Farmasains: Jurnal Ilmiah Ilmu Kefarmasian
Volume 12, Number 1, 2025, 22-33
ISSN:2621-9816 (Online); 2086-6968 (Print)
Journal homepage: https://journal.uhamka.ac.id/i\ndex.php/farmasains/

Research Article

Serum Formulation of *Thymus vulgaris* and *Syzygium aromaticum* Essential Oils and Antioxidant Activity Test

Formulasi Serum Thymus vulgaris dan Syzygium aromaticum serta Uji Aktivitas Antioksidan

Marcella Liem[1], Eva Monica[2*], Michael Resta Surya Yanuar[1]

[1]Program Studi Farmasi, Fakultas Ilmu Kesehatan, Universitas Ma Chung, VPT N-01, Malang, 65151, Jawa Timur, Indonesia
[2]Program Studi Pendidikan Profesi Apoteker, Fakultas Ilmu Kesehatan, Universitas Ma Chung, VPT N-01, Malang, 65151, Jawa Timur, Indonesia

Received:
August 23, 2024

Reviewed:
January 9, 2025

Accepted:
March 12, 2025

Published:
April 30, 2025

*Corresponding author:
eva.monica@machung.ac.id

ABSTRACT

Essential oils from thyme (*Thymus vulgaris*) and clove (*Syzygium aromaticum*) are natural antioxidants with significant potential to neutralize free radicals. This study aims to evaluate the antioxidant properties of a serum containing a blend of these essential oils using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. It also aims to determine which serum formulation meets the required physical quality standards. The assessment includes organoleptic analysis, pH measurement, viscosity, homogeneity, spreadability, adhesiveness, globule size distribution, stability, emulsion type, hedonic evaluation, and antioxidant activity. The results indicate that the serum is in liquid form, with a pH between 5.149±0.227 and 5.447±0.109, viscosity ranging from 817.1±3.8 to 2083±0.00 cPs, adhesiveness lasting 5.45±0.26 to 11.210±0.09 minutes, and spreadability between 2.32±0.016 and 2.71±0.053 cm. Hedonic testing revealed that F1 was the most preferred formulation. Antioxidant activity analysis showed moderate to very weak effects, with IC_{50} values of 145.628 ppm (F1), 413.658 ppm (F2), and 536.529 ppm (F3). F1, which contains 4.5% clove essential oil and 1.5% thyme essential oil, demonstrated the best balance of physical properties and antioxidant activity among the tested formulations.

Keywords: Antioxidant, Clove Essential Oil, Thyme Essential Oil, Serum

ABSTRAK

Minyak atsiri timi (Thymus vulgaris) dan minyak atsiri cengkeh (Syzygium aromaticum) merupakan contoh antioksidan alami yang berpotensi dalam menangkal radikal bebas. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan serum yang mengandung kombinasi minyak atsiri timi dan cengkeh menggunakan metode 2,2-difenil-1-pikrilhidrazil (DPPH), serta menentukan formula serum yang memenuhi parameter mutu fisik. Evaluasi yang dilakukan meliputi uji organoleptik, pH, viskositas, homogenitas, daya sebar, daya lekat, distribusi ukuran globul, stabilitas, tipe emulsi, hedonik, dan aktivitas antioksidan. Hasil penelitian menunjukkan serum berbentuk cairan dengan nilai pH 5,149±0,227 hingga 5,447±0,109; viskositas 817,1±3,8 hingga 2083±0,00 cPs; daya lekat 5,45±0,26 hingga 11,210±0,09 menit, daya sebar 2,32±0,016

sampai 2,71±0,053 cm. Uji hedonik menunjukkan bahwa F1 merupakan formula yang paling disukai. Analisis aktivitas antioksidan menunjukkan efek yang sedang hingga sangat lemah, dengan nilai IC_{50} sebesar 145,628 ppm (F1), 413,658 ppm (F2), dan 536,529 ppm (F3). Dari ketiga formulasi yang diuji, F1, yang mengandung 4,5% minyak atsiri cengkeh dan 1,5% minyak atsiri timi menunjukkan keseimbangan terbaik antara sifat fisik dan aktivitas antioksidan.

Kata Kunci: *Antioksidan, Minyak Atsiri Cengkeh, Minyak Atsiri Timi, Serum*

INTRODUCTION

As a tropical country, Indonesia experiences constant exposure to sunlight, increasing the risk of skin damage and premature ageing. Skin ageing is characterised by the appearance of wrinkles, pigmentation disorders (hypopigmentation/hyperpigmentation), and changes in skin texture, which becomes rough, dry, and dull, significantly reducing aesthetic appeal (1). Efforts to inhibit oxidative processes caused by free radicals have been pursued through antioxidant compounds (2). Although synthetic compounds such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butyl hydroquinone (TBHQ) are widely applied, their use is restricted by regulatory authorities due to potential carcinogenic effects when used excessively (3).

Previous studies have demonstrated that natural ingredients possess significant antioxidant potential, particularly thyme essential oil (*Thymus vulgaris*) and clove essential oil (*Syzygium aromaticum*). Thyme essential oil is dominated by thymol, a free radical scavenger with antimicrobial activity. In contrast, clove essential oil contains eugenol, which inhibits oxidation and displays anti-inflammatory properties (4–8). A study by Yeddes (2022) reported IC_{50} values of 29.20±0.12 µg/mL for thyme essential oil and 15.020±0.02 µg/mL for clove essential oil, indicating strong antioxidant potency (5). Furthermore, combining these oils at 2-4% concentrations has been proven to produce emulsions with good stability (9).

