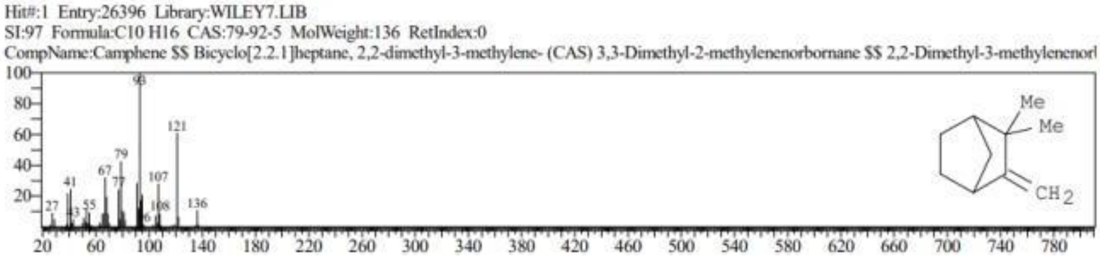

Image 4. Champhene compound

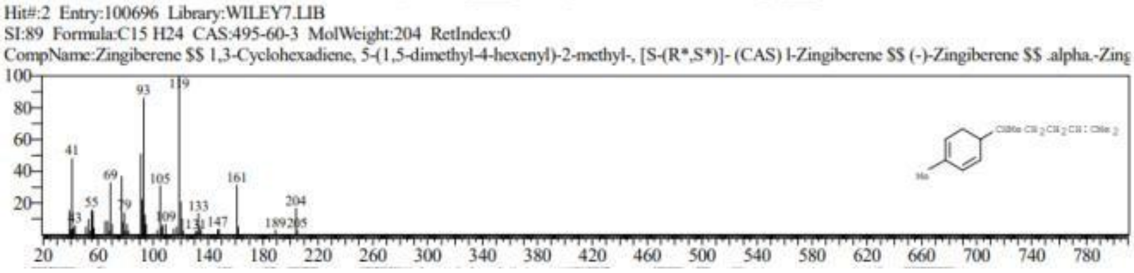

Image 5. Zingibrene compound

GC-MS test results prove that the chemical stability of red ginger essential oil is greatly influenced by storage conditions. Exposure to high temperatures can cause degradation of the main active compounds, so to maintain chemical stability, it is recommended to store essential oils at room temperature in dark, airtight containers. Based on the results of GCMS analysis of the essential oil components of red ginger (Zingiberis officinale var rum), the percentage of essential oil components can be determined, with the highest percentage being zingibrene at 27.69%, followed by β-sesquiphellandrene at 25.37%, cgampene (11.33%), and alpha pinene at 5.41%. According to the research results, zingiberene accounts for 27.69% of the pain-relieving compounds, and camphene accounts for 11.33%. According to Ar Razi et al. (2024), the active compounds in ginger include sesquiterpenes, bisapolene, zingiberol, zingiberene, and several essential oils that have sedative, antipyretic, analgesic, and antibacterial properties. According to research by Fajrin (2019), GC/MS analysis revealed that camphene is the highest-abundant compound in red ginger oil, which may be significant for its antihyperalgesic effects.

This study found that red ginger oil exhibits antihyperalgesic activity in mice with chronic pain and could be further developed for antihyperalgesic applications. The analgesic compound in red ginger essential oil, identified by GC/MS, is zingiberene, with the highest percentage at 27.69%, and camphene at 11.33%. The mechanism of action of camphene involves inhibiting the activity of the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor, a nerve receptor involved in detecting pain and heat. The mechanism of action of zingiberene, which has a percentage of 5.41%, involves inhibiting the activity of the cyclooxygenase (COX) enzyme, which is involved in prostaglandin production. Ginger contains several non-volatile compounds, including gingerene, gingerol, shogaol, and zingerone (Tritanti et al., 2019). The compound zingiberene is known to give ginger its aroma, while gingerol, shogaol, and zingerone give it its spicy, hot, and bitter taste. The chromatogram results of the essential oil components of red ginger obtained from three distillations did not detect gingerol, shogaol, and zingerone but contained gingerene at 2.28%. These chromatogram results indicate that the essential oil produced has a strong characteristic ginger aroma, but its taste is not very spicy or hot, as the three main ginger components—gingerol, shogaol, and zingerone—were not detected. The absence or undetectability of the main ginger components such as gingerol, shogaol, and zingerone in the chromatogram may be due to several factors, such as excessively high temperatures during the distillation process or the use of gas chromatography, which can cause spontaneous decomposition of ginger. Therefore, more extensive research using gas chromatography-mass spectrometry is needed to identify ginger compounds that may not have been identified previously (Tritanti et al., 2019).

CONCLUSION

The analgesic compound in red ginger was first identified by GC-MS, which showed that the analgesic compound obtained from the study contained 27.69% zingiberene and 11.33% camphene, out of 25 compounds contained in the GC-MS test. Based on the research results, the liniment should be stored at a stable room temperature and protected from direct sunlight exposure to maintain its quality and efficacy during storage.

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