

Research Article

In vitro evaluation of the sun protection factor of *Archidendron clypearia* extract and optimization of an anti-collagenase serum formula using a simplex lattice design

Devi Putri Anggraini, Nabila Julidia Deswita Putri, Supomo*, Yullia Sukawaty, and Eka Siswanto Syamsul

Sekolah Tinggi Ilmu Kesehatan Samarinda, 75124 Samarinda, East Kalimantan, Indonesia

*Corresponding author: fahmipomo@gmail.com

Received: 28 August 2025; Revised: 18 June 2026; Accepted: 14 August 2026; Published: 30 August 2026

Abstract

Skin aging is associated with reduced elasticity caused by collagen degradation mediated by collagenase enzymes, and this process is accelerated by ultraviolet (UV) exposure. Natural-based serums are increasingly explored as multifunctional cosmetic products because they may provide both photoprotective and anti-aging effects. This study aimed to evaluate the in vitro Sun Protection Factor (SPF) of the ethanol extract and n-hexane, ethyl acetate, and water fractions of *Archidendron clypearia* leaf and to optimize an anti-collagenase serum formulation using the Simplex Lattice Design (SLD). The effects of xanthan gum and triethanolamine concentrations on viscosity, spreadability, and pH were evaluated. At 400 ppm, the ethyl acetate fraction showed the highest SPF (38.44 ± 0.05 ; high), followed by the ethanol extract (33.40 ± 2.28 ; high), water fraction (21.75 ± 0.23 ; medium), and n-hexane fraction (8.54 ± 0.07 ; low). The optimized serum contained 1% xanthan gum and 0.5% triethanolamine and produced a desirability value of 1, viscosity of 1816.67 cPs, spreadability of 5.8 cm², and pH of 7.82. The deviation between predicted and experimental values was below 5%. The optimized serum containing 0.025% extract showed collagenase inhibitory activity with an IC₅₀ of 68.06 ppm. These findings indicate that *A. clypearia* leaf extract has potential as a natural cosmetic active ingredient; however, reference-controlled SPF testing, stability assessment, in vivo validation, and safety evaluation are required before practical use.

Keywords: *Archidendron clypearia*, collagenase, sun protection factor, simplex lattice design, serum

Introduction

Indonesia is located along the equatorial line and is classified as a tropical country with high sunlight intensity, which contributes to various skin problems caused by ultraviolet (UV) radiation [1]. Kalimantan is home to diverse ethnic groups and cultural backgrounds. Its communities significantly depend on natural resources obtained from home gardens and surrounding forests to meet daily needs, including food, traditional medicines, cosmetics, and other necessities [2]. A common plant used by the community of Kalimantan is *Archidendron clypearia* (known locally as *petai belalang*), a shrub or small tree with multiple branches that can reach up to 15 meters in height [3]. This plant has been traditionally used by the residents of Beringin Village, Sajad District, Sambas Regency, as a natural cosmetic ingredient [4]. Meanwhile, facial skin aging is a natural biological process that cannot be avoided, as it

includes a gradual decline in skin cell regeneration and collagen production. The process is also accompanied by weakening of the skin's internal support structures and natural protective mechanisms [5]. Visible signs typically begin around the age of 30 and are influenced by two primary factors, namely intrinsic and extrinsic aging. Intrinsic aging is driven by genetic changes associated with increasing age and is characterized by fine wrinkles, dryness, roughness, skin laxity, and epidermal thinning. However, extrinsic aging is predominantly caused by chronic exposure to UV radiation, and it accounts for approximately 90% of visible aging. It is manifested in hyperpigmentation and further worsened by environmental factors, such as cigarette smoke and air pollution, which contribute to oxidative skin damage [6, 7].

During the aging process, structural skin proteins,

such as collagen and elastin, undergo degradation due to the activation of collagenase and elastase enzymes [8]. In adults, the total collagen content of the skin decreases by approximately 1% per year [9]. Reduced collagenase activity leads to diminished collagen synthesis, causing a decline in collagen-producing fibroblasts. Consequently, the skin biomatrix deteriorates as the collagen scaffold progressively loses its strength and stability [10].

Excessive exposure to UV radiation induces erythema, premature aging, and even skin cancer [11], [12]. The use of sunscreens with an adequate SPF is essential for skin protection [13]. According to a previous study, the SPF value represents the effectiveness of a product or active compound in providing UV protection. Higher SPF values represent stronger photoprotective capacity against UV-induced damage [14]. Natural plant-derived compounds, particularly polyphenols with anti-inflammatory and antioxidant properties, have shown potential as sunscreen ingredients due to the UV-absorbing capacity and ability to neutralize free radicals [15].

