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Research Paper

Comparative Antioxidant Effects of Essential Oil Blends and Individual Oils Using the DPPH Radical-Scavenging Assay

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Abstract: Oxidative stress is recognized as a major contributor to conditions such as cancer, aging, and inflammation. Consequently, there is a growing interest in the use of natural antioxidants, particularly essential oils, owing to their safety benefits and potent antioxidant properties. The aim of this study was to evaluate the antioxidant effects of four essential oil blends (Refresh, Zest, Revitalizing, and Relax Oil), as well as those of the single essential component oils using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method. Essential oils diluted in distilled water (DW) were evaluated, and the absorbance was measured using a Flexstation 3 ELISA reader. Vitamin C was used as a positive control. In the DPPH radical-scavenging assay, the scavenging abilities of oil blends B1, B2, B3, and B4 were 92%, 93%, 95%, and 92%, respectively, with the negative control set at 100%. Meanwhile, individual oils, S1, S2, S3, and S4 exhibited scavenging abilities of 94%, 97%, 100%, and 98%, respectively. Overall, essential oil blends showed higher antioxidant activity than most individual essential oils, suggesting a possible synergistic effect among their antioxidant components. Therefore, blending can effectively maximize the antioxidant activity of individual oils, offering a valuable approach for maximizing the efficacy of natural antioxidants, with substantial implications for their potential application in various industries.

Keywords: 1,1 DPPH; Blended Oil; Aromatic Oil; Vitamin C; Antioxidant

Introduction

Essential oils are widely recognized for their potent antioxidant properties, rendering them potentially valuable in food preservation, pharmaceuticals, and cosmetics. Although previous research has primarily focused on the antioxidant activity of individual essential oils, few studies have systematically compared the effectiveness of blended formulations. Considering that blending essential oils could enhance antioxidant efficacy through synergistic effects, this study investigates and compares the antioxidant activity of essential oil blends with that of single oils. The aim was to provide insights into their practical applications as natural antioxidants across various industries.

Terpenoids and phenylpropanoids are the primary bioactive components of essential oils and are critical for antioxidant activity. Phenolic compounds, in particular, are recognized for their strong radical-scavenging properties (Amorati et al., 2013). Antioxidants reduce oxidative stress by neutralizing reactive oxygen species (ROS) and free radicals, preventing cellular damage that contributes to aging, cancer, cardiovascular diseases, and inflammatory disorders (Lobo et al., 2010; Halliwell, 2012). Consequently, natural antioxidant-based stress mitigation strategies have garnered increasing research interest.

With the growing demand for natural and sustainable antioxidants, essential oils have emerged as promising candidates owing to their rich concentrations of bioactive compounds, including

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phenols, terpenoids, aldehydes, and ketones. These compounds act as potent electron donors, enabling essential oils to effectively scavenge free radicals, as demonstrated in assays such as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Singh and Pulikkal, 2022). In addition to their antioxidant properties, essential oils are also recognized for their physiological benefits, including anti-aging effects, skin protection, and immunity enhancement.

Although previous studies (Miguel, 2010; Baj et al., 2023) have shown the antioxidant potential of individual essential oils and certain blended formulations, they have primarily focused on specific compounds rather than evaluating a range of blend compositions. For example, Miguel (2010) reported that a mixture of thyme and rosemary had enhanced antioxidant activity, indicating a possible synergistic effect between key bioactive compounds. However, these studies often lack systematic comparisons across multiple blends, rendering it challenging to determine whether blending consistently improves antioxidant activity or whether the effects are influenced by specific compositional ratios.

Moreover, previous research has often employed single-component approaches, focusing on isolated compounds such as thymol, carnosol, or linalool (Baj et al., 2023). Although these findings provide valuable insights into the antioxidant mechanisms of individual oils, they do not fully represent the complex interactions that occur within blended essential oils. The aim of this study was to address these gaps by systematically evaluating various essential oil blends, comparing their antioxidant activities with those of their single-component counterparts, and analyzing the potential influence of compositional interactions.