To date, no cosmetic preparation, particularly a serum, has combined these two active ingredients. Serums contain small molecules, enabling faster skin penetration compared to creams, especially when formulated with optimal stability and appropriate packaging to prevent active ingredient degradation (9). Therefore, this study employs essential oil concentrations ranging from 1.5% to 4.5%, with a total mixture of 6% and varying ratios of thyme to clove essential oil (1:3, 1:1, dan 3:1).

Based on this rationale, this study aims to evaluate the antioxidant activity of a serum combining thyme and clove essential oils by measuring its antioxidant parameters and determining the optimal formulation that meets physical quality test requirements. Thus, this research is expected to yield a safe, effective, and stable natural-based cosmetic preparation to address skin ageing concerns.

Materials and Methods

Materials

The materials used were thyme essential oil (*Thymus vulgaris*), obtained from Essential Formula with plant origin in India, and clove essential oil (*Syzygium aromaticum*), purchased from SESMU with plant origin in Indonesia. Additional components are carbomer 940 (Brataco), Span 20 (Brataco), propylene glycol (Brataco), methylparaben (Brataco), propylparaben (Brataco), distilled water, absolute ethanol (Merck), gallic acid (Merck), triethanolamine, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Merck), and Sudan III (Pudak Scientific).

Methods

1. Antioxidant Activity Assay of Thyme and Clove Essential Oils Using the DPPH Method (4)

A 40 ppm DPPH solution was prepared by weighing 4 mg of DPPH and dissolving it in absolute ethanol (*p.a.*) in a 100 mL volumetric flask. Sample preparation involved creating a concentration series of 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, and 600 ppm for thyme essential oil and 20 ppm,

30 ppm, 40 ppm, 50 ppm, 60 ppm, and 70 ppm for clove essential oil. A 10 ppm gallic acid reference solution was prepared by weighing 1 mg of gallic acid and dissolving it in absolute ethanol (*p.a.*) in a 5 mL volumetric flask to obtain a 200 ppm stock solution, followed by dilution to 10 ppm. The assay was performed by mixing 3.8 mL of DPPH solution with 0.2 mL of sample, incubating the mixture for 25 minutes, and measuring the absorbance using a UV-Vis spectrophotometer (Jasco V-760, Japan) at a 516 nm wavelength. The gallic acid reference was tested under identical conditions. The resulting absorbance values were used to calculate IC_{50} values through linear regression analysis, plotting % inhibition against concentration using the following equation (10):

$$\% \text{ Inhibition} = \frac{\text{Absorbance of the Sample - Absorbance of the Negative Control}}{\text{Absorbance of the Positive Control - Absorbance of the Negative Control}} \quad \text{.....(1)}$$

2. Preparation of Serum Formulation Containing Combined Thyme and Clove Essential Oils

All equipment and materials were sterilized and prepared in advance. Serum formulations were prepared in triplicate for each formulation (**Table 1**). In mixture 1, the carbomer was placed in a beaker glass, followed by adding distilled water and triethanolamine, then allowed to swell for 30 minutes. In mixture 2, methylparaben, propylparaben, propylene glycol, and Span 20 were homogenised thoroughly. Both mixtures were homogenized, and then the combined thyme essential oil, clove essential oil, and Span 20 were added. Finally, distilled water was added to achieve a final volume of 500 mL.

Table 1. Serum Formulation Composition

Ingredient	F0 (%w/w)	F1 (%w/w)	F2 (%w/w)	F3 (%w/w)
Thyme Essential Oil	0	1.5	3	4.5
Clove Essential Oil	0	4.5	3	1.5
Carbomer	0.125	0.125	0.125	0.125
Triethanolamine	0.125	0.125	0.125	0.125
Propylene Glycol	15	15	15	15
Span 20	0.5	0.5	0.5	0.5
Methylparaben	0.3	0.3	0.3	0.3
Propylparaben	0.6	0.6	0.6	0.6
Distilled water	up to 100	up to 100	up to 100	up to 100

3. Formulation Evaluation

a. Organoleptic Test

The test was carried out by observing the serum formulation's colour, odour, and physical form. Texture evaluation was conducted to assess user comfort and tactile sensation. Colour assessment was used to evaluate the visual appearance, while odour evaluation aimed to identify the characteristic scent of the prepared formulation. All test results were documented and repeated three times (11).

b. pH Test

The test was performed using a LAQUA PH2000 pH meter, which was calibrated using buffer solutions at pH 4 and pH 7 (11).

c. Viscosity and Flow Test

The test was conducted using a Brookfield viscometer (Metek DV2T). The formulation was placed in a beaker glass, and the spindle was immersed until fully submerged. Measurements were taken at a rotational 60 rpm speed (11).

d. Homogeneity Test

The test was performed using a glass slide. A sufficient amount of formulation was taken and evenly placed on the glass slide. Physical uniformity was evaluated visually (11).

e. Spreadability Test

The test was conducted using a spreadability tester. An amount of 0.5 grams of the formulation was weighed and placed on a microscope slide, followed by loading with 1, 3, 5, and 7-gram weights. After one minute, the diameter of the spread formulation was measured (11).

f. Adhesiveness Test

The test was performed using glass slides and weights. A 0.5-gram formulation was weighed, placed on glass slide 1, and covered with glass slide 2. The slides were pressed together with a 1 kg weight, and the duration of adhesion was measured using a stopwatch (11).

g. Emulsion Type Test

A small amount of formulation was mixed with Sudan III dye, placed on a glass slide, and observed under a microscope to determine the type of emulsion formed (11).

h. Globule Size Distribution Test

The test was performed by placing the formulation on a glass slide, covering it, and observing using an OptiLab microscope connected to the application. The results were measured using the Image Raster application (11).

i. Stability Test

The test was conducted using the cycling test method. Formulations were alternately stored at 4°C for 24 hours and 40°C, each for 24 hours, repeated over six complete cycles. Evaluated parameters included an organoleptic, pH, homogeneity, spreadability, and adhesiveness test (12).

j. Hedonic Test

A hedonic evaluation was conducted with 40 randomly selected panelists. Each participant completed a Google Form questionnaire evaluating the serum formulation's colour, odour, and texture (11).