According to previous studies [16], the leaf of *A. clypearia* contains secondary metabolites, such as terpenoids, steroids, and flavonoids, including robinetin, fisetin, gallic acid, and quercetin. Flavonoids act as potent antioxidants capable of scavenging free radicals and exhibiting anti-inflammatory activity. A subsequent study [17] reported antibacterial activity in *A. clypearia* leaf extracts. Sunscreen activity was also observed within the “ultra-protection” category in ethyl acetate fractions at 1000, 750, and 500 µg/mL, with SPF values of 30, 28, and 22, respectively [18]. The same study reported IC₅₀ values of 3.125 µg/mL (methanol extract), 4.272 µg/mL (ethyl acetate extract), and 34.570 µg/mL (n-hexane extract), suggesting very strong antioxidant activity. The ethyl acetate extract also showed tyrosinase inhibitory potential with an IC₅₀ of 78.125 µg/mL.

Serum is a formulation designed in either aqueous or oil-based systems, characterized by the ability to penetrate deeper skin layers to enhance the delivery and effectiveness of bioactive compounds [19]. The preparation of serum typically exhibits low to moderate viscosity, ensuring efficient absorption and ease of spreadability across the skin surface [20]. Furthermore, the composition of the gelling agent plays a crucial role in determining the viscosity of a serum formulation. Xanthan gum is commonly used as a gelling agent due to its non-toxic nature, compatibility with various pharmaceutical ingredients, as well as excellent stability and viscosity across a wide pH and temperature range. This gelling agent is readily soluble in both cold and hot water,

facilitating the absorption of water and consequently increasing the viscosity of the formulation. The typical concentration of xanthan gum used as a gelling agent ranges from 0.5% to 1% [21]. Alkalizing agents, such as triethanolamine, function as swelling enhancers, pH neutralizers for the base, and clarity-improving agents [22]. According to [23], the optimal concentration range of triethanolamine as an alkalizing agent is 0.4-1%.

Despite the traditional use of *A. clypearia* leaf by local communities, specific scientific data regarding the sunscreen potential of its extracts and fractions remain limited. Its suitability for development into serum preparations with anti-aging benefits has not been evaluated. The selection of serum as the dosage form is based on the effectiveness, practicality, and suitability for users across all age groups [24]. Meanwhile, a method widely used for determining the optimal concentration of formulation components to achieve desirable physical characteristics is the Simplex Lattice Design (SLD) [25]. Through optimizing serum formulations based on variations in xanthan gum and triethanolamine concentrations, this study aimed to determine the influence of the variations on the physical properties of the serum and the collagenase inhibitory activity.

The novelty of this study lies in integrating comparative in vitro SPF screening of the ethanol extract and three solvent fractions with SLD-based optimization of a serum and subsequent assessment of the optimized formulation's anti-collagenase activity within a single development framework. Through optimizing serum formulations based on variations in xanthan gum and triethanolamine concentrations, this study aimed to determine the influence of the variations on the physical properties of the serum and the collagenase inhibitory activity.

Materials and Methods

Materials

The instruments used in this study included a macerator, rotary evaporator, water bath, vacuum pump, Buchner funnel, porcelain crucibles, analytical balance, Brookfield viscometer, spreadability testing apparatus, pH meter, and beaker glass. Other instruments included measuring cylinders, watch glasses, a magnetic stirrer, a mortar and pestle, glass slides, a 96-well microplate, and a microplate reader.

Chemicals

Several chemicals were used to optimize serum formulations based on variations in xanthan gum and triethanolamine concentrations. These included *A. clypearia* leaf, ethanol, distilled water, 2N hydrochloric acid, Mayer's reagent, Dragendorff's reagent, Bouchardat's reagent, 5% ferric chloride

solution, magnesium powder, amyl alcohol, chloroform, acetic anhydride, concentrated sulfuric acid, xanthan gum, sodium benzoate, triethanolamine, propylene glycol, Na₂EDTA, and a Collagenase Activity Colorimetric Assay Kit.

