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Short Communication

Antioxidant Activity of Facial Wash, Moisturizer, and Serum Containing *Andrographis paniculata* Extract, *Syzygium aromaticum* Oil, and *Pogostemon cablin* Oil

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ABSTRACT

Oxidative stress is closely associated with the aging process, prompting an investigation into the therapeutic potential of Andrographis paniculata extract, Syzygium aromaticum oil, and Pogostemon cablin oil, herbs known for their widespread medicinal use. This study aimed to assess the antioxidant properties of skincare products, including facial wash, moisturizer, and serum formulated with these oild and extract. Utilizing the DPPH assay to evaluate antioxidant activity, we found that all products displayed antioxidant activity, with the serum showing the greatest efficacy (IC_{50} value of 2792.17 µg/mL) compared to the moisturizer (IC_{25} value of 2541.73 µg/mL) and facial wash (IC_{25} value of 2752.01 µg/mL). Notably, all products (with active ingredients) significantly enhanced radical scavenging activity over the product bases (products without active ingredients) at 5000 µg/mL, with percentage improvement as follows: serum 53.48 ± 1.50%, moisturizer 39.5 ± 2.18%, and facial wash 7.23 ± 2.20%. These findings suggest that facial wash, moisturizer, and serum containing the combination of Andrographis paniculata extract, Syzygium aromaticum oil, and Pogostemon cablin oil have the potential to become effective anti-aging treatments in the cosmetic market due to their antioxidant properties. Further studies are needed to assess their mechanism of action as anti-aging cosmetics.

***Keywords*:** 2,2-diphenyl-1-picrylhydrazyl, antioxidant, anti-aging, serum, moisturizer, facial wash

Introduction

Reactive oxygen species (ROS) significantly contribute to intrinsic and extrinsic aging pathways. Oxidative stress, resulting from an imbalance between the production of ROS and the antioxidant defense mechanism, plays a pivotal role in the aging process, including skin aging. Specifically, mitochondrial ROS are crucial in cellular aging by damaging cellular components and influencing signal transduction for biological processes. The skin, constantly exposed to environmental oxidants and possessing a high metabolic rate, experiences a higher ROS load than other organs, contributing to both intrinsic (chronological) and extrinsic (environmental, such as UV radiation) aging processes.[1-3] The oxidative stress mechanism in photoaging involves excessive ROS production from UV exposure, leading to cellular damage, which initiates a series of reactions, including matrix metalloproteinases (MMPs) activation, inflammation, immune responses, and weakened antioxidant defenses, ultimately resulting in the visible signs of photoaging.[2,3] This highlights the role of antioxidants in neutralizing ROS to slow the aging process and maintain skin health, thereby protecting against age-related damage and preserving a youthful appearance.

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Additionally The trend toward natural anti-aging cosmetics emphasizes the synergistic potential of combining various botanical extracts to enhance their efficacy. Research has explored innovative formulations, such as merging coconut oil with deer antler stem cell extract,[4] employing a mixture of green tea extracts with other botanicals to combat skin disorders,[5] and combining extracts from *Terminalia bellirica*, *Phyllanthus emblica*, Triphala, and *Carica papaya*, which have exhibited anti-aging effects, including improvement in skin wrinkles and elasticity.[6] The emphasis on extract combinations reflects a strategic shift in the cosmetic industry toward utilizing the synergistic antioxidant, anti-inflammatory, and skin-rejuvenating properties of natural ingredients. This approach aims to formulate more effective and holistic anti-aging skincare solutions, showcasing the commitment of the industry to natural, plant-based ingredients for combating the signs of skin aging.

In the pursuit of mitigating oxidative stress and its implications on aging, natural sources have gained attention for their cosmetic applications, offering a rich source of antioxidants. Incorporating antioxidants into anti-aging cosmetic products, with the goal of promoting a healthy and youthful skin appearance, offers significant benefits in dermatological practices by protecting the skin and potentially alleviating signs of aging.[7-10] The cosmetic industry has increasingly focused on natural ingredients, seeking alternatives to synthetic compounds due to their potential for lower side effects and their appeal to consumers seeking 'greener' products. Among these natural sources, king of bitters (*Andrographis paniculata*), clove (*Syzygium aromaticum*), and patchouli (*Pogostemon cablin*) are known for their antioxidant properties.[11-13] These plants contain bioactive compounds such as eugenol, which has been shown to possess antioxidant activity.[11,14,15] The antioxidant activity of clove essential oil has been extensively studied. It has been found to

scavenge free radicals and chelate metal ions, thereby exhibiting antioxidant properties.[14] The essential oil of *Syzygium aromaticum* has also demonstrated antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assays.[15] Additionally, eugenol, a major component of clove essential oil, has been shown to possess antioxidant activity.[11] *Andrographis paniculata* is another plant that has been studied for its antioxidant properties. It contains bioactive compounds such as andrographolide, which has been found to exhibit antioxidant activity.[16-17] Traditionally used as a medicinal plant, *Pogostemon cablin* has been investigated for its essential oil components, such as patchoulol,[18] and is reported to possess antioxidant properties.[13]