Data Analysis

Statistical analysis was performed using SPSS software version 29. Statistically evaluated parameters included pH, spreadability, adhesiveness, viscosity, and IC_{50} values, which were analysed using one-way ANOVA or Kruskal-Wallis test, depending on the data distribution. For stability testing, the dependent t-test and Wilcoxon signed-rank test were employed. These tests were performed to identify differences between treatments. A 95% confidence level was used to determine statistical significance.

RESULTS

Antioxidant activity testing of thyme and clove essential oils was conducted using the DPPH method to verify their antioxidant properties. The results showed that thyme essential oil had an IC_{50} value of 336.02±8.94 ppm (classified as a very weak antioxidant). In contrast, clove essential oil demonstrated significantly higher antioxidant activity, with an IC_{50} of 54.00±9.00 ppm (classified as a strong antioxidant) (13). Four serum formulations were prepared, each differing in the concentration ratios of thyme to clove essential oils as active ingredients. Thymol (38.16%) was identified as the major component of thyme oil, whereas eugenol (83.52%) dominated the composition of clove oil. Additional formulation components included carbomer (gelling agent), propylene glycol (humectant), Span 20 (emulsifier), methylparaben and propylparaben (preservatives), and distilled water (solvent) (14).

Organoleptic and homogeneity evaluations (**Table 2**) confirmed that all formulations were physically homogeneous, with no insoluble particles observed. The organoleptic assessment revealed slightly turbid appearances (yellowish/white), liquid textures, and distinctive aromas attributable to the essential oils.

Table 2. Results of Organoleptic Evaluation and Homogeneity Test of Serum

Formula	Colour	Texture	Aroma	Homogeneity	Image
0	Turbid	Slightly viscous liquid	Odourless	Homogeneous	
1	Turbid yellowish	Liquid	Characteristic clove aroma	Homogeneous	
2	Turbid yellowish	Liquid	Characteristic clove and thyme aroma	Homogeneous	
3	Turbid whitish	Liquid	Characteristic thyme aroma	Homogeneous	

The pH test was conducted to determine the acidity of the formulations, as skin-compatible pH values are essential to prevent irritation or dryness. A suitable serum pH ranges from 4.5 to 6.5 (15). All four formulations met the required skin pH range, as shown in **Table 3**. Statistical analysis using one-way ANOVA indicated no significant differences in pH values among the serum formulations ($p>0.05$).

Table 3. Results of pH, Viscosity, Adhesiveness, and Spreadability Tests of Serum

Formula	pH	Viscosity (cPs)	Adhesiveness (seconds)	Spreadability (cm)
0	5.4±0.1	2083.0±0.0*	11.2±0.1*	2.7±0.1
1	5.3±0.1	1149.0±3.5	7.3±0.2*	2.6±0.1
2	5.2±0.2	917.8±3.9	6.5±0.1*	2.6±0.1
3	5.2±0.2	871.1±3.8*	5.5±0.3*	2.3±0.0

Viscosity testing assessed each formulation's resistance to flow, a critical parameter for serum application. An ideal serum typically has a viscosity within 230-1150 cPs (16). Formulations F1, F2, and F3 met this requirement, whereas F0 did not, exhibiting a notably higher viscosity of 2083 cPs. Kruskal-Wallis test indicated significant differences in viscosity between formulations ($p<0.05$), prompting further post hoc testing. The additional test revealed a significant difference only between F0 and F3. Although no significant differences were observed between F0, F1, and F2, **Table 3** illustrates a downward trend in viscosity across the formulations. Flow properties were subsequently examined using various spindle speeds (30 rpm, 50 rpm, 60 rpm, 100 rpm, then decreasing to 60 rpm, 50 rpm, and 30 rpm). As shown in **Figure 1**, all four formulations exhibited plastic non-Newtonian flow behaviour. Yield value measurements indicated that F0 had the highest value (14.32 dyne/cm^2), followed by F1 (6.87 dyne/cm^2), F2 (5.26 dyne/cm^2), and F3 (4.96 dyne/cm^2). These results suggest varying shear stress requirements to initiate flow, closely related to the composition of each formulation. Higher yield values imply greater initial resistance to flow, potentially affecting the serum's rheological characteristics and skin application comfort.

The spreadability test was conducted to determine how easily each formulation could be applied across the skin surface. The acceptable spreadability range for semi-solid preparations is 5-7 cm (1). As shown in **Table 3**, none of the four formulations met this criterion. One-way ANOVA analysis indicated no significant differences in spreadability across the four formulations ($p>0.05$).