To address this gap, the aim of this study was to:

1. Compare the antioxidant activities of essential oil blends with those of their individual components to determine whether blending improves the efficacy
2. Assess potential synergistic interactions by evaluating compositional variations across different blends
3. Provide foundational data to inform the formulation of optimized essential oil-based antioxidants for commercial applications.

In this study, we used the DPPH radical-scavenging method, a widely accepted approach for assessing antioxidant activity, due to its simplicity, reproducibility, and effectiveness in evaluating free radical-scavenging properties (Martemucci et al., 2022). Using this method, we systematically analyzed four essential oil blends and their primary components to determine whether blending improves antioxidant performance.

Furthermore, we compared the antioxidant activity of the essential oil blends to that of vitamin C, a well-known synthetic antioxidant (Saleh et al., 2010). In contrast to previous studies that primarily focused on single essential oils, this research offers a comparative perspective on the impact of specific oil combinations on antioxidant efficacy.

To the best of our knowledge, the present study is the first to evaluate essential oil blends using the DPPH method within a comparative framework. Our findings are thus expected to contribute to the development of natural antioxidants across various disciplines, including natural product chemistry, food science, and cosmetics. Through the identification of the optimal essential oil blends that enhance antioxidant activity, this research provides foundational data for practical applications across various industries. These findings will serve as a valuable reference for future studies, assisting in the optimization of natural antioxidant formulations for commercial use.

Materials and Methods

Essential Oils

Four essential oil blends and their primary components (i.e., the individual essential oils in each blend) were used in this study. All oils were purchased from Herb Island (Pocheon, Republic of Korea). The test results of the authenticity of the oils are listed in Table (1). The essential oil blends were formulated based on existing literature regarding their antioxidant potential, as well as empirical knowledge. However, the proportions of oils in the blends were not optimized through a systematic experimental design. All oils were stored at room temperature (24°C) in the dark until use. The composition of each oil blend is shown in Table (2).

Reagents

DPPH reagent was purchased from Sigma-Aldrich (1707-75-1, St. Louis, MO, USA). Vitamin C (ascorbic acid, 50-81-7, Sigma-Aldrich) was used as a positive control. Essential oils were prepared for the experiments by adding them to distilled water (DW) and vortexing to achieve concentrations of 100 and 1,000 ppm.

Table 1. Authenticity of essential oils used in this study

Category	EINECS No.	CAS NO.	Certified by
Bergamot fruit oil	*616-915-9 / *614-687-5	8007-75-8/ 68648-33-9	KERFOOT
Eucalyptus oil	283-406-2	8000-48-4 / 84625-32-1	KERFOOT
Fennel oil	282-892-3	84455-29-8	KERFOOT
Frankincense oil	289-620-2 / 232-474-1	89957-98-2 / 8050-07-5	KERFOOT
Scented geranium flower oil	290-140-0	90082-51-2 / 8000-46-2	KERFOOT
Grapefruit peel oil	289-904-6	90045-43-5 / 8016-20-4	KERFOOT
Juniper berry oil	283-268-3	8002-68-4 / 73049-62-4 / 84603-69-0	KERFOOT
Lavender oil	90063-37-9	8000-28-0	TREATT
Orange peel oil	N/A	8028-48-6	KERFOOT
Peppermint oil	282-015-4	8006-90-4/ 84082-70-2	KERFOOT
Rosemary oil	283-291-9	84604-14-8 / 8000-25-7	KERFOOT
Sandalwood oil	N/A	8006-87-9	Moksha

* EC No. Indicates a substance without an existing EC number but which has been assigned a list number in the EC format

