Journal Syifa Sciences and Clinical Research (JSSCR)
Volume 8 Number 1, 2026
Journal Homepage: http://ejurnal.unq.ac.id/index.php/jsscr, E-ISSN: 2656-9612 P-ISSN:2656-8187
DOI: https://doi.org/10.37311/jsscr.v8i1.36162

Myristica fragrans Aromatherapy Oil: Formulation, Freeze-Thaw Stability, and Antistress Activity

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ABSTRACT

Nutmeg essential oil derived from *Myristica fragrans* is widely utilised in the food, pharmaceutical, perfumery, and cosmetic industries, and it contains bioactive constituents such as safrole and myristicin that may contribute to neurosensory responses relevant to aromatherapy. This study aimed to evaluate the physical stability of nutmeg essential oil formulated as an aromatherapy wind oil and to assess its antistress-like activity in white mice (*Mus musculus*). A post-test only control group design was employed using 15 mice randomly allocated into five groups with three mice per group, consisting of a negative control (K−), nutmeg aromatherapy oil formulations containing 2% (F1), 4% (F2), and 8% (F3) essential oil, and a positive control (K+) using Freshcare. Stability was assessed using a freeze–thaw cycling test followed by physical evaluations including organoleptic properties, homogeneity, pH, spreadability, and viscosity. Antistress-like activity was evaluated by measuring immobility time in the forced swimming test after 60 minutes of aromatherapy exposure. Immobility time decreased from 174.6667 seconds in K− to 149.0 seconds in F1, 133.6667 seconds in F2, and 101.3333 seconds in F3, with the lowest value observed in K+ at 87.3333 seconds. These findings indicate that the nutmeg aromatherapy oil remained physically stable under cycling conditions and produced a concentration-related reduction in immobility time, with statistically significant differences between groups at $p < 0.05$.

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Keywords:
Aromatherapy; *Myristica fragrans;* Anti-stress; Tail suspension test; Forced swim test

Received:	*Accepted:*	*Online:*
2025-12-15	*2026-01-16*	*2026-01-31*

1. Introduction

In Indonesian society, the term "masuk angin" is used to describe symptoms or complaints such as fever, congestion, muscle pain, stiffness, flatulence, and loss of appetite. Indonesians use topical products to reduce the symptoms of masuk angin. Treating the symptoms of masuk angin with medicated oil is one of the most common methods [1]. Aromatherapy essential oils use primary ingredients derived from essential oils extracted from plants and are effective in improving mental and physical health. Products containing aromatherapy can improve your health by relaxing the body,

refreshing the mind, improving mood, and aiding in the physiological healing of diseases [2].

Stress is defined as the physical, mental, and chemical reaction of the body to frightening, surprising, confusing, dangerous, or disturbing situations [1]. Stress management uses pharmacological and non-pharmacological methods. One non-pharmacological way to reduce stress is to do things that make you feel better, such as laughter therapy and aromatherapy [3].

Traditional medicine is becoming increasingly popular in society. This is because it is inexpensive, easily accessible, and has few side effects. Many plants in our environment have not been utilised properly, and some are even considered useless. More than a thousand types of plants are classified as medicinal plants, one of which is nutmeg [4]. Nutmeg is an antioxidant that is very important for boosting immunity during autumn and summer, helping to prevent flu and other long-term illnesses. Antioxidants protect cells from free radicals, antioxidants protect cells from free radicals. Aromatherapy is linked to free radical prevention because the essential oils used contain natural antioxidant compounds, such as phenols, terpenes, and flavonoids, which help neutralize free radicals and protect cells from oxidative stress [5].

Based on the above description, this study is interested in examining the production of aromatherapy oil preparations and evaluating the preparations as well as determining the anti-stress effects on mice *(Mus Musculus*) using the immobility time test of aromatherapy oil from nutmeg seed oil (*Myristica Fragrans)*.

2. Methods

Sample Collection and Processing

Nutmeg seeds were collected from Fogi Village, North Maluku Sub-district, Sula Islands Regency. The seeds were harvested directly from nutmeg trees and washed under clean running water to remove adhering dirt. The nutmeg fruits were then chopped and separated into kernels, husks, and seeds, followed by sun-drying to facilitate separation of the seeds from the shells and to reduce moisture prior to distillation.