The combination of *Andrographis paniculata*, *Syzygium aromaticum*, and *Pogostemon cablin* is of particular interest due to the synergistic effects that may enhance their antioxidant capabilities. This synergy could potentially offer a more potent defense against oxidative stress, thereby providing a more effective anti-aging solution when compared to the use of individual extracts. Integrated into skincare products, such as facial wash, moisturizer, and serum, these extracts aim to counteract skin aging by acting as antioxidant. Highlighting the importance of product-based research, Liu *et al*.,[19] demonstrated that the LUMIVIVE® System (LVS), a day and night serum duo with antioxidants and peptides, alongside a basic moisturizer, reduced pigmentation, redness, and maintained sebum levels, especially in pollution-exposed populations.

This study highlights the potential of combining *Andrographis paniculata*, *Syzygium aromaticum*, and *Pogostemon cablin* in anti-aging cosmetics, focusing on the antioxidant activity of developed skincare products such as facial wash, moisturizer, and serum. Previous research has explored the benefits of these individual plant extracts, but their combination in skincare formulations represents an innovative approach that has not been widely investigated. This study aims to assess the antioxidant potential of facial wash, moisturizer, and serum containing *Andrographis paniculata* extract, *Syzygium aromaticum* oil, and *Pogostemon cablin* oil using DPPH assay, providing a framework for assessing the efficacy of these formulations.

Materials and methods

Materials

The series of anti-aging products that tested, including facial wash, moisturizer, and serum containing Andrographis paniculata extract, Syzygium aromaticum oil, and Pogostemon cablin oil, were developed by PT Skinpharma Teknologi Indonesia (6°34'21.4896"S 106°44'42.4428"E) with a manufacturing date on September 18th, 2023. Due to confidentiality agreements with the cosmetic manufacturer as a research partner in our research grant collaboration, the exact compositions of these products cannot be disclosed. DPPH (Cat. No. D4313) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Methanol (Cat. No. 1.06009. 2500) of pro analytical grade was purchased from Merck & Co. (Darmstadt, Germany).

Antioxidant activity evaluation using DPPH assay

Each product was tested using a DPPH technique according to the method described by Zhao et al.,[20] with some modifications. The analyses were conducted using a UV-Vis spectrophotometer (UV-1800 240 V, Shimadzu, Kyoto, Japan). A total of 15 milligrams of DPPH were dissolved in 500 mL of methanol to obtain a 30 µg/mL DPPH solution. The absorbance of the DPPH solution was aimed in the range of 0.6-0.8. In a test tube, 5 mL of DPPH working solutions were combined with 1 mL of sample solution (facial wash, moisturizer, and serum products were priorly diluted into 1500, 2500, 3500, 4500, and 5000 µg/mL). The test tubes were then mixed for 10 seconds and incubated in complete darkness for 30 minutes. The same procedure was repeated to compare the radical scavenging capability of the products with active ingredients and product bases (without active ingredients) at a concentration of 5000 µg/mL. The following formula was used to calculate the percentage of antioxidants or radical scavenging activity (RSA):

$$\%\ of\ antioxidant\ activity = \frac{Ac - As}{Ac} x100 \qquad 1)^{21}$$

where Ac and As represent the control reaction absorbance and testing specimen absorbance, respectively.

Statistical analysis

All experiments were performed in triplicate, and the data were expressed as the mean ± standard deviation (SD). Statistical analysis was conducted using IBM SPSS Statistics 24 for Windows 10, employing one-way ANOVA and Tukey's HSD test.

Results and discussion

The results of this study highlight the significant antioxidant activity of facial wash, moisturizer, and serum containing *Andrographis paniculata*, *Syzygium aromaticum*, and *Pogostemon cablin*, as assessed by the DPPH radical scavenging assay, which is widely used to assess the antioxidant capacity of compounds. In this method, the stable DPPH radical reacts with test substances (such as natural antioxidants). When an antioxidant donates an electron to DPPH, the radical becomes colorless, allowing quantification of scavenging activity. The efficacy of antioxidants is also quantified by the IC_{50} value, indicating the concentration needed to inhibit 50% of the initial DPPH radicals, providing a comparative basis of radical scavenging abilities from different antioxidants. This assay, despite its simplicity, remains valuable in antioxidant research.[22]