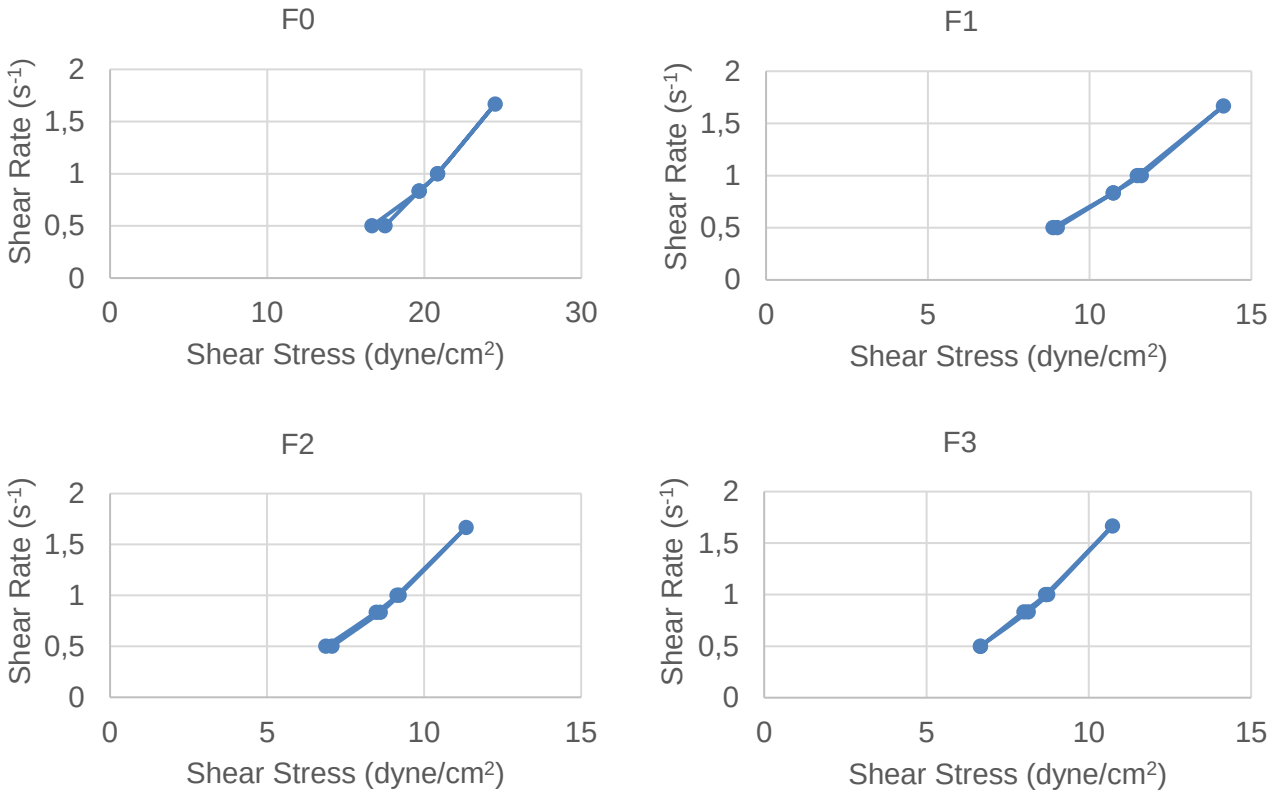
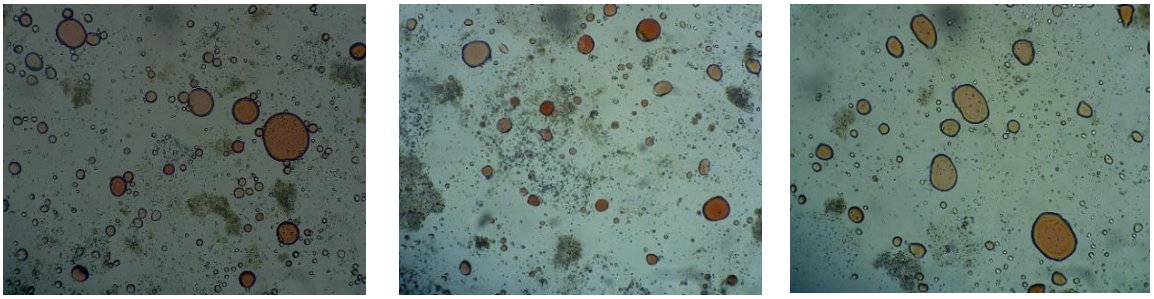


Figure 1. Flow properties of serums F0, F1, F2, and F3 indicate non-Newtonian plastic type flow behaviour

The adhesion test evaluated each serum's ability to adhere to the skin. A good adhesion time is higher than 4 seconds (17). All formulations successfully met this requirement. One-way ANOVA revealed significant differences in adhesion among the formulations, followed by Least Significant Difference (LSD) post-hoc analysis, which showed significant differences in adhesion across all formulations ($p<0.05$). The emulsion type test determined whether each serum was an oil-in-water or water-in-oil emulsion. As shown in **Figure 2**, all formulations were identified as oil-in-water (O/W) emulsions, thus meeting the required specifications (18).



(a) **(b)** **(c)**

Figure 2. Emulsion type test results of the serum (O/W). (a) F1; (b) F2; (c) F3

Globule size distribution was tested using a globule size distribution curve to determine the average globule size rangein each formulation. The average globule sizes for formulations F1 to F3 were 37.56 µm, 38.10 µm, and 32.13 µm, respectively. Statistical analysis showed that these results did not differ significantly among the formulations. These results are considered acceptable, as they fall within the typical emulsion globule size range of 1.5-100 µm (19). Furthermore, the globule property can be determined through the antilog of the SD calculation values. An antilog SD value below 1.2 indicates a monodisperse system, while values above 1.2 suggest polydispersity. According to **Table**

4, all formulations exhibited antilog SD values above 1.2, indicating that all three formulas were categorised as polydisperse. These findings demonstrate that the globule sizes were appropriate for emulsions. However, the globule properties did not yet meet the desired monodisperse characteristic.

Table 4. Results of the Globule Size Distribution Test of the Serum

Formula	Globule Size (μm)	SD (μm)	Antilog
1	37.56	8.94	20.90
2	38.10	0.26	20.60
3	32.13	7.42	17.14

Stability testing was conducted using the cycling test method, which involved repeated evaluations of organoleptic properties, homogeneity, pH, spreadability, and adhesiveness evaluations over multiple cycles (20). Based on organoleptic assessment results, no changes in colour and aroma were recorded. The colours remained consistent—white, cloudy white, or cloudy yellowish-white—and the aromas were characteristic of clove, thyme, or a combination thereof. However, a slight change in consistency was observed, with the formulations appearing somewhat more fluid after testing. Homogeneity testing revealed no phase separation throughout all six cycles, indicating good formulation stability.