Nutmeg Seed Distillation

Nutmeg essential oil was obtained by distillation as previously described [6]. Briefly, the distillation apparatus was assembled using a stand, clamps, condenser, and a heating mantle. A round-bottom flask containing 1 L of distilled water was placed on the heater, and a second flask was filled with 500 g of nutmeg seeds. The condenser was connected, and cooling water was circulated through the condenser hoses. Distillation was carried out at 100°C for 5 h. The distillate was transferred into a separating funnel and allowed to stand until two layers formed (aqueous and oil layers). The oil layer was collected, dried using anhydrous sodium sulfate to remove residual water, and stored in a closed container until formulation [6].

Formulation of Nutmeg Aromatherapy Oil

The aromatherapy oil was prepared by weighing peppermint and camphor into a mortar, followed by addition of VCO (virgin coconut oil) and trituration until a homogeneous mixture was obtained. Nutmeg essential oil (*Myristica fragrans*) was then incorporated according to the assigned formulation concentration (F1–F3) and mixed thoroughly until a uniform preparation was achieved. The detailed composition of each 10 mL formulation, including the negative control (K−), nutmeg essential oil concentrations, and the positive control (K+), is presented in **Table 1**. The final product was packaged in roll-on bottles or suitable containers. In this formulation, essential oils

provide the characteristic aroma and aromatherapy benefits, whereas peppermint/menthol and camphor contribute a warm–cool sensory perception on the skin [7].

Table 1. Composition of nutmeg (*Myristica fragrans*) aromatherapy oil per 10 mL

Ingredient	K− (vehicle)	F1	F2	F3	K+	Function
Nutmeg essential oil / nutmeg active ingredient*	–	2%	4%	8%	–	Active ingredient
Peppermint	10%	10%	10%	10%	–	Coolant / aromatiser
Camphor	5%	5%	5%	5%	–	Warming agent / counterirritant
VCO (virgin coconut oil)	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	–	Vehicle / moisturiser
Positive control product	–	–	–	–	Freshcare oil	Positive control

Evaluation of Formulations

Organoleptic Test

The testing conducted aims to examine the physical properties of the formulation, including its form, and any changes in colour and odour that occur in the sample after formulation [8].

Homogeneity Test

Homogeneity was assessed to confirm the absence of coarse particles or visible aggregates, indicating that the preparation was uniform and did not show incompatibility [8].

pH Test

The pH test was conducted to ensure that each formulation was compatible with skin pH and met the acceptable pH range for topical preparations [8]. A solution was prepared by dissolving 1 g of sample in 25 mL of distilled water, and pH was determined using pH indicator paper applied directly to the prepared solution [9].

Spreadability Test

Spreadability was evaluated because good spreadability facilitates application on the skin and may enhance topical performance [8]. Approximately 1 g of sample was placed between two circular glass plates. A load of 50 g was added incrementally each minute up to 200 g, and the diameter of spreading was measured using a ruler to evaluate the effect of applied load [10].

Viscosity Test

Viscosity was measured to characterise flow resistance of the oil preparation and to ensure the product remained sufficiently viscous while still pourable. Measurements were performed using a Brookfield viscometer with spindle no. 6. Viscosity (centipoise, cps) was calculated from the dial reading multiplied by the corresponding spindle correction factor. Flow behaviour could be further described by plotting shear stress versus shear rate [11].

Preference Test

A preference (hedonic) test was conducted to assess respondents' acceptance of the product based on organoleptic attributes, including colour, aroma, and sensory perception on the skin. These attributes reflect perceived quality because they are evaluated directly by human senses and influence product presentation and user acceptance [1] .

Irritation Test

Skin irritation potential was assessed to determine whether topical application elicited observable cutaneous reactions. Skin tolerability was evaluated using a patch test with a 24–48 h observation period and graded erythema/edema scoring, as previously described [12]. No signs of erythema, edema, itching, or stinging were observed for any formulation, indicating the absence of irritation under the study conditions.

Cycling Test (Freeze–Thaw Stability)

A freeze–thaw cycling method was applied to assess physical stability [13]. The oil preparation was stored at 4 ± 2°C for 24 h and then transferred to 25 ± 2°C for the next 24 h, constituting one cycle. The procedure was repeated for six cycles. At the end of each cycle, physical evaluations were performed, including organoleptic properties, homogeneity, pH, spreadability, and viscosity [13] .

