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Formulation and Efficacy Test of Antistress Aromatherapy Candles Containing Peppermint Leaf Essential Oil (Mentha Piperita L) and Clove Leaf Essential Oil (Syzygium Aromaticum L) on Mice (Mus Musculus)

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ABSTRACT
Purpose: This study aims to determine the effectiveness of aromatherapy candles containing peppermint (Mentha piperita L.) and clove (Syzygium aromaticum L.) essential oils at concentrations of 3%, 5%, and 7% on mice.
Research Method: This study employed a laboratory experimental method with a post-test-only control group design. The formula used in this study was a combination of peppermint and clove with concentrations FII (2%:1%), FIII (3%:2%), and FIV (4%:3%), as well as negative and positive controls (lavender). The evaluation was conducted on physical tests of the candles, including organoleptic tests, burn time tests, and melting point tests. The antistress effectiveness testing was divided into five groups, each consisting of 5 mice. The first treatment involved the Tail Suspension Test to induce stress in the mice. Subsequently, the mice were exposed to aromatherapy candles, and the Forced Swimming Test was conducted. The test results were analyzed using the One-Way ANOVA test.
Results and Discussion: All aromatherapy candle formulas met the physical evaluation test requirements. Formulas FII and FIII demonstrated antistress efficacy with a significance value of less than 0.05.
Implications: These results indicate that the combination of peppermint leaf essential oil and clove leaf essential oil has potential as an active ingredient in anti-stress aromatherapy candles. Further research is needed to support its effectiveness on a broader scale.

Keywords: antistress; peppermint leaves (mentha piperita l.); clove leaves (syzygium aromaticum l.); aromatherapy candles; essential oils.

Introduction

Aromatic plants have long been utilized in various traditional medicines, primarily due to the essential oils derived from plant parts, including leaves, flowers, stems, and roots. Essential oils possess volatile properties that enable their use in aromatherapy, a complementary therapy that utilizes scents to stimulate the central nervous system and produce calming effects (Sundara *et al.*, 2022). One popular form of preparation is aromatherapy candles, which not only serve as a therapeutic medium but also as

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room fresheners and mood enhancers (Ariani *et al.,* 2024; Fitrian *et al.,* 2024). Aromatherapy candles work by releasing the aroma of essential oils into the air when burned, which is then inhaled and can stimulate the limbic system in the brain, providing a relaxing effect and reducing stress and anxiety (Azizi et al., 2024). The problem that forms the basis of this study is the high prevalence of stress, especially among students. Stress occurs when individuals feel that environmental demands exceed their capacity to cope, causing physical and mental disorders. In Indonesia, the prevalence of academic stress among students is high, ranging from 36.7% to 71.6% (Fikri *et al.,* 2024). The use of pharmacological therapy carries the risk of long-term side effects, making non-pharmacological therapies such as aromatherapy a safer alternative. The combination of peppermint (Mentha piperita L.) and clove (Syzygium aromaticum L.) essential oils is believed to have strong anti-stress potential; however, its effects in an aromatherapy candle formulation have not been extensively studied (Pradana *et al.,* 2024; Purwaningsih *et al.,* 2023).

Research on aromatherapy continues to evolve, particularly in the context of stress management through non-pharmacological approaches. Aromatherapy has been scientifically proven to stimulate the limbic system in the brain through olfaction, which in turn influences an individual's emotional and physiological state. In this context, the use of peppermint essential oil (Mentha piperita L.) and clove essential oil (Syzygium aromaticum L.) is relevant because both contain active compounds with sedative and relaxing effects. The main components of peppermint essential oil, namely menthol (30–55%) and menthone (14–32%), are known to provide a refreshing effect, soothe the throat, and support the body's relaxation process by increasing lung oxygenation (Purwaningsih et al., 2023; Zuladh Gonzhary & NHerawati, 2023). These compounds work by reducing muscle tension and providing a soothing, cooling sensation. On the other hand, essential oil from clove leaves contains high concentrations of eugenol (75.04–77.54%), along with eugenyl acetate and β-caryophyllene, which contribute to its analgesic and anticonvulsant effects (Pradana *et al.,* 2024; Tampubolon *et al.,* 2024). Clove leaves have been used in traditional medicine for centuries to treat coughs, dizziness, sleep disorders, and even as a mental stimulant (Ticoalu *et al.,* 2024). Previous studies have supported the effectiveness of aromatherapy as an antistress agent. For example, research by Azizi *et al.,* (2024) showed that aromatherapy candles made from lemongrass oil reduced stress in mice at doses of 2%, 4%, and 6%. Purnomo *et al.,* (2021) found that the use of peppermint aromatherapy was effective in reducing anxiety among final-year university students under pressure to write their thesis. This study highlights the role of menthol and menthone compounds in creating a calming effect. Additionally, Lusiana & Indawati, (2023) developed an aromatherapy candle formulation based on a combination of peppermint and lemon, concluding that Formula I (2:3 %) is the most optimal formulation, as it contains sufficient essential oil content while maintaining the physical stability of the candle.