Table 5. Results of Serum pH Stability Test

Formula		Before Freeze-Thaw Cycle	After Freeze-Thaw Cycle
0	pH	5.45	5.30
	SD	0.11	0.12
1	pH	5.29	5.25
	SD	0.05	0.20
2	pH	5.23	5.11
	SD	0.17	0.03
3	pH	5.15	5.10
	SD	0.23	0.02

Regarding pH stability, values across cycles are presented in **Table 5**. Statistical analysis was conducted using SPSS software, beginning with a normality test using Shapiro-Wilk, revealing that all formulas were normally distributed with significance values $p>0.05$. A Levene's test was used to assess homogeneity of variance, showing that the data were homogeneous with significance values $p>0.05$. The data met both assumptions, so a dependent t-test was used for further analysis. The results of the dependent t-test showed $p>0.05$ for all formulas, indicating no significant differences in pH values across stability cycles.

Table 6. Results of Serum Spreadability Stability Test

Formula	Cycles	Spreadability (cm)	SD
0	0	2.71*	0.06
	6	2.79*	
1	0	2.50	0.22
	6	2.81	
2	0	2.56*	0.13
	6	2.74*	
3	0	2.32*	0.38
	6	2.85*	

From the stability spreadability values presented in **Table 6**, statistical analysis was also performed using SPSS software. The analysis started with the Shapiro-Wilk normality test, which found that F1, F2, and F3 were normally distributed with significance values $p>0.05$. Levene's test was then conducted, revealing that the data were homogeneous with significance values $p>0.05$. Since the assumptions of normality and homogeneity were met, a dependent t-test was performed. The results

showed a p<0.05 (0.035) for F1, indicating a significant difference in spreadability across cycles. In contrast, F2 and F3 had p>0.05 (0.184 and 0.122, respectively), suggesting no significant differences in spreadability between cycles. As the F0 data did not meet the homogeneity assumption, the Wilcoxon signed-rank test was used instead. The Wilcoxon test results showed that F0 exhibited no significant difference in spreadability between cycles.

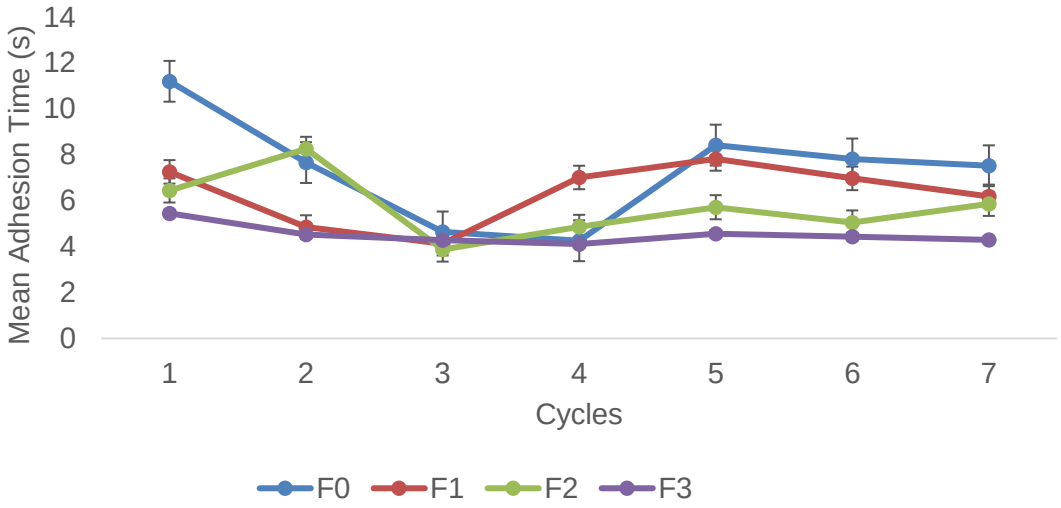


Figure 3. Stability graph of serum adhesiveness test using the cycling test

From the adhesiveness values in **Figure 3**, statistical analysis was also conducted using SPSS software. The Shapiro-Wilk test indicated that F0, F2, and F3 were normally distributed with significance values p>0.05. Levene's test further confirmed homogeneity of variance significance values p>0.05. As the data were normally distributed and homogeneous, a dependent t-test was performed. The results showed that F0, F2, and F3 had p<0.05 (0.00, 0.013, and 0.024, respectively), indicating significant differences in adhesiveness across cycles. For F1, which did not meet the homogeneity assumption, the Wilcoxon signed-rank test was used. This analysis showed no significant difference in adhesiveness across cycles.

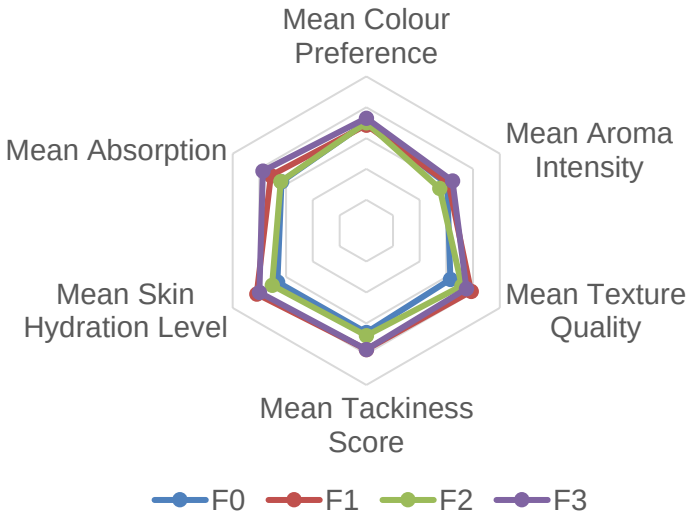


Figure 4. Hedonic test chart of the serum

Hedonic testing was conducted to determine respondents' preferences toward the various formulations. As illustrated in the radar graph in **Figure 4**, F3 was the most preferred formulation based

on overall sensory attributes. However, according to Google Form results regarding the most preferred formula, F1 received the highest preference percentage, with 47.5% of respondents selecting it.

