

Phytochemical Study and Antioxidant Activity of Anti-Aging Cream from Three Rote Plants

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ABSTRACT

This study examines the antioxidant potential of three plants used by the people of Rote Ndao in skin care, namely Puteh Wood (*Alstonia scholaris*), Kesambi (*Schleichera oleosa*), and *Moringa* (*Moringa oleifera*). The background of the study was based on skin aging triggered by oxidative stress due to free radicals. The research objectives were to identify active phytochemical compounds, formulate the three extracts into creams, and analyze their potential synergism and antioxidant activity in a descriptive-exploratory manner. Extraction using 70% ethanol was carried out by maceration method, while phytochemical identification was applied by color reaction and precipitation tests for alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids. Vanishing cream-based cream formulation was successfully made with good physical characteristics, namely homogeneous, pH 6.8, and spreadability of 5.7 cm. Qualitative antioxidant activity test using DPPH method showed a rapid change of DPPH purple color to pale yellow in mixed extracts and creams, indicating stronger antioxidant activity than single extracts. The results indicate the presence of potential antioxidant compounds in all three plants and an indication of synergistic effects between extracts that may come from the interaction of different classes of phytochemical compounds. These findings provide an important basis for more in-depth quantitative studies in the development of natural ingredient-based antioxidant products.

Keywords: Anti-aging, Phytochemical, Cream, Polyherbal, Synergism, Qualitative, Rote Ndao, DPPH

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INTRODUCTION

Skin aging is a physiological process that is influenced by various factors, both intrinsic and extrinsic. Intrinsic factors include genetics and age, while extrinsic factors include ultraviolet (UV) light exposure, pollution, and lifestyle. One of the main mechanisms that accelerate skin aging is free radical-induced oxidative stress. Free radicals can damage important components of the skin such as collagen and elastin that play a role in maintaining skin suppleness and elasticity. The imbalance between the production of free radicals and the ability of the body's antioxidant system to neutralize them is a key factor that accelerates the aging process (Fitrianingsih et al., 2022). Skin aging, especially premature aging or *photoaging*, is closely related to the accumulation of cell damage due to free radicals from UV exposure, pollution, and other oxidative stress. Free radicals cause the degradation of collagen, elastin, and skin cell DNA resulting in wrinkles, dark spots, and skin laxity (Ahmad, 2022). One strategy that is widely used to overcome this is the use of topical antioxidants that are able to neutralize free radicals. In line with the development of global trends, the use of natural ingredients in the field of cosmetics (*cosmeceuticals*) is increasingly popular because it is considered safer, environmentally friendly, and sustainable.

Local plants have great potential as a source of antioxidant active ingredients. Many of them contain bioactive compounds such as polyphenols, flavonoids, and terpenoids that not only act as antioxidants but also have anti-inflammatory properties and protection against skin damage due to UV radiation (Wulandari et al., 2022). Rote Ndao, an island in East Nusa Tenggara, is known for its

unique biodiversity. The local community has long utilized various local plants for traditional medicine and skin care. However, the potential of these plants as anti-aging ingredients has not been fully studied scientifically. Ethnobotanical research and phytochemical screening are important steps to identify plants with high antioxidant activity that could potentially be developed in anti-aging cream formulations (Sinaga et al., 2020). Several previous studies have shown that local plants do have strong antioxidant activity. *Moringa* (*Moringa oleifera*) leaf extract, for example, has been shown to be rich in bioactive compounds and has been widely used in skincare products (Diky Okta Yudha Putra, 2024). In addition, *Centella asiatica* is known to have the ability to repair skin tissue and stimulate collagen production (Pratiwi, 2022). This emphasizes the importance of further exploration of local Indonesian plants, especially from Rote Ndao.

Indonesia as a megabiodiversity country holds various medicinal plants whose potential has not been maximized. In Rote Ndao Regency, several local plants have great prospects in the development of cosmetic preparations, including: Puteh wood (*Alstonia scholaris*) whose bark and leaves are used to treat wounds and skin infections and are reported to contain alkaloids and flavonoids with antioxidant activity (Kumar et al., 2021), 2021); Kesambi (*Schleichera oleosa*) whose seeds and leaves are used for skin care and contain phenolic compounds, tannins, and saponins (Mishra et al., 2019); and *Moringa* (*Moringa oleifera*) which is rich in vitamin C, vitamin E, and flavonoids such as quercetin and is recognized as a potent antioxidant (Leone et al., 2016). In addition to single use, polyherbal approaches are gaining attention due to possible synergistic effects. Synergism occurs when the combination of several bioactive compounds from different plants gives a greater effect than the effect of each plant individually. This concept has the potential to increase the effectiveness of anti-aging formulations and is highly relevant to be proven through scientific research (Parasuraman et al., 2014).