Table 2. Contents of essential oil blends

Oil blend	Category	Content (%)
B1 (Refresh)	Peppermint oil (Single 1)	67.0
	Lavender oil	10.0
	Pine needle oil	10.0
	Eucalyptus oil	5.0
	Juniper berry oil	4.0
	Rosemary oil	4.0
B2 (Zest)	Rosemary oil (Single 2)	26.0
	Scented geranium flower oil	20.0
	Fennel oil	15.0
	Juniper berry oil	15.0
	Bergamot fruit oil	11.0
	Grapefruit peel oil	9.0
	Pine leaf oil	4.0
B3 (Revitalizing)	Bergamot fruit oil (Single 3)	45.00
	Lavender oil	35.00
	Scented geranium flower oil	10.00
	Mastic thyme flower oil	9.00
	Frankincense oil	0.50
	Sandalwood oil	0.50
B4 (Relax)	Orange peel oil (Single 4)	48.0
	Bergamot fruit oil	20.0
	Scented geranium flower oil	16.0
	Lavender oil	14.0
	Sandalwood oil	2.0

Standard Particulate Matter

Standard atmospheric dissolved particulate matter samples (PM10 LIKE; Certificate of Analysis: ERM-CZ120) provided by the Joint Research Centre Institute for Reference Materials and Measurements (Geel, Belgium) were used as the negative control.

Equipment

A Flexstation 3 ELISA reader from Molecular Devices (San Jose, CA, USA) was used to measure antioxidant activity.

Evaluation of DPPH Radical-Scavenging Activity

A DPPH solution (0.1 mM) was prepared by dissolving DPPH in ethanol. Each essential oil sample and the vitamin C positive control were mixed with the DPPH solution (final mixture: 50 µL of sample and 50 µL of DPPH solution in a total volume of 100 µL). The mixtures were allowed to react for 30 minutes in the dark at room temperature (24°C). Measurement of absorbance and scavenging activity. Both oil samples and vitamin C were diluted in DW to a concentration of 0.5 mg/mL. To evaluate DPPH radical-scavenging activity, 100 µL of the oil sample or vitamin C solution was mixed with 100 µL of the DPPH solution, resulting in a final mixture volume of 200 µL. The mixture was allowed to react for 30 min in the dark at room temperature to ensure complete radical scavenging. After the reaction was terminated, absorbance at 517 nm was measured using the Flexstation 3 ELISA Reader (Singleton and Rossi, 1965). The DPPH radical-scavenging activity (%) was calculated using the following formula (Kedare and Singh, 2011):

$$DPPH\ radical - scavenging\ activity\ (\%) =$$

$$(1 - Abssample/Abscontrol) \times 100$$

Where Abssample is the absorbance of each sample (essential oil or vitamin C) after mixing with the DPPH solution, and Abscontrol represents the absorbance of the negative control containing only the DPPH solution. Each experiment was conducted in duplicate, and the average of the results was used as the Abs value.

Data and Statistical Analysis

Each experiment was performed in triplicate for each condition, and means, as well as standard deviations, were calculated. Statistical analyses were performed using SPSS Statistics 27 (IBM). Initially, multiple comparison tests, such as Bonferroni correction and Tukey's HSD test, were applied to compare the antioxidant activities of single essential oils and blended oils. However, these tests revealed no statistically significant differences among the groups. Consequently, we opted to use the Student's *t*-test to compare the antioxidant activity of each oil (both single and blended) against Distilled Water (DW) as a control. This approach enabled us to assess the individual antioxidant effects of each essential oil more effectively. A p-value of less than 0.05 was considered statistically significant.

Results

In this study, we evaluated the antioxidant activities of both essential oil blends and individual essential oils using the DPPH radical-scavenging method. Note that lower DPPH values indicate greater radical-scavenging ability. Our results showed that both essential oil blends and individual essential oils exhibited antioxidant activity relative to distilled water (DW), which was set as the control at 100%.