Table 7. Results of Antioxidant Activity Test of Serum

Formula	IC_{50} (ppm)	SD (ppm)	Category
1	145.63	17.45	Moderate
2	413.66	9.07	Very Weak
3	536.53	13.94	Very Weak

The antioxidant activity of the formulations was evaluated using the DPPH method, with results summarized in **Table 7**. Antioxidant activity was evaluated based on the IC_{50} value, where lower values indicate stronger activity. The calculated IC_{50} values were 145.63 ppm for F1, 413.69 ppm for F2, and 520.61 ppm for F3, indicating that F1 exhibited the highest antioxidant activity among the tested formulations.

DISCUSSION

Essential oils are complex mixtures of bioactive compounds that play a crucial role in cosmetic products' antioxidant activity and sensory properties. In this study, the antioxidant activity of thyme (*Thymus vulgaris*) and clove (*Syzygium aromaticum*) essential oils was assessed using the DPPH method. This method evaluated the ability of antioxidant compounds to donate electrons or hydrogen atoms to reduce DPPH free radicals, as indicated by a colour change and absorbance measured by UV-Vis spectrophotometry. Both essential oils contain bioactive compounds such as thymol, eugenol, and carvacrol, which exhibit high antioxidant activity. These compounds function to neutralise free radicals through hydrogen or electron transfer mechanisms (5). The antioxidant mechanism of thymol and eugenol against DPPH involves the donation of hydrogen atoms to DPPH, converting it into its reduced form (DPPH-H). Subsequently, eugenol or thymol resonates into an intermediate form, wherein two radicals interact and stabilise each other through bond formation (21).

This study showed that the thyme essential oil used exhibited relatively low antioxidant activity, whereas the clove essential oil demonstrated high antioxidant activity. This comparison differs from previous findings that reported high antioxidant activity for both oils. Differences in the concentration of active compounds may explain this discrepancy. Previous studies recorded thymol and eugenol contents of 78.54% and 87.3% (5), while the certificate of analysis for the oils used in this study reported thymol at 30% and eugenol at 83.52%. These variations in active compound concentrations likely influenced the activity of the antioxidant outcomes observed in this research.

Although thyme essential oil showed relatively low antioxidant activity, its inclusion in anti-ageing serum formulations remains relevant due to its well-documented antimicrobial and anti-inflammatory properties. Furthermore, thyme oil has the potential to enhance product stability by preventing oxidation and degradation of active ingredients, particularly through synergistic effects when formulated with other antioxidants. These findings align with the study by Abdelhamed *et al*., which reported significant antibacterial activity against *Cutibacterium acnes* and *Staphylococcus epidermidis* (21), as well as research by Choi *et al*. (2019), which indicated that thymol could inhibit tyrosinase expression in mouse melanoma cells stimulated by Alpha-Melanocyte Stimulating Hormone (α-MSH) (22).

Organoleptic and homogeneity tests showed that the serum formulation met the desired specifications. Organoleptically, the serum was characterised by a liquid texture—attributed to low viscosity—and displayed colour and odour consistent with the essential oil profiles used (23). The homogeneity test confirmed the even distribution of all components, ensuring optimal dispersion of active ingredients for maximum therapeutic efficacy (11). pH adjustment was also conducted to ensure values within the 4.5-6.5 range, consistent with skin pH, to minimise irritation risk and maintain the skin barrier balance (15).

Viscosity testing revealed a decrease in viscosity values across formulations, influenced by the pH-dependent nature of carbomer, whose viscosity tends to increase under more alkaline conditions (13). Although most formulations met the specified viscosity range, variations in certain formulations may impact adhesion properties (24). Flow property testing indicated that the serum exhibits plastic non-Newtonian flow behaviour, which is commonly observed in pharmaceutical preparations. Plastic

flow, as one type of non-Newtonian flow, means the material does not flow until the applied shear stress exceeds the yield value. In topical application, the yield value is a key parameter that defines the minimum shear stress required to initiate flow. This parameter indicates the initial force needed to overcome internal structural resistance and enable uniform spreading on the skin. Formulations with relatively higher yield values and plastic behaviour require greater force to disrupt the internal structure and spread evenly, which may benefit storage stability but reduce the ease of application. Conversely, lower yield values facilitate spreading with minimal force, enhancing application uniformity, although too low values present challenges for formulation stability (25).

Based on the research data, the spreadability test of the semi-solid formulation yielded values that did not meet the established requirements. However, the viscosity and adhesion parameters measured remained within the expected specification range, indicating that the suboptimal spreadability did not significantly diminish the overall performance of the formulation. Spreadability, a crucial parameter in active ingredient delivery, is theoretically inversely related to viscosity (24). However, in this study, no significant differences were observed between the formulations, indicating that the composition of excipients and the interactions among components also play a role in influencing this phenomenon. Formulations with higher viscosity tended to exhibit better adhesiveness, thereby enhancing the retention of active ingredients on the skin surface and potentially prolonging therapeutic effects (26).