Sample preparation

In this study, the samples used were *A. clypearia* leaf obtained from the Special Purpose Forest Area (KDKT), West Samboja District, Kutai Kartanegara Regency, East Kalimantan. Samples were examined to confirm the authenticity at Herbarium Wanariset (WAN), Samboja Environmental and Forestry Instrument Standard Implementation Center.

Phytochemical screening

Test for alkaloids

A total of 0.5 g of simplicia powder was weighed and mixed with 1 mL of 2 N hydrochloric acid and 9 mL of distilled water, followed by heating in a water bath for 2 minutes. The mixture was cooled, filtered, and the resulting filtrate was subjected to alkaloid testing using Mayer's, Dragendorff's, and Bouchardat's reagents. The sample was considered to contain alkaloids when a minimum of two out of the three tests produced positive results [26].

Test for phenolic

Ten drops of the extract solution were placed into a test tube, followed by the addition of three drops of 5% FeCl₃ reagent. The formation of a dark purple coloration represented the presence of phenolic compounds [27].

Test for flavonoids

A total of 5 mL of the extract solution was placed into a test tube, followed by the addition of 0.1 g of magnesium powder, 1 mL of concentrated hydrochloric acid, and 2 mL of amyl alcohol. After shaking, the mixture was allowed to separate into layers. A red, yellow, or orange precipitate appearing in the amyl alcohol layer represented the presence of flavonoid compounds [28].

Test for saponins

A total of 0.5 g of the extract was placed into a test tube and mixed with 10 mL of hot water, then shaken for approximately 1 minute. A stable foam layer measuring 1–10 cm in height and persisting for at least 10 minutes represented the presence of saponins. The foam remained after the addition of one drop of 2 N HCl [29].

Test for steroid-terpenoid

A total of 2 mL of the extract solution was transferred into a test tube and mixed with 2 mL of chloroform, followed by the addition of 10 and 3 drops of acetic anhydride and concentrated H₂SO₄, respectively. The formation of a red coloration implied a positive reaction for terpenoids. Meanwhile, the appearance of a green coloration represented a positive reaction for steroids [30].

Determination of in vitro SPF

Stock solutions of the extract and fractions were prepared by weighing 50 mg of each sample and transferring the sample into separate 50-mL volumetric flasks. The samples were then diluted with 70% ethanol to the calibration mark to obtain a concentration of 5000 ppm. Serial dilutions were subsequently prepared from the stock solution. Aliquots of 1 mL, 2 mL, and 3 mL of the 5000-ppm stock solution were transferred into 10-mL volumetric flasks and diluted to volume with 70% ethanol. This process led to the final concentrations of 500 ppm, 1000 ppm, and 1500 ppm, respectively.

The Sun Protection Factor (SPF) was determined in vitro using a UV–Vis spectrophotometer. Before the measurement, the instrument was calibrated with 1 mL of 70% ethanol. The test solutions were placed into quartz cuvettes, and absorbance readings were recorded in the wavelength range of 290–320 nm at 5-nm intervals. A 70% ethanol solution was used as the blank. All measurements were carried out in triplicate to ensure accuracy and reliability. The SPF value was then calculated using the following equation [31]:

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda) \quad (1)$$

CF = Correction Factor (10), EE = Erythema Effect Spectrum, Abs = Absorbance, and I = Intensity of UV light at wavelength λ nm

The erythemal effectiveness (EE) values were constants, and each absorbance was multiplied by the corresponding EE $\times$ I in **Table 1**. The resulting sum was multiplied by a correction factor (CF = 10) to obtain the final SPF value [31, 32].

A standard sunscreen reference was not included in this preliminary screening. Therefore, the reported SPF values are interpreted as within-study in vitro estimates for comparing the extract and fractions and not as direct equivalence to a commercial sunscreen product.

Table 1. EE x I values

Wavelength (nm)	EE x I
290	0.015
295	0.081
300	0.287
305	0.327
310	0.186
315	0.083
320	0.018
Total	1

Table 2. Formulation of face serum

Material	Function	Formula Concentration (%)					Range (%)
		F1	F2	F3	F4	F5	
<i>A. clypearia</i> leaf extract	Active substance	0.025	0.025	0.025	0.025	0.025	-
Xanthan gum	Serum base	1	0.5	0.75	0.625	0.875	0.5-1 [21]
TEA	Alkalizing agent	0.5	1	0.75	0.875	0.625	0.4-1 [23]
Sodium benzoate	Preservative	0.1	0.1	0.1	0.1	0.1	0.1-0.5 [33]
Propylen glycol	Humectant	15	15	15	15	15	≈15 [33]
Na ₂ EDTA	Chelating agent	0.05	0.05	0.05	0.05	0.05	0.005-0.1 [33]
Aquadest Ad	Solvent (mL)	30	30	30	30	30	-

Determination of serum formula

Serum formulation was optimized using the SLD method with Design Expert software version 12 (**Table 2**). The variables optimized were the concentrations of xanthan gum and triethanolamine, with upper and lower limits of 1% and 0.5%, respectively, resulting in a total formulation range of 1.5%. The concentration ratios were generated based on SLD recommendations, comprising three simplex and two augment design points without replication.