Each product was evaluated at various concentrations ranging from 1500 to 5000 µg/mL. Table 1 displays the antioxidant activity as DPPH radical percent inhibition for facial wash, moisturizer, and serum. At the highest concentration tested (5000 µg/mL), the facial wash, moisturizer, and serum demonstrated DPPH radical inhibition of 33.65%, 47.20%, and 67.12%, respectively. The findings demonstrated that serum showing the highest activity, followed by the moisturizer and facial wash. The IC_{50} values further support the superior antioxidant efficacy of the serum, which had an IC_{50} value of 2792.17 µg/mL compared to the facial wash and moisturizer, both of which exceeded 5000 µg/mL, indicating that the serum is more effective at lower concentrations compared to the moisturizer and facial wash (Figure 1). At concentrations of 4500 and 5000 µg/mL, each product exhibits significantly different DPPH inhibition percentages. At lower concentrations, only the serum shows a significant difference in DPPH inhibition compared to both the moisturizer and facial wash.

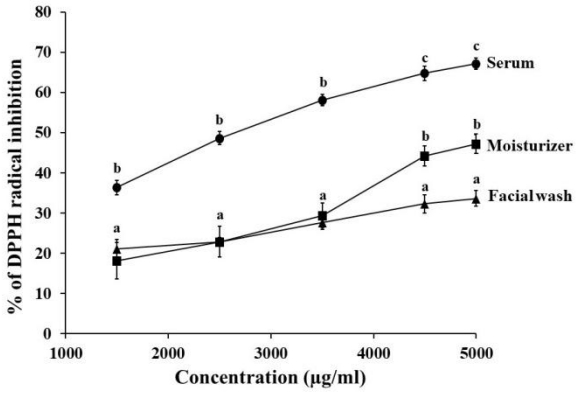

Figure 1: DPPH radical scavenging activity percentage of moisturizer, serum and facial wash containing *Andrographis paniculata* extract, *Syzygium aromaticum* oil, and *Pogostemon cablin* oil. Different letters indicate significant different between products at the same concentration ($p < 0.05$)

Figure 2 that demonstrates the significant enhancement in antioxidant activity provided by *Andrographis paniculata*, *Syzygium aromaticum*, and *Pogostemon cablin* in the products. The products (with active

ingredients) significantly enhanced radical scavenging activity over the product bases (products without active ingredients) at 5000 µg/mL with percentage improvement of serum, moisturizer, and facial was 53.48 ± 1.50%, 39.5 ± 2.18%, and 7.23 ± 2.20%, respectively as shown in. This enhancement was most pronounced in the serum, which exhibited the highest increase in DPPH radical inhibition, followed by the moisturizer and facial wash. This suggests a synergistic effect of the combined extracts in enhancing the antioxidant capacity of the formulations.

Table 1: Percentage of DPPH radical inhibition

Concentrations (µg/mL)	**% of DPPH radical inhibition**		
	Facial Wash	**Moisturizer**	**Serum**
1500	21.15 ± 2.25[a]	18.14 ± 4.49[a]	36.33 ± 1.78[b]
2500	22.83 ± 1.14[a]	22.87 ± 3.88[a]	48.66 ± 1.71[b]
3500	27.65 ± 1.65[a]	29.38 ± 3.05[a]	58.12 ± 1.44[b]
4500	32.29 ± 2.25[a]	44.20 ± 2.45[b]	64.80 ± 1.80[c]
5000	33.65 ± 2.00[a]	47.20 ± 2.39[b]	67.12 ± 1.42[c]
IC_{50} value	**More than 5000 µg/mL, IC_{25} is 2752.01 µg/ mL**	**More than 5000 µg/mL, IC_{25} is 2541.73 µg/mL**	**2792.17 µg/mL**

Values in the same row followed by different letters indicate significant differences ($p < 0.05$)

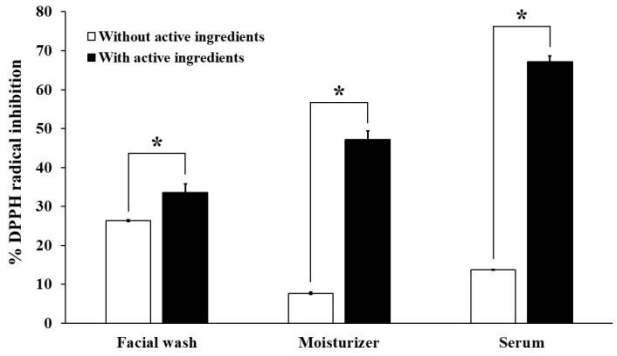

Figure 2: Improvement of DPPH radical scavenging activity of moisturizer, serum and facial wash containing containing *Andrographis paniculata* extract, *Syzygium aromaticum* oil, and *Pogostemon cablin* oil at 5000 µg/mL product concentration. * indicate significant differences between product with and without active ingredients ($p < 0.05$)