The essential oil blends generally showed comparable antioxidant activity to the individual oils (Fig. 1). Specifically, oil blends B1, B2, B3, and B4 exhibited scavenging abilities of 92%, 93%, 95%, and 92%, respectively. Regarding the individual oils, S1, S2, S3, and S4 showed scavenging abilities of 94%, 97%, 100% (equivalent to the antioxidant capacity of DW), and 98%, respectively. Although both blends and individual oils exhibited antioxidant activity, the scavenging rates of certain oil blends (B1 and B4) were slightly lower than those of the individual oils at the same concentration (0.5 mg/mL).

Table (3) presents the t-test results comparing the antioxidant activities of the various oil blends and individual oils against DW. Among the samples, only B1 showed a significant difference ($p = 0.003$, $t = -243.667$), indicating a notable deviation from the control. Other samples, such as B2, B3, B4, and the individual oils (S1, S2, and S4), did not demonstrate significant differences ($p > 0.05$). Although no statistically significant differences were observed between most blends and individual oils, potential interactions between bioactive compounds in the blends could still contribute to variations in antioxidant performance.

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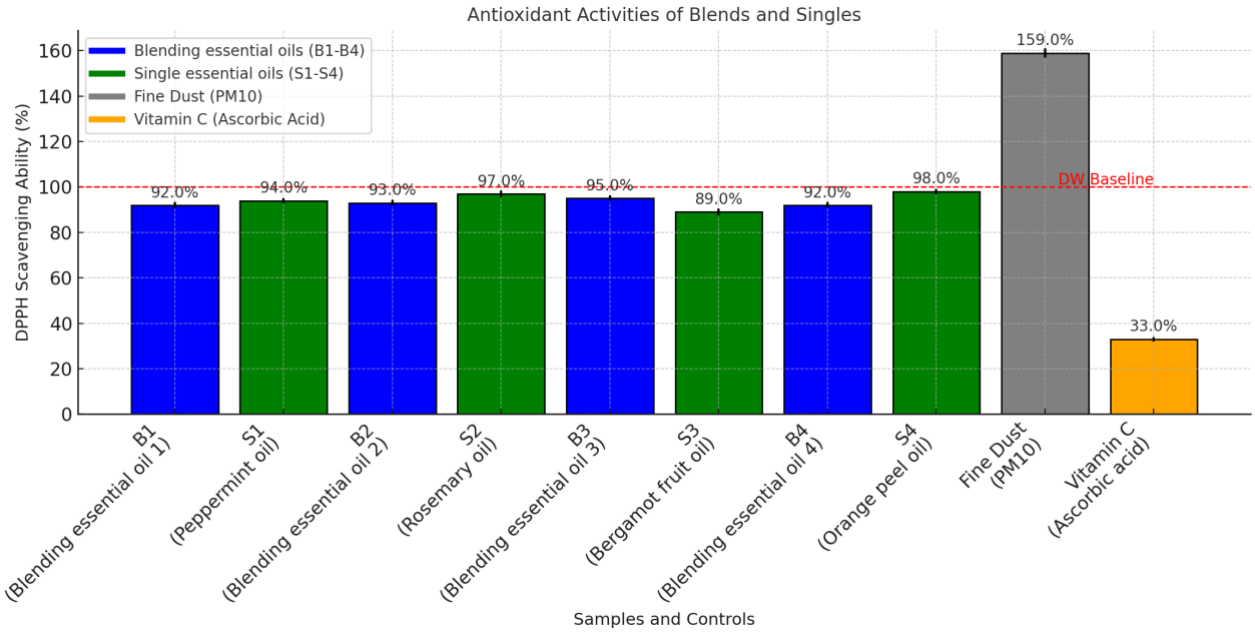

Fig. 1. Data are presented as mean ± SD from duplicate experiments. The red dashed line represents DW's baseline. Most samples showed similar or higher activity than DW, whereas fine dust promoted oxidation (155.1%), and vitamin C showed lower activity (33.3%)