Testing using Sudan III dye indicated that the formulation belongs to an oil-in-water (O/W) emulsion system, which is preferable for easy spreading and active ingredient absorption by the skin. However, globule size distribution analysis revealed a polydisperse nature (antilog SD>1.2), indicating particle size heterogeneity. This condition is less than ideal, as it may increase particle aggregation (27), compromising emulsion stability. Therefore, improving the formulation by achieving a more uniform (monodisperse) globule distribution is necessary to enhance the stability of the system and the effectiveness of active ingredient delivery.

Stability testing was conducted using a cycling test method over six cycles, evaluating organoleptic properties, homogeneity, spreadability, adhesiveness, and pH at each cycle. The test results showed that colour and odour remained stable, although changes in texture were observed. Limitations in viscosity testing, due to the minimum sample volume of 250 mL required for the Brookfield viscometer, constrained further analysis. Temperature variations during the test cycles also affected the results of adhesiveness, pH, and spreadability tests; however, these changes remained within acceptable limits, except for the spreadability test, which showed more significant variability.

Hedonic testing revealed that although F3 received the highest overall sensory rating, 47.5% of respondents preferred F1. F1, which had a higher average moisture level, also received positive responses regarding aroma, where the higher percentage of clove essential oil resulted in a dominant scent. These findings underscore the crucial role of aroma in influencing sensory perception, as a strong fragrance can enhance comfort and user experience in cosmetic product applications (28).

The antioxidant activity test of the serum indicated that the product falls into the category of moderate antioxidant activity. These findings support the hypothesis that increasing the concentration of clove essential oil enhances antioxidant activity. However, thyme essential oil did not exhibit the expected synergistic effect when combined with clove essential oil, possibly due to the low thymol content in the thyme oil used (9). This result is supported by antioxidant activity data showing that the thyme essential oil demonstrated weak activity. Therefore, adjustments to the concentration of active compounds, particularly in the thyme essential oil, are recommended to improve the synergistic effectiveness in formulating an anti-ageing serum.

CONCLUSION

The findings of this study indicate that F1 possesses moderate antioxidant activity, as reflected by its IC_{50} value of 145.628 ppm. F1 also demonstrated superior physical quality attributes, meeting the requisite standards for pH, organoleptic characteristics, adhesiveness, viscosity, emulsion type, and globule size distribution. Furthermore, F1 emerged as the most preferred formulation among respondents in the hedonic assessment. Future investigations are recommended to explore the formulation's long-term stability and evaluate its potential antibacterial properties, particularly those arising from the synergistic effects of thyme and clove essential oils.

REFERENCES

1. Wahid H, Karim SF, Sari N. Formulasi Sediaan Krim Anti-aging dari Ekstrak Kolagen Limbah Sisik Ikan Bandeng (*Chanos chanos*). *J Sains Kes*. 2022;4(4):428-436. https://doi.org/10.25026/jsk.v4i4.1289.
2. Apristasari O, Yuliyani SH, Rahmanto D, Srifiana Y. Famiku (Face Mist-ku) yang Memanfaatkan Ekstrak Kubis Ungu dan Bengkoang sebagai Antioksidan dan Pelembab Wajah. *Farmasains: Jurnal Ilmiah Ilmu Kefarmasian*. 2018;5(2):35–40. https://doi.org/10.22236/farmasains.v5i2.1446.
3. Wulansari AN. Alternatif Cantigi Ungu (*Vaccinium varingiaefolium*) sebagai Antioksidan Alami : Review. *Farmaka*. 2018;16(2):419–429. https://doi.org/10.24198/jf.v16i2.17574.
4. Aljabeili HS, Barakat H, Abdel-Rahman HA. Chemical Composition, Antibacterial and Antioxidant Activities of Thyme Essential Oil (*Thymus vulgaris*). *Food and Nutrition Sciences*. 2018;09(05):433–446. https://doi.org/10.4236/fns.2018.95034.
5. Yeddes W, Mejri I, Grati-Affes T, Khammassi S, Hammami M, Aidi-Wannes W, et al. Synergistic Effect of Cloves (*Syzygium aromaticum*), Thyme (*Thymus vulgaris*) and Lemon (*Citrus limon*) Blended Essential Oils Optimized by Mixture Design for Improving the Antioxidant Activity. *Avicenna Journal of Medical Biochemistry*. 2022;10(1):20–29. https://doi.org/10.34172/ajmb.2022.03.
6. Mehrabi A, Mahmoudi R, Morasa HK, Mosavi S, Kazeminia M, Rezaei FA, et al. Study of Chemical Composition, Antibacterial and Antioxidant Activity of Thyme Leaves and Stems Essential Oil. *Journal of Medicinal Plants and By-Products*. 2022;11(2):253–263. https://doi.org/10.22092/JMPB.2021.353440.1323.
7. Aprotosoaie AC, Miron A, Ciocârlan N, Brebu M, Roşu CM, Trifan A, et al. Essential Oils of Moldavian Thymus Species: Chemical Composition, Antioxidant, Anti-Aspergillus and Antigenotoxic Activities. *Flavour and Fragrance Journal*. 2019;34(3):175–186. https://doi.org/10.1002/ffj.3490.
8. Kurniawan MF, Sugihartini N, Yuwono T. Uji Iritasi serta Evaluasi Sifat Fisik Emulgel Minyak Atsiri Bunga Cengkeh dengan Metode Simplex Lattice Design. *Farmasains: Jurnal Ilmiah Ilmu Kefarmasian*. 2018;5(1):1–8. https://doi.org/10.22236/farmasains.v5i1.1111.
9. Karsli GT, Sahin S, Oztop MH. High-Pressure-Homogenized Clove and Thyme Oil Emulsions: Formulation, Stability, and Antioxidant Capacity. *ACS Food Science and Technology*. 2022;2(12):1832–1839. https://doi.org/10.1021/acsfoodscitech.2c00231.
10. de Menezes BB, Frescura LM, Duarte R, Villetti MA, da Rosa MB. A Critical Examination of the DPPH Method: Mistakes and Inconsistencies in Stoichiometry and IC_{50} determination by UV–Vis Spectroscopy. *Analytica Chimica Acta*. 2021;1157:338398. https://doi.org/10.1016/j.aca.2021.338398.
11. Hikmah FN, Malahayati S, Nugraha DF. Formulasi dan Evaluasi Sediaan Serum Gel Ekstrak Bunga Melati (*Jasminum sambac* L.). *Journal Pharmaceutical Care and Sciences*. 2023;3(2):93–108. https://doi.org/10.33859/jpcs.v3i2.248.
12. Wardani D, Nurul N, Sujana D, Nugraha RY, Nurseha R. Formulasi Krim Ekstrak Etanol Daun Reunceu (*Staurogyne elongata* (Blume) O.Kuntze) dengan Variasi Konsentrasi Parafin Cair dan Setil Alkohol. *PHARMA XPLORE*. 2021;6(2):36-46. https://doi.org/10.36805/farmasi.v6i2.1940.
13. Sheskey PJ, Cook WG, Cable CG. *Handbook of Pharmaceutical Excipients 8th*. Pharmaceutical Press; 2017.
14. Gulcin İ, Alwasel SH. DPPH Radical Scavenging Assay. *Processes*. 2023;11(8). https://doi.org/10.3390/pr11082248.
15. Tarigan IL, Latief M. *Antibakteri: Potensi Tanaman Jambi*. Tasikmalaya: EDU Publisher; 2021.
16. Liandhajani, Fitria N, Ratu AP. Karakteristik dan Stabilitas Sediaan Serum Ekstrak Buah Kersen (Muntingia calabura L.) dengan Variasi Konsentrasi. *Jurnal Farmamedika (Pharmamedica Journal)*. 2022; 7(1):17-27. https://doi.org/10.47219/ath.v7i1.140.
17. Pogaga E, Yamlean PVY, Lebang JS. Formulasi dan Uji Aktivitas Antioksidan Krim Ekstrak Etanol Daun Murbei (*Morus alba* L.) menggunakan Metode DPPH (*1,1-Diphenyl-2-Picrylhydrazyl*). *Pharmacon*. 2020;9(3):349-356. https://doi.org/10.35799/pha.9.2020.30018.