Previous studies have demonstrated the benefits of peppermint and clove essential oils separately as stress-relieving agents. For example, research by Purnomo *et al.,* (2021) showed that peppermint aromatherapy can reduce anxiety levels in final-year students. The menthol and menthone content in peppermint has a significant calming effect on psychological conditions. Meanwhile, studies by (Ticoalu *et al.,* 2024; Pradana *et al.,* 2024) have outlined the benefits of eugenol in clove essential oil, which is used in the health sector as a treatment for coughs, dizziness, and as a mental stimulant. Additionally, research by Azizi *et al.,* (2024) using aromatherapy candles made from lemongrass on mice demonstrated the effectiveness of this method as an anti-stress therapy. Lusiana & Indawati, (2023) also developed a candle formulation combining peppermint and lemon, with a primary focus on the physical formulation aspects of the candle, such as its melting point and burning time. However, there is a significant gap in the literature regarding the combination of peppermint and clove essential oils in a single aromatherapy candle formulation and its experimental testing for effectiveness. Most previous studies have only tested a single type of essential oil or have not employed a post-test-only control group approach in their test animals. Additionally, most studies have focused on organoleptic testing, physical formulation, or human subjective perception, rather than objective testing of biological responses. Therefore, there is an urgent need to explore the empirical aspects of combining two essential oils in candle form to address the theoretical and practical gaps in the development of more effective non-pharmacological antistress therapies.

The novelty of this study lies in the development of an aromatherapy candle formulation that combines two types of natural essential oils, namely peppermint (Mentha piperita L.) and clove (Syzygium aromaticum L.), each of which has been proven to have antistress effects but has never been studied in combination in a single candle formulation. Additionally, this study employs an experimental laboratory approach with a post-test-only control group design, using laboratory mice (Mus musculus), which enables the objective measurement of effectiveness based on biological responses to stress. Unlike previous studies that focused solely on sensory aspects, physical formulation, or human subjective responses, this study provides empirical laboratory-based evidence of the impact of aromatherapy. This study also considers sustainability by using soy wax as the base material for the candles, which is a more environmentally friendly alternative to paraffin wax. Thus, the primary objective of this study is to formulate the optimal concentration combination of peppermint and clove essential oils in the form of aromatherapy candles and to test their effectiveness in reducing stress in mice. The results obtained are expected to contribute both scientifically and practically to the development of more efficient, safe, and sustainable natural-based aromatherapy products as an alternative non-pharmacological therapy for stress conditions.

Literature Review and Hypothesis Development

Peppermint leaves (Mentha Piperita L.)

Peppermint leaves (Mentha piperita L.) are herbal plants from the Lamiaceae family, widely known for their rich essential oil content, which is particularly high in bioactive compounds, including menthol and menthone. These compounds provide significant pharmacological effects, including anti-inflammatory, antimicrobial, analgesic, and sedative activities. Research by Zhao *et al.,* (2022) indicates that peppermint essential oil has high potential as a therapeutic agent, particularly in the treatment of central nervous system disorders and as a natural stress-relieving agent. These pharmacological activities are supported by the presence of other active compounds such as neomenthol, isomenthone, limonene, and 1,8-cineole, which synergistically enhance the relaxing and calming effects on the body. (Kehili *et al.,* 2020) also confirmed that peppermint has strong antinociceptive and anti-inflammatory effects, making it an ideal candidate for plant-based alternative therapy. Additionally, menthol is known to stimulate cold receptors on the skin and mucous membranes, providing a characteristic refreshing sensation that is often utilized in topical formulations and inhalations. In the context of aromatherapy, these pharmacological effects are highly relevant, as they can be utilized to alleviate stress through the olfactory pathway, which influences the limbic system —the brain's emotional regulation center. The use of peppermint leaves in aromatherapy has shown positive results in several clinical and experimental studies. (Eftekhari *et al.,* 2021) confirm that Mentha spp., including peppermint, have significant potential in modern phytotherapy due to their ability to improve psychological and physiological

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balance in the body. Its sharp and calming aroma plays a role in reducing tension and anxiety, especially when applied in the form of inhalation oil or aromatherapy candles. (Mahdavikian et al., 2020) found in their study that the use of peppermint aromatherapy significantly improved sleep quality in patients with cardiovascular disease, who often experience sleep disturbances due to anxiety. These results indicate that peppermint's effects are not limited to temporary relaxation but also improve overall psychological well-being. Even in modern health product innovations, peppermint has been incorporated into various pharmaceutical and non-pharmaceutical formulations, including functional textiles, as developed by Tariq *et al.,* (2024). These researchers successfully applied peppermint oil through microencapsulation technology to fabric, producing sustained aromatherapy effects. These findings demonstrate that peppermint leaves are not merely ordinary natural ingredients but possess significant scientific and practical value in the development of stress-relief products and holistic therapy.

Clove leaves (Syzygium Aromaticum L.)

Clove leaves (Syzygium aromaticum L.) are a part of the plant that has high therapeutic value due to its main active compound, eugenol. This plant belongs to the Myrtaceae family and is widely known in tropical regions, including Indonesia, as both a spice and a medicinal plant. The essential oil derived from clove leaves is chemically rich in eugenol, which has been found to reach concentrations of 75% to 80% in some studies, as well as β-caryophyllene and eugenyl acetate in smaller amounts. These compounds are responsible for biological activities, including antiseptic, analgesic, anti-inflammatory, and antimicrobial effects (Selles *et al.,* 2020). In their study, they confirmed that clove leaf essential oil exhibits significant antibacterial and antioxidant activity against various test pathogens, demonstrating great potential in pharmaceutical and aromatherapy applications. Eugenol, the dominant component, also demonstrates anti-inflammatory properties by inhibiting prostaglandin synthesis and reducing nerve activity that causes pain and inflammation (Nisar *et al.,* 2021). Through this mechanism, eugenol derived from clove leaves can be utilized as an active ingredient in the development of non-pharmacological therapies for anxiety and stress disorders.