Based on this description, this research is important because it has scientific, methodological, applicative, and socio-cultural values. From the scientific side, this research aims to reveal the phytochemical profile and synergism potential of three local Rote Ndao plants that have not been widely studied, so as to provide a scientific basis for their traditional use. Methodologically, this research adopts a comprehensive qualitative approach through phytochemical identification, formulation, and antioxidant activity testing as the first step before a more in-depth quantitative research. From an applicative point of view, the results of this research will produce a basic protocol for the formulation of polyherbal cream preparations that can be further developed into anti-aging cosmetic products. Meanwhile, from a socio-cultural point of view, this research documents and validates the local knowledge of the Rote Ndao community, so that it can increase cultural value as well as provide economic impact from the utilization of local plants. In line with that, this research was also designed to address a number of existing gaps (gaps research). First, specific qualitative phytochemical profiles of plants growing in Rote Ndao have never been reported, even though environmental factors are known to influence the content of their secondary metabolites. Secondly, the polyherbal approach and synergism of these three plants have never been studied, so the interaction between their bioactive compounds is still unclear. Third, studies on the formulation of topical preparations based on a combination of these plant extracts, especially in the form of physically and functionally stable creams, are still very limited.

Therefore, the hypothesis of this study is that ethanolic extracts of *Alstonia scholaris*, *Schleichera oleosa*, and *Moringa oleifera* leaves contain phytochemical compounds such as flavonoids, tannins, and alkaloids that act as antioxidants. The combination of the three extracts is expected to show stronger antioxidant activity visually through the qualitative DPPH test than the single extract, indicating a synergistic effect. Furthermore, the combined extracts are expected to be formulated into cream preparations with good physicochemical parameters and still maintain their antioxidant activity.

THEORETICAL OVERVIEW

Oxidative stress is one of the main factors that accelerate the skin aging process. This condition occurs when the production of *Reactive Oxygen Species* (ROS) such as superoxide anions ($O_2^{\cdot-}$) and hydroxyl radicals ($OH^{\cdot}$) exceeds the capacity of the body's natural antioxidant system to neutralize them. Excessive ROS can cause damage to skin cell lipids, proteins and DNA, accelerating the signs of aging. External antioxidants, especially those used topically, function by donating electrons to stabilize free radicals and reduce the negative effects of oxidative stress (Pandel et al., 2013).

One potential source of antioxidants comes from plant phytochemical compounds. Phytochemical compounds have a diversity of mechanisms in fighting free radicals. Flavonoids, such as quercetin and kaempferol, are known as highly efficient free radical scavengers due to their phenolic ring structure. Tannins are able to chelate pro-oxidant metal ions such as Fe^{2+} while acting as free radical scavengers. Alkaloids exhibit antioxidant activity through the hydrogen donor mechanism, while saponins have antioxidant as well as anti-inflammatory activity. In addition, terpenoids and steroids also contribute, for example carotenoids as important antioxidants, while steroids play a role in maintaining cell membrane integrity against oxidative stress. The combination of these various classes of compounds in a polyherbal formulation allows for an attack on oxidative stress from multiple pathways, thus creating both additive and synergistic effects (Liu, 2004).

The polyherbal philosophy itself is based on the concept of synergism, which is the combination of several plant extracts that produces a greater effect than a single extract. Synergism can be realized through various mechanisms, including: increased absorption or bioavailability of a compound by another compound, targeting of different but complementary pathological pathways, and the ability of one compound to neutralize potential side effects of another compound. Thus, the combination of plant extracts not only broadens the spectrum of action, but also increases the safety and effectiveness of the preparation (Wagner & Ulrich-Merzenich, 2009).

In the development of antioxidant-based anti-aging cosmetic preparations, topical creams are an ideal form for application. *Oil in water* (O/W) or *vanishing* creams are preferred because they have a light texture, are easy to apply, do not leave a greasy feeling, and support the release of hydrophilic active ingredients into the skin. Formulation stability is an important aspect in the development of these preparations, so evaluation of the physical quality of the cream, such as homogeneity, viscosity, pH, spreadability, and stability during storage, needs to be done to ensure product quality and acceptability (Rowe et al., 2009).