Antistress Activity Test and Forced Swimming Test

Mice were stressed using a tail suspension procedure by suspending the animals at a height of 60 cm for 3 consecutive days, for 3 min each day. Animals were randomly allocated into five groups (K−, F1, F2, F3, and K+), with three mice per group. Each group was exposed to the assigned aromatherapy oil in a glass jar for 60 min. Subsequently, the forced swimming test (FST) was performed by placing mice in a water-filled container and recording immobility time during the test period, with a total observation duration of 6 min [6].

The forced swimming test is a widely used rodent behavioural assay to evaluate antidepressant-like activity and depression-like states by measuring escape-related mobility behaviour in an inescapable water environment. Implementation requires careful adherence to procedural details and minimisation of unnecessary stressors that may confound behavioural interpretation [14].

Data Analysis

Anti-stress effects among groups were analysed using one-way ANOVA. Prior to ANOVA, data distribution was assessed using a normality test to confirm that the immobility time data in each group met the normality assumption required for parametric inference [15] .

Ethical Considerations

This research has obtained ethical approval from the Health Research Ethics Committee/Animal Research Ethics Committee at the relevant institution. The ethical permit letter was issued by Makassar Health Polytechnic, with the number 1006/M/KEPK-PTKMS/V/2025.All research procedures involving mice were conducted in accordance with the 3R principles (Replacement, Reduction, and Refinement) and adhered to international ethical guidelines for the use of laboratory animals. Efforts were made to minimize the number of test animals and minimize pain, stress, and discomfort during the research process.

3. Results and Discussion

Organoleptic properties after freeze–thaw cycling

Table 2 presents the organoleptic characteristics (colour, smell, and texture) of the aromatherapy essential oil preparations before and after the cycling test. Overall, all groups maintained an oily texture before and after cycling, indicating that the preparations retained their basic physical form under temperature stress. The negative control (K−) remained cloudy white before and after cycling, while formulations containing nutmeg essential oil (F1–F3) exhibited pale-to-light yellow colouration that did not change after cycling. Regarding odour, F1–F3 consistently retained the characteristic smell of nutmeg seeds after cycling, suggesting that the cycling procedure did not cause an apparent deterioration of sensory attributes within the observation limits of the test [16].

Table 2. Organoleptic test results of aromatherapy essential oil preparations before and after cycling

Formula	Colour (Before)	Smell (Before)	Texture (Before)	Colour (After)	Smell (After)	Texture (After)
K−	Cloudy white	Characteristic of peppermint	Oil	Cloudy white	Characteristic of nutmeg seeds*	Oil
F1	Pale yellow	Characteristic of nutmeg seeds	Oil	Pale yellow	Characteristic of nutmeg seeds	Oil
F2	Light yellow	Characteristic of nutmeg seeds	Oil	Light yellow	Characteristic of nutmeg seeds	Oil
F3	Light yellow	Characteristic of nutmeg seeds	Oil	Light yellow	Characteristic of nutmeg seeds	Oil

Homogeneity after freeze–thaw cycling

Homogeneity testing was performed to confirm that each preparation formed a uniform system without visible coarse particles, and to verify that temperature stress during cycling did not induce phase separation or reappearance of aggregates. As shown in **Table 3**, all formulations (K−, F1, F2, and F3) remained homogeneous before and after the cycling test, indicating that the preparations retained adequate uniformity under the storage-stress simulation. This finding is important because loss of homogeneity during temperature fluctuation can compromise dose uniformity and user acceptability, particularly for topical oil products [16] .

Table 3. Homogeneity test results of aromatherapy essential oil preparations before and after cycling

Formula	Before cycling	After cycling	Requirement/Acceptance criteria
K−	Homogeneous	Homogeneous	No visible coarse particles
F1	Homogeneous	Homogeneous	No visible coarse particles
F2	Homogeneous	Homogeneous	No visible coarse particles
F3	Homogeneous	Homogeneous	No visible coarse particles

pH stability

The pH values before and after cycling are summarised in **Table 4**. After cycling, pH decreased in K−, F1, and F2, while F3 showed a slight increase. Importantly, all values remained within the acceptable pH range for topical preparations (4.5–6.5), indicating that the formulations maintained suitable acidity for skin application after the stability challenge [17]. Minor pH shifts may be influenced by temperature and light exposure during testing, as fluctuating temperatures can trigger chemical and physical changes in formulation components that alter acidity/alkalinity, and light exposure may also affect aromatherapy oils and contribute to pH variation [8] .