In modern aromatherapy practice, clove leaves are one of the most promising sources of essential oils for further development. Its strong and distinctive aroma can stimulate the central nervous system, particularly the limbic system, which plays a role in regulating emotions and mood. Nisar *et al.* (2021) state that eugenol can stabilize neuronal activity in the brain, which physiologically reduces tension and produces a mild sedative effect. This makes clove leaf essential oil a potential candidate for use in the formulation of aromatherapy products, such as candles, massage oils, or inhalers (Lv *et al.,* 2025). Add that eugenol also plays a role in maintaining tissue integrity and accelerating the body's recovery from oxidative stress, which is often associated with psychological disorders. In the development of natural product technology, clove leaf oil has been utilized in the creation of topical formulations and functional textiles with relaxing and skin-protective properties. With their good volatility and stability of aromatic compounds, clove leaves have an advantage over other plant parts in producing long-lasting therapeutic effects.

Aromatherapy Candles

Aromatherapy candles are a form of complementary therapy that utilizes a combination of wax and essential oils to create therapeutic effects through the process of aroma inhalation. When the candle is lit, the heat from the flame warms the essential oils contained in the wax matrix, releasing volatile

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compounds that can be inhaled and stimulate the central nervous system through the olfactory pathway. This process stimulates the limbic system in the brain, which plays an important role in regulating emotions, mood, and responses to stress. Nisar *et al.,* (2021) explain that active compounds in essential oils, such as eugenol, can provide sedative and anxiolytic effects through complex neurophysiological mechanisms, making them potential ingredients in the development of anti-stress products. The effectiveness of aromatherapy candles in improving sleep quality and reducing anxiety is also supported by the findings of Ng *et al.,* (2022), which confirm that inhalation-based aromatherapy has a significant impact on the psychoemotional balance of individuals in a non-pharmacological therapy context.

From a formulation aspect, the success of aromatherapy candles depends heavily on the choice of base wax used. Paraffin is the most commonly used material because it is easy to shape and inexpensive; however, its use has raised concerns due to its potential to produce harmful compounds, such as formaldehyde and benzene, when burned. A study by Salthammer *et al.,* (2021) found that burning paraffin-based candles can produce pollutant particles that are harmful to respiratory health in the long term. To address this challenge, the use of natural waxes such as soy wax has been widely developed due to their more environmentally friendly and safe properties. (Junio *et al.,* 2021) stated that soy wax not only produces cleaner combustion but also maintains the volatility and stability of aromatic compounds longer than paraffin, thereby enhancing the effectiveness of the aroma produced and additionally, using soy wax as an aroma-releasing medium allows aromatherapy candle formulations to last longer and provide more consistent therapeutic effects. In industrial practice, combining soy wax with essential oils such as peppermint, lavender, or clove has become a popular formulation because it not only promotes health but also aligns with market trends that prioritize sustainability and safety.

Essential oil

Essential oils are natural aromatic compounds produced by plants through various parts, including leaves, flowers, bark, roots, and seeds. The main components of essential oils include monoterpenes, sesquiterpenes, phenols, esters, aldehydes, and other volatile compounds that easily evaporate at room temperature. These compounds are known to exhibit high biological activity and have been widely utilized in both traditional and modern medicine. In a study conducted by Bhavaniramya et al. (2019), essential oils were found to have strong antimicrobial and antioxidant effects, making them highly potential for use as therapeutic agents and protectants against oxidation in various applications, including health and food. Additionally, according to Elshafie et al. (2024), essential oils from various plants such as Origanum vulgare, Syzygium aromaticum, and Mentha piperita demonstrate high effectiveness in inhibiting the growth of harmful pathogens, indicating their potential as biological protective agents. In the context of their use in pharmaceuticals and complementary health, the active compounds in essential oils can interact with the central nervous system through the olfactory pathway, providing sedative, anxiolytic, and mood-balancing effects.

In addition to their antimicrobial and anti-inflammatory properties, essential oils also have advantages in aromatherapy applications. Through complex mechanisms, volatile compounds from inhaled essential oils stimulate the limbic system in the brain, which is directly linked to emotion regulation and the regulation of stress hormones. (Nisar *et al.,* 2021) highlight the role of eugenol—the primary component in clove essential oil, which has been shown to stabilize neural activity and alleviate mental tension. The use of essential oils in aromatherapy has been supported within the framework of

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complementary and integrative therapy, as explained by Ng *et al.,* (2022), who note that essential oils contribute to the emotional recovery of patients and enhance comfort in non-pharmacological therapy. Beyond clinical applications, the use of essential oils is rapidly expanding in food technology and industry, particularly in the form of microcapsules to enhance stability and efficacy. Hassid *et al.,* (2025) explain that the nanoencapsulation process of essential oils can protect bioactive compounds from degradation and enhance their efficiency in biological systems. Thus, essential oils are not merely aromatic or natural fragrances but possess extensive functional and therapeutic value, encompassing physical health, emotional well-being, and biological protection.

Research Method

This research employed a laboratory experimental method with a post-test-only control group design, using mice (Mus musculus) as test animals. The research was conducted at the Pharmacology Laboratory of Muhammadiyah University Kudus. The population in this study consisted of fresh peppermint leaves (Mentha piperita L.) and clove leaves (Syzygium aromaticum L.) sourced from Nalumsari Village, Jepara Regency, Central Java. In this study, the researcher conducted direct observations on the test animals to collect data on the antistress effects of aromatherapy candles. This study has received ethical approval from the Ethics Committee of Muhammadiyah University of Purwokerto with Registration Number: KEPK/UMP/389/III/2025.