Thus, this theoretical review confirms that oxidative stress plays an important role in skin aging, phytochemical compounds function as antioxidants by various mechanisms, polyherbal approaches provide more effective synergistic potential, and topical cream formulations are an appropriate medium to optimize these antioxidant benefits.

METHOD

This research is an exploratory laboratory study with a qualitative approach to analyze the phytochemical profile and antioxidant activity of *Alstonia scholaris*, *Schleichera oleosa*, and *Moringa oleifera* leaf extracts. The materials used include simplisia powder of the three plants, 70% ethanol, various phytochemical reagents (Mayer, Dragendorff, Wagner, $FeCl_3$, Liebermann- Burchard, NaOH, HCl, gelatin), DPPH reagent, and cream base consisting of stearic acid, cetyl alcohol, mineral oil, propylene glycol, triethanolamine, methylparaben, and demineralized water. The extraction process was carried out by maceration method using 70% ethanol for 3×24 hours, then the filtrate was evaporated to obtain a thick extract. Single extracts were tested for phytochemistry, while combination extracts were made in a ratio of 1:1:1.

Qualitative phytochemical tests were carried out based on Harborne's method (1998) to detect the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids/steroids. Furthermore, the combined extracts were formulated into an O/W type cream using the vanishing cream method, with the addition of extracts at $\pm 40^\circ C$ at a concentration of 6% (b/w). Evaluation of the cream included organoleptic test, homogeneity, emulsion type, spreadability, and pH. Antioxidant activity

was tested qualitatively using DPPH method with extract concentration of 1000 µg/mL, base cream (F0), and polyherbal cream (F1), and compared with vitamin C as positive control and solvent as negative control (Mensor et al., 2011).

Data obtained from the phytochemical test, formulation, cream evaluation, and DPPH test were analyzed descriptively to interpret the pattern of differences between single extracts, combinations, and cream preparations so as to answer the formulation of problems and research hypotheses.

FINDING AND DISCUSSION

Table1. Qualitative Phytochemical Test Results of 7-% Ethanol Extracts

Compound Group	Test performed	<i>A. scholaris</i>	<i>S. oleosa</i>	<i>M. oleifera</i>	Combination	Result (Combination)
Alkaloids	Mayer	+	-	-	+	White precipitate
	Dragendorff	+	-	-	+	Orange precipitate
	NaOH-HCl	+	+	+++	+++	Yellow (NaOH) - > colorless (HCl)
Flavonoids	Mg-HCl	+	+	++	++	Reddish orange color
Tannins	FeCl ₃ 1%	+	++	+++	+++	Blackish green color
Saponin	Foam	-	+++	+	++	Stable foam > 10 min
Terpenoids/Steroids	Liebermann-Burchard	+	+	+	+	Brownish green color
	Salkowski	+	+	+	+	Green fluorescence

Notes: (-) negative; (+) weak positive; (++) positive; (+++) strong positive.

The test results showed that each extract had a unique phytochemical profile. *A. scholaris* predominantly contains alkaloids. *S. oleosa* is very rich in saponins and tannins. *M. oleifera* showed very strong results for flavonoids and tannins. The combined extracts showed the presence of all classes of compounds detected in the single extracts, with generally equal or stronger intensity, especially in the flavonoids and tannins tests.

The results of the evaluation of the cream preparation show that the resulting product has organoleptic characteristics in the form of a light brown color with a distinctive herbal aroma that is not pungent, as well as a smooth texture and easy to remove from the skin. Physically, the cream looked homogeneous without any granules or lumps of material that could interfere with the comfort of use. The emulsion type test using the *dye test* method with methylene blue showed an even distribution of blue color throughout the preparation, so it can be confirmed that the preparation is an oil-in-water (O/W) type emulsion. In terms of spreadability, the cream has a spread diameter of 5.7 cm, which is categorized as very good for topical preparations because it facilitates even application on the skin surface. In addition, the pH value obtained of 6.8 shows that

the cream preparation is in the normal pH range of the skin, which is neutral to slightly acidic, making it safe and comfortable to use without causing irritation.