Extensive research has documented the potent antioxidant properties of *Andrographis paniculata, Syzygium aromaticum, and Pogostemon cablin*. Andrographolide, a bioactive compound from *Andrographis paniculata*, exhibits antioxidant activity by scavenging ROS, inhibiting enzymes like nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase that produce free radicals, restoring activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), and modulating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway to regulate genes involved in detoxification and antioxidant defense.[16] Rao *et al*.,[23] highlighted that the tertiary butanol phase of *Andrographis paniculata* leaves has an IC_{50} value of 52.10 ± 1.53 µg/mL, indicating potent antioxidant activity. Similarly, the essential oil of *Syzygium aromaticum*, primarily composed of eugenol, β-caryophyllene, and α-humulene, has been shown to possess significant antioxidant properties.[24] Selles *et al*.,[15] demonstrated that IC_{50} for *Syzygium aromaticum* essential oil was $4.82 \pm 0.06 \times 10^{-2}$ µg/mL, which was higher than IC_{50} of isolated quercetin, gallic acid, and ascorbic acid. *Pogostemon cablin,* with its high content of patchoulol and other terpenes, also exhibits notable antioxidant activity, further supporting the findings of this study. The IC_{50} of *Pogostemon cablin* extract obtained by supercritical fluid extraction was determined to be 1.21 ± 0.17 mg/mL with a patchoulol concentration of 0.2953 g/g oil.[25] This antioxidant activity may be the result of major compounds of essential oil, mainly monoterpenes (patchoulol and seychellene) and sesquiterpene (α-bulnesene and α-guaiene).[26]

Chronic and repetitive exposure to UV radiation (UVR) could accelerate skin aging by triggering the formation of ROS through activation of enzymes like catalase and upregulation of nitric oxide synthase (NOS). ROS encompasses both oxygen-derived free radicals and non-radicals originating from both endogenous and exogenous sources. Predominantly, endogenous ROS are produced by mitochondria during oxygen reduction for ATP production. The body possesses a natural antioxidant defense mechanism. However, when the production of ROS exceeds the capacity of this system, it leads to oxidative stress, resulting in cellular damage, apoptosis, and ultimately, cell death.[27] Research has established a correlation between elevated ROS levels and oxidative stress contributing to skin aging. Antioxidants may prevent or slow the cellular damage caused by free radicals, suggesting that their use could help delay skin aging.[28] Topical antioxidants have been reported to protect the skin from free radical damage and may even reverse photodamage. Natural products commonly had antioxidant activity by radical scavenging mechanisms. ROS was reduced, which led to ROS reduction by breaking down the oxidative chain reaction, therefore preventing biomolecular damage and skin aging, and could act as anti-aging mechanism.[29] Based on these findings, products containing *Andrographis paniculata*, *Syzygium aromaticum*, and *Pogostemon cablin* show promise as potential ingredients for developing anti-aging skincare products.

The facial serum, when used together with the wash and moisturizer, proved to be effective and well-tolerated for treating photodamaged skin, demonstrating notable improvements in fine lines, wrinkles, and other evaluation metrics within just four weeks of use.[30] Several studies emphasize the importance of rigorous clinical testing of dermatological products across diverse populations, focusing on anti-aging effects like wrinkle reduction and improved skin elasticity using antioxidants and plant extracts. They also explore environmental impacts, such as air pollution, on skin health and the protective roles of skincare products. Employing methods from randomized controlled trials to blinded assessments ensures the reliability of results, advancing the scientific foundation for skincare development and validating efficacy claims.[19,31-34]

This study highlights the antioxidant potential of combining *Andrographis paniculata*, *Syzygium aromaticum*, and *Pogostemon cablin* in anti-aging products. However, due to the variety of free

radicals, antioxidant activity should ideally be evaluated using multiple methods, such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP), to provide a more comprehensive understanding of the antioxidant potential. This reliance on the DPPH assay alone is a limitation that future research should be addressed. Additionally, future efforts should include testing for skin allergies to ensure the safety of these products for consumer use. Therefore, while this study demonstrates the antioxidant benefits of these skincare formulations, it emphasizes the need for more extensive testing to validate their efficacy and safety.

Conclusion

The study evaluated a series of anti-aging products, including facial wash, moisturizer, and serum containing *Andrographis paniculata* extract, *Syzygium aromaticum* oil, and *Pogostemon cablin* oil, demonstrated significant antioxidant activity. Among the products, the serum exhibited the highest antioxidant activity, followed by the moisturizer and facial wash. Future research should focus on conducting clinical trials to validate the effectiveness and safety of these formulations on human skin. Additionally, enhancing formulation strategies to improve the stability and skin penetration of these herbal compounds could significantly advance the development of effective natural anti-aging skincare solutions.

Conflict of interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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