Tools and Materials

The equipment required for this study includes a round-bottom flask (Iwaki Pyrex), condenser, clamps, stand, heating mantle, hot plate, stopwatch, aquarium, thermometer, moisture balance (Ohaus), beaker glass (Iwaki Pyrex), Erlenmeyer flask (Iwaki Pyrex), analytical balance (Ohaus), measuring glass (Iwaki Pyrex), separating funnel (Iwaki Pyrex), wax mold, dropper, spatula, porcelain dish, and stirring rod. The materials used in this study were soy wax, stearic acid, candle wicks, distilled water, concentrated sulfuric acid (H_2SO_4), glacial acetic acid, Mayer's reagent, Wagner's reagent, Dragendroff's reagent, mice (Mus musculus), peppermint leaves (Mentha piperita L.), and clove leaves (Syzygium aromaticum L.).

Research Procedures

Preparation of Peppermint and Clove Leaf Samples

The samples used in this study were dried peppermint leaves (Mentha piperita L.) and clove leaves (Syzygium aromaticum L.). Fresh peppermint leaves and clove leaves were processed into crude drugs through several stages, starting with the collection of raw materials, followed by wet sorting, washing, chopping, drying, and dry sorting. After that, 1000 grams of dried samples were used.

Distillation of Peppermint Leaves and Clove Leaves

In the steam distillation process, the equipment and materials are prepared first. Next, 1000 grams of peppermint leaves and cloves are used, and the distillation process is carried out using distilled water as a solvent for approximately 6 hours at a steam pressure exceeding 1 atm and a temperature exceeding 100°C.

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Phytochemical Screening Test

Flavonoid Test

Essential oil is added dropwise (3-7 drops) into a test tube, followed by a few drops of concentrated sulfuric acid solution (H_2SO_4). The color change is observed; if the solution turns dark red or yellow, this indicates the presence of flavonoids (Ropiqa *et al.,* 2023).

Triterpenoid

Essential oil is added dropwise (3-7 drops) to a test tube, followed by 1-2 drops of glacial acetic acid solution and 1-2 drops of concentrated sulfuric acid solution (H_2SO_4). The color change is observed; if the solution turns red or orange, it indicates the presence of triterpenoid compounds (Ropiqa *et al.,* 2023).

Alkaloid Test

Peppermint leaf essential oil was added in an amount of 2 mL to 1 mL of 2N sulfuric acid, and then heated for 30 minutes. After heating, the solution was allowed to separate, and the sulfuric acid layer was divided into three test tubes. In the first tube, two drops of Wagner's reagent are added, and the formation of a brown precipitate indicates a positive result. The second tube is given two drops of Mayer's reagent, with a positive result appearing as a white precipitate. The third tube is supplemented with two drops of Dragendorff's reagent, and the formation of an orange-colored precipitate indicates a positive result (Ropiqa *et al.,* 2023).

Making Aromatherapy Candles

The process of creating the wax base begins with preparing the tools and materials, followed by weighing each ingredient according to the specified requirements. Soy wax and stearic acid are melted separately at 100°C using porcelain bowls on a hot plate. Once melted, the two ingredients are mixed while stirring until homogeneous, and the active ingredient is added when the temperature reaches 60°C. The finished wax base mixture is then poured into prepared molds with wicks and left to harden. The formulation used in this study is presented in Table 1.

Table 1. Formulation of Aromatherapy Candle with Peppermint Leaf and Clove Leaf Essential Oils

Materials	Formula				
	F1	F2	F3	Negative Control	Positive Control
Peppermint Essential Oil	2	3	4	-	Lavender aromatherapy candle
Clove Essential Oil	1	2	3	-	
Stearic acid	20	20	20	25	
Soy wax	Ad 100	Ad 100	Ad 100	Ad 100	

Source: Processed primary data (2025)

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Physical Properties Test

Organoleptic Test: Testing is conducted by observing the candle's visual color, its physical shape, and its aroma. The candle must have the same color, be free of cracks, defects, and breaks (Chotimah *et al.,* 2024).

Burning Time Test: The candle burn time test is conducted by lighting the candle wick and running a stopwatch until the wick is completely burned and the candle extinguishes. The burn time is calculated from the difference between the initial lighting time and the time when the wick is completely burned. The standard burn time is 378-614 minutes (Chotimah *et al.,* 2024).

Melting Point Test: The test was conducted by using a dropper to apply wax and storing it in a refrigerator for 16 hours at a temperature of 4 °C to 10 °C. After that, a pan containing 500 mL of water was heated with an empty beaker placed inside it. The dropper containing wax was placed in the beaker, and a thermometer was attached to the mouth of the dropper. The temperature on the thermometer was recorded as the wax melted and dripped into the beaker. The standard melting point according to SNI 0386-1989-A/SII 0348-1980 is within the temperature range of 50°C to 58°C (Oktarina *et al.,* 2021).

Treatment of Test Animals

Acclimatization of test animals must be carried out before starting treatment. This process aims to help animals adapt to their new environment. The time required for acclimatization ranges from 3 to 14 days. Mice were fed pellets mixed with corn of medium texture, neither too hard nor too soft (Khairani *et al.,* 2024). Following the completion of the adaptation treatment, the mice were divided into five groups, with each group consisting of 5 mice. The grouping of experimental animals is presented in Table 2.