Table 3. T-test results comparing the antioxidant activity of oil blends and individual oils with DW at a concentration of 0.5 mg/mL;Concentration = 0.5 mg/mL DW

Oil blend or component	Mean absorbance	Standard deviation	t-value	p-value	Cohen's d
B1	0.919	0.0084	-6.19489	0.025	6.194
B2	0.946	0.0325	-1.34342	0.311	1.343
B3	0.981	0.0014	0.330409	0.772	0.330
B4	0.962	0.0629	-0.35455	0.756	0.354
S1	0.902	0.0035	-9.61332	0.010	9.613
S2	0.909	0.0190	-4.46791	0.046	4.467
S3	0.933	0.0749	-0.85002	0.484	0.850
S4	0.900	0.0219	-4.52983	0.045	4.529

Table 4. Comparison of antioxidant activity between single oils and blended oils at 0.5 mg/mL

Oil blend + separate component	Mean	Standard deviation	t-value	p-value	Cohen's d
B1-S1	0.01650	0.00495	40.714	0.133	3.333
B2-S2	0.03650	0.05162	10.000	0.500	0.707
B3-S3	0.04800	0.07637	0.889	0.537	0.628
B4-S4	0.06200	0.04101	20.138	0.279	1.511

Sample S3 was excluded because of incomplete data. Multiple comparison tests (Bonferroni and Tukey's HSD) were initially applied to compare the antioxidant activities of single essential oils and blended oils, but no statistically significant differences were found ($p > 0.05$). As a result, a Student's *t*-test was conducted to compare the antioxidant activities of each oil (both single and blended) against distilled water (DW). The t-test results demonstrated that several essential oils exhibited significantly higher antioxidant activity compared to DW, confirming their effectiveness. These findings are summarized in Table (4). The B1-S1 pair showed the smallest mean difference of 0.01650 ($p = 0.133$). Other pairs, such as

B2-S2, B3-S3, and B4-S4, also demonstrated no significant differences, suggesting a minimal impact of blending on antioxidant activity.

As a control, vitamin C exhibited a scavenging ability of 33% at a concentration of 0.5 mg/mL, which was markedly lower than that of both blends and individual oils. In contrast, fine dust promoted oxidation, registering a scavenging rate of 155%.

Discussion

Synergistic Effects of Essential Oil Blends

The differences in antioxidant activity between essential oil blends and individual oils may be attributed to synergistic interactions among their bioactive compounds. Previous studies indicate that combining multiple antioxidants can enhance overall activity that exceeds the combined contributions of their individual components, particularly when phenolic and terpenoid compounds interact to improve free radical-scavenging efficiency (Sacchetti et al., 2005). In the present study, the blended oils exhibited lower residual DPPH radical-scavenging values than the individual oils, suggesting that blending promotes interactions between antioxidant components, potentially leading to synergistic effects (Chen et al., 2023; Liu et al., 2023).

These results are consistent with previous findings showing that blending thyme and rosemary oils results in superior antioxidant properties compared to using either oil separately (Tural and Turhan, 2017; Saricaoglu and Turhan, 2018). The observed enhancement in antioxidant activity suggests that intermolecular interactions among the antioxidant components may improve overall efficacy. However, without quantitative confirmation using interaction indices, this remains a hypothesis rather than a definitive conclusion.

Certain compounds, such as thymol and eugenol, reportedly exhibit enhanced antioxidant capacity when combined with components from other essential oils (Ouedrhiri et al., 2021; Baj et al., 2023; Tit and Bungau, 2023). These findings suggest that optimized oil blends could be more cost-effective and potent at lower concentrations, potentially reducing the side effects associated with high doses of individual oils. Therefore, essential oil blends offer promising natural antioxidant solutions for applications in food preservation, pharmaceuticals, and cosmetics.