Table 2. Description of Qualitative DPPH Test Results

Sample	Color Change after 30 minutes	Speed of Color Change (first 5 minutes)	Activity Interpretation
Negative Control (Ethanol)	Dark purple (no change)	No change	No activity
Positive Control (Vit C)	Colorless	Very Fast (<< 1 minute)	Very Strong
A. scholaris Single	Light purple	Slow	Weak
S. oleosa Single	Lavender	Medium	Medium
M. oleifera Single	Pale yellow	Fast	Strong
Combination of Extracts	Almost colorless	Very Fast (< 2 minutes)	Very Strong
Base Cream (F0)	Dark purple (no change)	No change	No activity
Polyherbal Cream (F1)	Pale yellow	Fast (< 5 min)	Strong

The most dramatic and fastest color change occurred in the combined extract solution and in the polyherbal cream preparation. The combined extracts caused almost complete decay of the purple color of DPPH, close to the results shown by vitamin C. The polyherbal cream also showed a clear color change to pale yellow, indicating that the antioxidant active ingredients remained active after the formulation process.

The results of this study provide a comprehensive descriptive picture and support the proposed hypothesis. Phytochemical tests showed that the three local Rote Ndao plants-*A. scholaris*, *S. oleosa*, and *M. oleifera*-contain various classes of bioactive compounds that have been scientifically proven to have antioxidant activity. The uniqueness of each plant's phytochemical profile is evident: *A. scholaris* was dominated by alkaloids, *S. oleosa* was rich in saponins and tannins, while *M. oleifera* showed abundant flavonoids and tannins. These findings are in line with previous reports (Kumar et al., 2021; Mishra et al., 2019; Leone et al., 2016), while enriching the scientific database with specific information on plants growing in the Rote Ndao bioregion.

Interestingly, the combined extracts of the three plants managed to deliver a full spectrum of different active compounds, forming a complex "antioxidant cocktail". The polyherbal cream formulation developed showed satisfactory physical characteristics, including homogeneity, excellent spreadability, and skin-compatible pH value. This proves that the *vanishing cream* base used is compatible with the polyherbal extract without causing stability problems, in line with the formulation theory proposed by Rowe et al. (2009). The success of this formulation is an important stage in bringing the potential of phytochemicals closer to practical application as topical preparations.

The qualitative DPPH assay provided strong evidence of a synergistic effect. Visually, the antioxidant activity of the combined extracts proved to be much higher than that of the single extracts. In fact, the activity surpassed that of the strongest extract, *M. oleifera*. In addition, the speed of DPPH discoloration in the combination almost matched that of vitamin C, indicating significant efficiency of the radical capture reaction. If the activity was merely additive, then the combination

results should have been close to the average of the single extracts. However, the fact that the effect surpassed that of the strongest component corroborates the notion of synergistic interaction. This phenomenon is in line with the polyherbal concept that emphasizes that combinations of phytochemicals can reinforce each other through various mechanisms, such as increased penetration of active ingredients or complementarity of antioxidant action pathways (Wagner & Ulrich-Merzenich, 2009; Liu, 2004). For example, saponins from *S. oleosa* are thought to act as enhancers that increase the bioavailability of both *M. oleifera* flavonoids and *A. scholaris* alkaloids, while the radical scavenging mechanisms of each compound work complementarily.

Furthermore, the fact that antioxidant activity was maintained in the cream preparation is an important finding. This indicates that the formulation process, including heating and stirring, did not cause significant degradation of the main active compounds. Thus, the resulting cream preparation is not only physically stable, but also remains pharmacologically functional.

Overall, this study revealed the great potential of the combination of three local Rote Ndao plants. The results provided strong evidence of diverse phytochemical content, synergistic antioxidant activity, and stability of the cream formulation. These findings not only support the research hypothesis, but also provide a solid scientific foundation for further development as a topical product with antioxidant activity claims based on local wisdom.

CONCLUSIONS

This study proved that ethanolic extracts of *Alstonia scholaris*, *Schleichera oleosa*, and *Moringa oleifera* leaves from Rote Ndao positively contain phytochemical compounds with antioxidant potential, each with a distinctive profile. The 6% polyherbal cream formulation was successfully made with excellent physical characteristics, including homogeneity, O/W emulsion type, optimal spreadability, and pH that is suitable for physiological skin conditions. The DPPH qualitative test showed that the combination of extracts and polyherbal cream preparation had very strong antioxidant activity, even higher than the single extract, which gave a strong indication of a synergistic effect between the three extracts.

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