Table 2. Treatment of Test Animals

Group	Formula	Number of mice	Treatment	Minimum weight
I	F1	5	Peppermint 2% dan Cengkeh 1%	20 grams
II	F2	5	Peppermint 3% dan Cengkeh 2%	20 grams
III	F3	5	Peppermint 4% dan Cengkeh 3%	20 grams
IV	Negative Control	5	Negative Control	20 grams
V	Positive Control	5	Positive Control	20 grams
Total		25		

Source: Processed primary data (2025)

Antistress Effectiveness Test

Tail Suspension Test: Test animals were suspended by their tails using adhesive attached to the tip, ensuring they could not escape or touch the surrounding surface. The tail suspension test (TST) lasted for six minutes, with the last four minutes used for analysis, as the first two minutes were typically spent by the test animals trying to escape from the apparatus. The primary parameter measured in this test is immobility time, which is the period during which the test animals hang without any active movement. Immobility time is considered the primary indicator of despair behavior (UCSF, 2021).

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Force Swimming Test: During the testing phase, mice were exposed for 1 hour to aromatherapy candles containing peppermint and clove essential oils at a predetermined concentration. Then, a force swimming test was conducted by placing the mice in an aquarium (diameter 18 cm and height 40 cm) filled with water to a depth of 15 cm at a temperature of 25°C. The mice were allowed to swim, and the immobility time was recorded using a stopwatch for 7 minutes (Inaku *et al.,* 2023).

Data Analysis Methods

Immobility time data analysis in mice was performed using a One-Way Analysis of Variance (ANOVA) statistical test, preceded by a data normality test, followed by a homogeneity test, and continued with the ANOVA statistical analysis. To determine significant differences between treatments, the Post Hoc Tukey test was performed (Inaku *et al.,* 2023).

Results and Discussion

Analysis Result

Plant Determination

In this study, peppermint leaves and clove leaves were identified at the Biology Laboratory, Faculty of Science and Applied Technology, Ahmad Dahlan University, with identification numbers 546/Lab.Bio/B/XII/2024 and 547/Lab.Bio/B/XII/2024. The identification results indicate that the samples used are peppermint leaves (Mentha piperita L.) and clove leaves (Syzygium aromaticum L.).

Sample Processing

Peppermint leaves and clove leaves are sourced from Nalumsari Village, Jepara Regency, Central Java. Fresh leaves are collected and processed into crude drugs through several stages, including wet sorting to remove impurities and foreign objects from the leaves, followed by washing with running water. After washing and draining, the leaves are chopped into small pieces. The chopping process aims to accelerate the drying process. The thinner the leaves are, the faster they will dry. The drying process is then carried out under sunlight, covered with a black cloth. The dried leaves are first sorted to remove foreign objects and impurities that may still be adhering to the dried crude drug. The crude drug is then ground using a blender. This process aims to standardize particle size and obtain a fine powder of the crude drug.

Seven thousand grams of dried peppermint leaves yielded 1,532 grams of dried simplisia, while clove leaves yielded 1,218 grams. Peppermint leaves experienced a drying shrinkage of 78.1%. Meanwhile, clove leaves experienced a drying shrinkage of 75.6%. Drying shrinkage is used to determine the limit of compounds lost during the drying process. The moisture content in peppermint leaves and clove leaves was measured using a moisture balance. The measurement results for peppermint leaves were 3.33% and for clove leaves, 3.87%. The dried materials met the requirements, as their moisture content did not exceed 10%—the moisture content and weight of the material decrease as the duration of the drying process increases. Several factors influencing the drying rate include temperature, air humidity, and volumetric airflow rate. Additionally, the characteristics of the dried material, such as size and initial moisture content, also play a role in the drying process (Rosyidah et al., 2024). In this study, mint leaves were smaller in size compared to clove leaves, resulting in a faster drying process.

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Steam Distillation

In the extraction process of peppermint leaves (Mentha piperita L.) and clove leaves (Syzygium aromaticum L.), steam distillation was employed, and distilled water was used as the solvent in this study. Distilled water was used as a solvent because its polarity differs from that of essential oils. This difference facilitates the separation of essential oils from the distillate. Additionally, distilled water does not mix with essential oils and has a lower boiling point than essential oils. The ground peppermint and clove leaves are then placed into the distillation flask. The sample-to-solvent ratio used is 1:2, with 1000 grams of sample and 2000 mL of distilled water. The essential oil is extracted through a heating process until it reaches a temperature of 100°C, the boiling point of water, to produce steam. The steam carries the components from the leaves. The steam then condenses into two liquids, namely water and oil, in the condenser. Next, the essential oil is separated from the distillate using a separating funnel.

The distillation product obtained is peppermint essential oil extract, characterized by a clear, yellowish color, and clove leaf essential oil, which has a pale yellow color. Both products meet the quality standards for essential oils, as outlined in ISBN: 979-548-026-X. The weight of the essential oil is presented in Table 3.

Table 3. Steam Distillation Results

Sample	Sample Weight (g)	Distillation Results (ml)	Yield (%)
Peppermint Leaf	1000	13 ml	1,3%
Clove leaves	1000	20 ml	2,0%

Source: Processed primary data (2025)

Based on Table 3, the extraction of peppermint leaf essential oil yielded 13 mL of essential oil, corresponding to a yield of 1.3%. In contrast, clove leaves yielded 20 mL of essential oil, corresponding to a yield of 2.0%. Several factors, including plant species, cultivation methods, harvesting time and methods, and the quality of raw materials, influence the yield of essential oil (Sasongko *et al.,* 2022). The study (Qolbi *et al.,* 2024) also mentions environmental factors, such as harvesting temperature, soil characteristics, and elevation, which also play a role in influencing the yield of essential oil produced by plants.