Table 4. Observation Data Results of the Essential Oil Preparation Test from Nutmeg Seeds (Myristica fragrans). Aromatherapy Essential Oil

Formula	pH Before Cycling	pH After Cycling	Requirements
K−	5.22	4.64	4.5–6.5
F1	4.84	4.55	4.5–6.5
F2	4.72	4.67	4.5–6.5
F3	4.51	4.71	4.5–6.5

Spreadability

Spreadability results are presented in **Table 5**. After cycling, spreadability decreased in K− and F1, slightly increased in F2, and remained unchanged in F3. Nevertheless, all formulations stayed within the specified spreadability requirement of 5–7 cm, indicating that application performance remained acceptable following cycling 5 [17]. Variations observed before and after cycling may be associated with temperature and light factors, where high temperature can accelerate reactions within the formula and light exposure (especially UV) can affect the stability of certain constituents, thereby influencing spreading behaviour [8].

Table 5. Data from the Observation Test of the Spreadability of Nutmeg Seed Essential Oil (Myristica fragrans) Formulation. Aromatherapy Essential Oil

Formula	Before Cycling	After Cycling	Requirements
K−	7.0 cm	6.0 cm	5–7 cm
F1	6.0 cm	5.8 cm	5–7 cm
F2	5.5 cm	5.6 cm	5–7 cm
F3	5.5 cm	5.5 cm	5–7 cm

Viscosity

Viscosity outcomes are shown in **Table 6**. After cycling, viscosity increased in F1–F3, while K− showed a decrease. These changes can be explained by the sensitivity of oil systems to temperature and light: increasing temperature generally reduces viscosity, whereas decreasing temperature increases viscosity; additionally, light exposure may promote oxidation/peroxidation reactions that can modify oil molecular structure and consequently alter viscosity [8]. Despite these changes, the values remained within the stated requirement as presented in Table 6 [17].

Table 6. Data from the Observation of Viscosity Tests on Aromatherapy Essential Oil Formulations

Formula	Before Cycling	After Cycling	Requirements
K−	48.00	45.00	46.29
F1	44.00	46.00	46.29
F2	42.50	48.00	46.29
F3	48.00	49.00	46.29

Preference test

A preference (hedonic) test involving 30 volunteers was performed and the distribution of responses is presented in **Table 7**. Overall, higher acceptance was observed for nutmeg essential oil formulations, particularly F3, which received the highest "really like" responses among F1–F3. The positive control (K+) showed the highest overall preference, while K− exhibited the lowest acceptance profile. These results indicate that formulation aroma characteristics substantially influence user acceptance [8].

Table 7. Data from the Observation Test of Preference for Nutmeg Seed Essential Oil (Myristica fragrans). Aromatherapy Oil

Formula	N	Dislike	Somewhat dislike	Neutral	Like	Really like
K−	30	7	20	3	0	0
F1	30	6	3	8	13	0
F2	30	0	2	6	10	12
F3	30	0	0	1	4	25
K+	30	0	3	0	0	27

Irritation test

In the irritation test on 30 volunteers for 30 minutes (**Table 8**), no irritation reactions such as itching, redness, skin swelling, or pain were observed in any group. Thus, under the study conditions, the preparations were considered safe for topical use as aromatherapy oil on human skin [18].

Table 8. Data from the Observation Test of Nutmeg Seed Essential Oil (Myristica fragrans). Aromatherapy Oil

Formula	Itching	Redness	Skin Swelling	Pain
K−	None	None	None	None
F1	None	None	None	None
F2	None	None	None	None
F3	None	None	None	None
K+	None	None	None	None

Antistress activity (immobility time)

Immobility time data are shown in **Table 9**. The negative control (K−) demonstrated the highest immobility time, whereas the positive control (K+) produced the lowest immobility time. Formulations containing nutmeg essential oil (F1–F3) reduced immobility time compared with K−, suggesting an antistress-like effect that increased with concentration under the experimental conditions. One-way ANOVA indicated a significant difference in immobility time among groups ($p < 0.05$); therefore, Tukey's post hoc test was applied to identify pairwise differences.