18. Sopianti DS, Ricki A, Haque AF. Variasi Ekstrak Etanol Biji Kebiul (*Caesalpinia bonduc* (L). Roxb) pada Formulasi Sediaan Emulsi M/A. *Jurnal Ilmiah Ibnu Sina (JIIS): Ilmu Farmasi dan Kesehatan*. 2021;6(1):11–20. https://doi.org/10.36387/jiis.v6i1.568.
19. Hisprastin Y, Nuwarda RJ. Review: Perbedaan Emulsi dan Mikroemulsi pada Minyak Nabati. *Farmaka*. 2018;16(1):133–140. https://doi.org/10.24198/jf.v16i1.17424.
20. Qomara WF, Musfiroh I, Wijayanti R. Review: Evaluasi Stabilitas dan Inkompatibilitas Sediaan Oral Liquid. *Majalah Farmasetika*. 2023;8(3):209–223. https://doi.org/10.24198/mfarmasetika.v8i3.44346.
21. Abdelhamed FM, Abdeltawab NF, ElRakaiby MT, Shamma RN, Moneib NA. Antibacterial and Anti-Inflammatory Activities of *Thymus vulgaris* Essential Oil Nanoemulsion on Acne Vulgaris. *Microorganisms*. 2022;10(9). https://doi.org/10.3390/microorganisms10091874.
22. Choi DG, Park CI, Lee SM, Baek JI. Anti-Melanogenic Effect of Thymol, a Major Odorant in Essential Oils of Family Lamiaceae. *The Korea Journal of Herbology*. 2019;34(4):19–25. http://doi.org/10.6116/kjh.2019.34.4.19.
23. Shejul TS, Kudale K. Facial Serum: Its Formulation, Usage, Special Ingredients, Various Types and Benefits. *International Journal of Pharmaceutical Research and Applications*. 2023;8(2):680-692. https://doi.org/10.35629/7781-0802680692.
24. Irianto IDK, Purwanto P, Mardan MT. Aktivitas Antibakteri dan Uji Sifat Fisik Sediaan Gel Dekokta Sirih Hijau (*Piper betle* L.) sebagai Alternatif Pengobatan Mastitis Sapi. *Majalah Farmaseutik*. 2020;16(2):202-210. https://doi.org/10.22146/farmaseutik.v16i2.53793.
25. Sinko PJ**.** *Martin's Physical Pharmacy and Pharmaceutical Sciences: Physical Chemical and Biopharmaceutical Principles in the Pharmaceutical Sciences*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2011.
26. Sghier K, Mur M, Veiga F, Paiva-Santos AC, Pires PC. Novel Therapeutic Hybrid Systems Using Hydrogels and Nanotechnology: A Focus on Nanoemulgels for the Treatment of Skin Diseases. *Gels*. 2024;10(1). https://doi.org/10.3390/gels10010045.
27. Amyliana NA, Agustini R. Formulasi dan Karakterisasi Nanoenkapsulasi *Yeast* Beras Hitam dengan Metode Sonikasi menggunakan Poloxamer. *Unesa Journal of Chemistry*. 2021;10(2):184–191. https://doi.org/10.26740/ujc.v10n2.p184-191.
28. Sowndhararajan K, Kim S. Influence of Fragrances on Human Psychophysiological Activity: with Special Reference to Human Electroencephalographic Response. *Scientia Pharmaceutica*. 2016;84(4):724–751. https://doi.org/10.3390/scipharm84040724.