Phytochemical Screening Test

The essential oil produced from the extraction process is then subjected to phytochemical screening. Phytochemical screening is conducted to identify the presence of secondary metabolites in the extract. Phytochemical testing plays a crucial role in identifying active compounds that may cause toxic effects or provide biological benefits. Through the identification of secondary metabolites contained in plant extracts, the potential pharmacological benefits of the plant can be determined (Khafid *et al.,* 2023).

The tests conducted on clove leaf and peppermint leaf essential oils included tests for flavonoids, triterpenoids, and alkaloids. The results of phytochemical screening of peppermint and clove leaf essential oils showed positive results for flavonoids, as evidenced by the formation of a red color when a few drops of concentrated sulfuric acid (H2SO4) solution were added. Flavonoids are a group of phenolic compounds commonly found in plant tissues and function as antioxidants. Triterpenoids are a group of secondary metabolites belonging to the terpenoid group (Nurcholis *et al.,* 2022).

Triterpenoids offer various pharmacological benefits, including enhancing mental function, providing a calming effect, and promoting smooth blood flow to the brain by revitalizing blood vessels. In tests on peppermint and clove leaf essential oils, a red color formed when glacial acetic acid solution and concentrated sulfuric acid solution (H_2SO_4) were added. Furthermore, alkaloid testing on peppermint and clove leaf essential oils did not yield positive results when treated with Wagner, Mayer, and Dragendroff reagents, as no precipitates formed. This is due to the alkaloid compounds being unstable at high temperatures during the distillation process, resulting in degradation and their absence in the results (Handayani & Fatmawati, 2021).

Aromatherapy Candle Formulation

Aromatherapy candle formulations are made in several concentrations, namely FI (2%:1%), FII (3%:2%), FIII (4%:3%), and Negative Control. In the process of making aromatherapy candles, the initial step is to prepare and weigh the ingredients according to the specified formula concentration. Soy wax and stearic acid are heated together on a hot plate until melted. Once both ingredients have melted, they are mixed and stirred until a homogeneous mixture is formed. Then, the essential oil is added according to the formulated amount. After all components are mixed homogeneously, the mixture is poured into small glass containers equipped with a wick in the center. The molded candles are left to harden for 24 hours. Soy wax is an environmentally friendly candle base made from soybean oil, which is easily obtainable and does not pollute the environment. Another advantage is its clean-burning process, which produces no smoke or unpleasant odors, making it safer and more comfortable to use (Utami & Tjandrawibawa, 2020).

Figure 1. Aromatherapy Candle Formulation

The selection of aromatherapy candles in this study was based on the use of essential oils that have properties to help alleviate various health problems, including anxiety relief. Aromatherapy candles are a non-pharmacological complementary therapy technique. The use of aromatherapy candles offers various benefits for the body, promoting relaxation, reducing stress levels, creating a pleasant atmosphere, enhancing sleep quality, and aiding in overcoming mental fatigue (Riani et al., 2023). Peppermint leaves were chosen because previous research (Purnomo et al., 2021) has shown that peppermint aromatherapy can alleviate burnout syndrome in final-year students completing their thesis. Another study also (Lestari et al., 2025) proved that aromatherapy is effective in reducing anxiety before tooth extraction. Meanwhile, the selection of clove leaves is supported by research (Anas et al.,

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2019) which found that the active compounds in the terpenoid group of ethanol extracts from clove flowers and leaves have stimulant properties by increasing locomotor activity in Swiss male mice. Research conducted (Sanggor et al., 2024) also demonstrated that the eugenol compound in cloves has antidepressant efficacy.

Physical Properties Test

Organoleptic Test

The formulated wax preparations were then physically tested to assess their quality. Organoleptic testing included observations of shape, color, and aroma. Based on Table 4, the evaluation results of the aromatherapy wax preparations in F1, F2, F3, Positive Control, and the Negative Control indicate that the formula meets the physical characteristics required by SNI 0386-1989-A/SII 0348-1980. In these standards, candles that meet quality requirements are characterized by uniform color, an intact surface without cracks or breaks, and a wick positioned in the center (Riani et al., 2023). The solid texture of the candles is attributed to the addition of base materials, such as stearic acid and soy wax. These two materials harden as the temperature decreases, giving the candles their solid texture (Oktarina et al., 2021; Rahmawati et al., 2024). This result is consistent with the organoleptic evaluation conducted, where all five formulas exhibit physical characteristics that meet the quality criteria.

Table 4. Organoleptic Test Results

Formula	Color	Shape	Fragrance
F1	Vaginal discharge cream	Dense and crack-free	Distinctive scent of peppermint and cloves.
F2	Vaginal discharge cream	Dense and crack-free	Distinctive scent of peppermint and cloves.
F3	Vaginal discharge cream	Dense and crack-free	Distinctive scent of peppermint and cloves.
Negative Control	Vaginal discharge cream	Dense and crack-free	Odorless
Positive Control	Vaginal discharge cream	Dense and crack-free	The distinctive scent of lavender

Source: Processed primary data (2025)

Based on Table 4, the results of the physical evaluation of aroma characteristics in F1 showed a weak aromatic odor, F2 showed a moderate aromatic odor, F3 showed a strong aromatic odor, and Negative Control had no odor because no essential oil was added. Positive Control had a characteristic lavender odor. Of all the formulas, F3 had a distinctly pungent odor due to the increased essential oil concentration, which intensified the aroma (Simorangkir, 2020).