Table 9. Results of Immobility Time Data in Mice After Exposure to Aromatherapy Oil

Formula	N	Average Immobility Time (Seconds)	Standard Deviation
K -	3	174.6667	5.131601
F1	3	149	4.582576
F2	3	133.6667	6.806859
F3	3	101.3333	4.163332
K +	3	87.3333	2.081666

The ANOVA procedure was used to evaluate whether the mean immobility times differed across groups. The correct interpretation is that when the Sig. value is less than 0.05, the group means are significantly different, whereas when Sig. is greater than 0.05, the difference is not statistically significant [19]. Based on the ANOVA results indicating a difference, the analysis was continued using Tukey's post hoc test to determine pairwise differences. The results showed that K− differed significantly compared with F1, F2, F3, and K+ (Sig = 0.000). F1 differed significantly compared with F2 (Sig = 0.003). F2 differed significantly compared with F3 and K+ (Sig = 0.000). F3 differed significantly compared with K+ (Sig = 0.005). All p-values met the significance criterion (Sig < 0.05), supporting that the aromatherapy oil formulations significantly affected immobility time relative to controls.

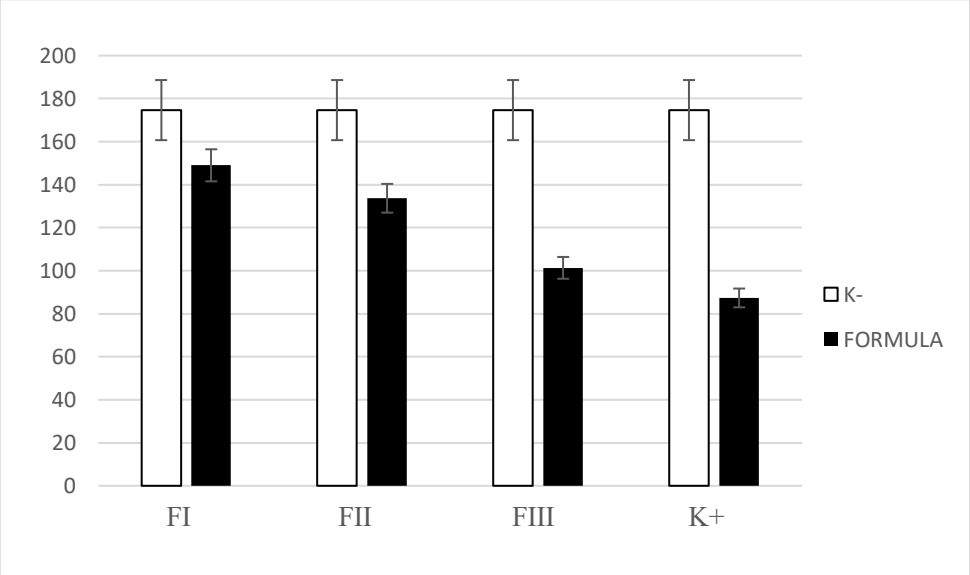

Figure 1. Average immobility time results for mice

This study has several limitations that should be considered when interpreting the results. First, using a stress mouse model limits the generalisability to humans. Second, the study primarily evaluated behavioural outcomes and general physicochemical parameters and did not examine molecular or biochemical mechanisms underlying the observed antistress-like effects. Third, although no irritation was observed in the short observation period, more comprehensive safety assessment is still needed, including delayed irritation/sensitisation potential and evaluation of possible interactions with additional excipients typically used in medicated oil formulations

4. Conclusion

The immobility time decreased steadily from the negative control group K− at 174.6667 seconds to the nutmeg essential oil formulations, namely F1 at 149.0 seconds, F2 at 133.6667 seconds, and F3 at 101.3333 seconds. The lowest immobility time was observed in the positive control group K+ at 87.3333 seconds. This trend indicates a concentration related reduction in immobility time following exposure to nutmeg aromatherapy oil and supports an antistress like effect under the present experimental conditions, with statistically significant differences between groups at $p < 0.05$. Further studies are needed to strengthen the safety profile through extended dermal tolerability assessment and toxicity testing, including subchronic and chronic evaluations, as well as investigation of potential systemic effects, considering that nutmeg contains bioactive constituents such as myristicin that may exert pronounced pharmacological activity at certain exposure levels.

Acknowledgements:

The authors thank the Department of Pharmacy, Faculty of Pharmacy, Megarezky University, Makassar, for facility support, as well as the laboratory and animal facility staff for technical assistance. We also thank all volunteers for their participation in the preference and irritation tests.

Conflicts of Interest:

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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