Antioxidant Effects of Single Essential Oils

Although single essential oils exhibited antioxidant activity, their efficacy was generally lower than that of blended oils. For example, S3 showed a DPPH residual rate of 0% at 0.5 mg/mL, suggesting limited antioxidant efficiency compared to blended oils, probably because of the absence of synergistic interactions among antioxidant components (Bag and Chattopadhyay, 2015). These findings highlight the importance of combining essential oils to maximize their antioxidant potential.

Industrial Implications of this Study

This study provides experimental evidence supporting the potential for essential oil blends to exhibit higher antioxidant activity than individual oils, reinforcing the importance of synergy in antioxidant research. Furthermore, these findings underscore the applicability of essential oil blends as natural antioxidants, laying the groundwork for future development of antioxidant-based formulations (Liu et al., 2023).

The demonstrated efficacy of the blended oils suggests they could be incorporated into various industrial applications, including:

- Food industry: as natural preservatives to extend shelf life and prevent lipid oxidation
- Cosmetic formulations: for enhanced oxidative protection and skin benefits
- Pharmaceuticals: for potential health benefits linked to antioxidant mechanisms

Given the increasing demand for natural and sustainable products, developing efficient, naturally derived antioxidant formulations is of considerable importance (Chen et al., 2023; Shah et al., 2022).

Limitations and Future Research Prospects

Although this study provides valuable insights, several limitations should be acknowledged:

Lack of Chemical Composition Analysis:

- The absence of GC-MS or HPLC-based profiling limits the precise identification and quantification of antioxidant compounds in the oil blends
- Future studies should quantitatively analyze the key bioactive compounds to correlate specific components with observed antioxidant activity.
- Use of a Single Antioxidant Assay (DPPH)
- The DPPH assay primarily evaluates hydrogen-donating antioxidant mechanisms but does not

capture other antioxidant properties, such as metal ion chelation or lipid peroxidation inhibition.

- Future studies should employ a broader panel of assays, including ABTS, FRAP, and ORAC, to provide a more comprehensive assessment of antioxidant activity.
- Lack of Quantitative Synergy Confirmation
- Although certain oil blends exhibited promising antioxidant activity, interaction indices (e.g., Chou-Talalay method) were not applied to rigorously assess synergy.
- Future research should employ synergy models to confirm and quantify the synergistic effects observed in specific blends.
- Non-Optimized Blend Ratios
- The blend ratios in this study were based on existing literature and empirical knowledge rather than systematic experimental design.
- Future studies should use response surface methodology (RSM) or mixture design models to determine optimal blend ratios for maximizing antioxidant efficacy.
- In Vitro vs. In Vivo Testing
- This study assessed antioxidant activity in vitro. However, its biological effectiveness in more complex systems remains uncertain.
- Future research should evaluate antioxidant efficacy in cellular and animal models to validate in vivo applicability and potential bioavailability.
- Solvent Choice: Potential Influence of Distilled Water
- Given the hydrophobic nature of essential oils, the use of distilled water as a solvent may have limited their solubility, potentially affecting assay accuracy
- Future studies should incorporate alternative solvents or emulsification techniques to improve oil solubility and ensure a more accurate assessment of antioxidant activity

By addressing these limitations, future research can provide a more systematic and comprehensive evaluation of essential oil blends, strengthening the scientific evidence base to support their diverse applications.

Conclusion

The results of this study demonstrate that essential oil blends exhibit greater antioxidant activity than individual oils, supporting their potential as effective natural antioxidants. These findings highlight the importance of synergy among bioactive compounds and suggest that optimized blending strategies can maximize antioxidant performance.

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Author Contributions

Jae Kyung Kim: Designed the research plan, organized the study, coordinated data analysis, and reviewed the manuscript.

Sunghun JANG: Conducted the experiments and contributed to manuscript writing.

Ethics

This study was approved by the Institutional Review Board of Dankook University (IRB No. DKU NON2024-004). Human participants were not involved.

Data Availability

The datasets used and examined in this study can be obtained from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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