Burning time testing

Burning time testing was conducted by observing the duration of the aromatherapy candle's burn from the moment it was lit until the entire candle was consumed (Kireina & Maulina, 2024). Based on the burn time test results in Table 5, it is evident that Positive Control exhibits the fastest burn time, at 386 minutes. The longest burn time is 593 minutes for Negative Control. The Positive Control has the fastest burn time because it weighs 75 grams compared to the other formulas, which weigh 100 grams.

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Among the three candles formulated in F3 (4%:3%), the fastest burn time is 493 minutes. Additionally, F2 (3%; 2%) has a burning time of 517 minutes, and F1 (2%; 1%) has a burning time. The differences in burning time among the candle formulas are influenced by the concentration of essential oil used. The higher the essential oil content in the formulation, the faster the candle tends to burn. This is due to the nature of essential oils, which easily evaporate during the burning process (Hilmarni *et al.,* 2021). Waxes containing higher amounts of stearic acid tend to have longer burn times compared to those with lower stearic acid content. This can be observed in Negative Control, which has a higher concentration of stearic acid compared to the other formulations. Several factors, including the position of the wick, the composition of the ingredients, and the type of container used, also influence the burn time of wax. If the wick is centered, heat distribution during combustion becomes more even, causing the wax to melt evenly and resulting in better durability. The addition of stearic acid in the candle formulation causes the wax structure to become denser, more complex, and form crystals. This structure slows down the melting process because heat from combustion has difficulty penetrating the dense and hard wax (Oktarina *et al.,* 2021).

Table 5. Burning Time Test Results

Formula	Time Details		Test Results	Description
	Start	Finish		
F1	09.00	18.05	545 minute	Meets burn time standards Requirements: 378-614 minutes
F2	09.00	17.37	517 minute	
F3	09.00	17.13	493 minute	
Negative Control	09.00	18.53	593 minute	
Positive Control	09.00	15.26	386 minute	

Source: Processed primary data (2025)

Figure 2. Burning Time Test

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Melting point test

Melting point testing is conducted to determine the temperature at which wax begins to melt or liquefy.

Table 6. Melting Point Test Results

Formula	Melting Point Test Results	Description
F1	54°C	Compliant with SNI 0386-1989-A/SII 0348-1980 Requirements: 50°C- 58°C
F2	53°C	
F3	51°C	
Negative Control	56°C	
Positive Control	52°C	

Source: Processed primary data (2025)

Melting point testing in Table 6 indicates that all formulas fall within the range of 51 °C to 55 °C. This range still meets the physical properties evaluation standards for waxes based on SNI, which is 50 °C to 58 °C. The formula with the highest melting point is Negative Control at 56°C. The Positive Control has a melting point of 52°C, followed by F1 at 54°C and F2 at 53°C. Meanwhile, F3 shows the lowest melting point at 51°C. This is due to the addition of essential oils during the candle-making process, which can affect the melting point. The higher the amount of essential oil added, the lower the melting point tends to be (Lusiana & Indawati, 2023). The research results align with the study by Oktarina et al. (2021), which attributed these differences to the influence of the base material and the proportions of ingredients used.

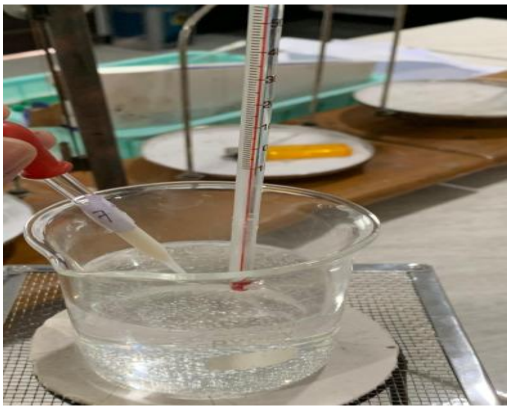

Figure 3. Melting Point Test

Antistress Effectiveness Test

The subsequent treatment involved testing the effectiveness of aromatherapy candles on male mice (Mus musculus) using the forced swimming test method. Before treatment, the mice were first adapted for seven days. A total of 25 mice were used in this study, divided into five groups, with five mice in each group. After the adaptation period, the mice were subjected to a tail suspension test for six minutes to induce stress. This method was performed by suspending the mice by their tails using adhesive tape attached to the tip of their tails, preventing them from escaping or touching the surrounding surface. Next, the mice were exposed to aromatherapy candles according to the concentration of each group, namely F1 (2%; 1%), F2 (3%; 2%), F3 (4%; 3%), Positive Control, and

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Negative Control. After exposure, the mice were placed in water to undergo a forced swimming test, with a maximum duration of 7 minutes, to observe the immobility time. This is the time the mice remain motionless, indicating the level of despair in their efforts to stay afloat.

Based on Table 7, the negative control group exhibited the highest average immobility time of 220.2 seconds, which served as a comparison for the other aromatherapy groups. The positive control group showed a decrease in average immobility time of 91.8 seconds. Group F1, with a concentration of (2%:1%), recorded an average immobility time of 157.6 seconds. Meanwhile, the F2 group with a concentration of (3%:2%) showed a decrease in average immobility time of 102.4 seconds, which is still relatively low. The lowest decrease occurred in the F3 group with a concentration of (4%:3%), with an average immobility time of 75 seconds. The stress reduction occurred because the aromatic components in essential oils tend to react quickly when inhaled, as the peppermint leaves, containing menthol, and the clove leaves, containing eugenol, can directly interact with the central nervous system through the olfactory system (smell). This system then sends stimuli to the nervous tissue in the brain. When compounds enter the body, they affect the limbic system, which plays a role in regulating emotions. Compounds in essential oils can influence locomotor activity, thereby reducing immobility time in mice (Kaluku et al., 2021).

The research findings align with the notion (Lestari *et al.*, 2025) that inhaling peppermint essential oil aromatherapy stimulates the nasal nerves, which in turn affect the brain and nervous system. These stimuli can promote the release of neurotransmitters that play a crucial role in regulating psychological conditions, including emotions, mood, thoughts, and desires. Research also (Simorangkir, 2020) indicates that eugenol has a depressive effect by inhibiting the oxidative degradation of catecholamines in mitochondria. As a result of this mechanism, levels of neurotransmitters such as norepinephrine, epinephrine, and serotonin in the brain increase.

Table 7. Antistress Effectiveness Test Results

Group	Formula	Number of mice	Treatment	Average Immobility Time (Seconds)	SD value
I	F1	5	Peppermint 2% and cloves 1%	157.6	47.4
II	F2	5	Peppermint 3% and cloves 2%	102.4	52.9
III	F3	5	Peppermint 4% and cloves 3%	75	18.5
IV	Negative Control	5	Negative Control	220.6	66.1
V	Positive Control	5	Positive Control	91.8	41.3

Source: Processed primary data (2025)

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Figure 4. Aromatherapy Candle Effectiveness Test

SPSS Test Results

The research data were analyzed using ANOVA statistical tests to evaluate the effect of five types of aromatherapy candles on mice experiencing stress. If the significance value (Sig.) is less than 0.05, it can be concluded that there is a significant difference in the average immobility time between groups (Azizi *et al.*, 2024).

- Statistical test results using ANOVA showed that there were significant differences between groups, so the analysis was continued with a Tukey Post Hoc test to determine which formulations differed from one another.
- Based on the test results, F1 did not show significant differences from the other groups, namely F2 (Sig = 0.390), F3 (Sig = 0.085), Negative Control (Sig = 0.267), and Positive Control (Sig = 0.231) because all significance values (greater than 0.05) were significant, thus concluding that there were no significant differences.
- In F2, there was a significant difference only with the Negative Control (Sig = 0.007 less than 0.05), but no significant difference with F1 (Sig = 0.390), F3 (Sig = 0.892), and Positive Control (Sig = 0.997).
- F3 showed a significant difference from the Negative Control (Sig = 0.001 less than 0.05) but was not significantly different from F1 (Sig = 0.085), F2 (Sig = 0.892), or the Positive Control (Sig = 0.980).
- In Negative Control, there were significant differences with F2 (Sig = 0.007), F3 (Sig = 0.001), and Positive Control (Sig = 0.003). The results showed Sig. (less than 0.05), indicating a significant difference, and no significant difference with F1 (Sig = 0.267, greater than 0.05).
- Positive control showed a significant difference compared to negative control (Sig = 0.003, less than 0.05) compared to F1 (Sig = 0.231), F2 (Sig = 0.997), and F3 (Sig = 0.980), which means there was no significant difference (greater than 0.05).

These results indicate that aromatherapy candles containing peppermint (Mentha piperita L.) and clove (Syzygium aromaticum L.) essential oils at concentrations of 5% and 7% have antistress efficacy comparable to that of lavender aromatherapy candles, which served as a positive control.

Conclusion

This study aims to formulate an aromatherapy candle using peppermint (Mentha piperita L.) and clove (Syzygium aromaticum L.) essential oils, and to test its effectiveness in reducing stress in mice

(Mus musculus) using a post-test-only control group experimental design. Based on organoleptic testing, melting point, and burn time, all candle concentrations (3%, 5%, and 7%) met the physical quality criteria for aromatherapy candles. However, only the 5% and 7% concentrations demonstrated effectiveness in reducing stress, with the 7% concentration being the most optimal formulation based on the reduction in immobility time of the mice. These results address the research question regarding the most effective concentration of the combination of peppermint and clove essential oils in aromatherapy candle formulations.

Scientifically, this study provides an original contribution to the development of natural-based aromatherapy products that combine two types of essential oils with complementary therapeutic characteristics. This study also reinforces the relevance of non-pharmacological approaches in safely and efficiently managing stress, while highlighting the potential of soy wax as an environmentally friendly base material for candles. Practically, the developed aromatherapy candle formulation could serve as an effective alternative for stress relief in society, particularly in the fields of pharmacy, psychology, and the natural health industry. From a managerial perspective, the results of this study can serve as a reference for the development of commercially viable aromatherapy products based on laboratory research. This study has limitations as it did not evaluate physiological stress parameters such as cortisol levels or changes in nervous system activity. Additionally, the study only used male test animals in a controlled laboratory setting.

To enrich understanding, further research is recommended to explore a broader range of dosage variables, incorporate stress biomarker measurements, and test the effectiveness of this aromatherapy candle on human populations in both clinical and community settings. Additional studies are also encouraged to assess the stability and shelf life of the candle formulation, as well as user responses to the aroma and comfort aspects during long-term use.

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