Serum preparation commenced with three initial solutions, namely the *A. clypearia* leaf extract, sodium benzoate, and Na₂EDTA, each of which was dissolved in an adequate amount of distilled water [34]. The serum base was then prepared by dispersing xanthan gum in hot distilled water until a homogeneous gel was formed [20]. After obtaining the base, the Na₂EDTA and sodium benzoate solutions were gradually incorporated, followed by the addition of triethanolamine. The *A. clypearia* extract was subsequently added in small portions with continuous mixing to ensure uniform distribution, and propylene glycol was added to obtain a homogeneous serum formulation [34].

A concentration of 0.025% extract was selected based on previous results suggesting that the methanolic extract of *A. clypearia* exhibited very strong antioxidant activity even at low concentrations. This enabled the extract to maintain high biological effectiveness without requiring a large amount of extract [18]. A study by [35] reported that increasing extract concentrations could influence the color intensity and texture of the formulation. In this case, excessively high concentrations could alter the appearance and physical stability of the serum. Therefore, a concentration of 0.025% was considered optimal to ensure product stability while maintaining biological activity.

Determination of serum evaluation

Test for organoleptic

Organoleptic was evaluated using visual observation of the serum's consistency, color, and odor [36].

Test for homogeneity

The homogeneity of the serum was evaluated using two glass object plates. A small amount of serum was placed on one plate, evenly spread, and then covered

with the second plate. The procedure was carried out in triplicate, and the serum was considered homogeneous when no coarse particles were observed [37].

Test for viscosity

Viscosity testing was conducted by placing the serum into a viscometer, after which the spindle was immersed completely in the sample. Measurements were taken at a rotation speed of 60 rpm using spindle number 4 [38]. The acceptable viscosity range for serum formulations was 230 - 3000 cPs [39].

Test for spreadability

Spreadability was assessed by placing 0.5 g of the serum on a circular glass plate and covering the sample with another glass surface. A 125 g weight was placed on the top plate and allowed to stand for 1 minute before the diameter of the spread was measured. For topical preparations, an acceptable spreadability range was 5 - 7 cm [40].

Test for pH

The pH of the serum formulation was measured using a pH meter. A total of 3 g of serum was dispersed in 30 mL of water [36]. The pH meter was calibrated before measurement using buffer solutions of pH 4.00, 6.86, and 9.18. Calibration was performed by immersing the electrode sequentially in each buffer and wiping the material with tissue between readings. The electrode was then immersed in the serum sample, and the pH value was recorded [22]. The normal pH range of human skin was approximately 5.0 - 6.8. Acceptable pH values for topical formulations fell within the range of 4.5 - 8.0. [41].

Determination of anti-collagenase

The inhibition of collagenase activity was evaluated using a Colorimetric Assay Kit following the protocol provided by the distributor. The test reagents consisted of:

- (1) Sample solution, prepared by mixing 10 μ L of the sample with collagenase assay buffer to a final volume of 100 μ L.
- (2) Positive control, prepared by adding 10 μ L of collagenase to the assay buffer to a final volume of 100 μ L.
- (3) Inhibitor control, prepared by mixing 10 μ L of collagenase with 2 μ L of the inhibitor (1,10-phenanthroline) and adjusting the volume to 100 μ L with assay buffer.
- (4) Background control, consisting of 100 μ L of collagenase assay buffer.

Each test solution was combined with a reaction mixture consisting of 40 μ L of the collagenase substrate (FALGPA) and 60 μ L of assay buffer.

Subsequently, the mixture was transferred into individual wells of a 96-well microplate. Absorbance was measured at a wavelength of 345 nm using a microplate reader. Readings were taken at 37°C over a period of 5 - 15 minutes. The anti-collagenase activity was calculated using the following equation [42]:

$$\% \text{ Inhibition} = \frac{A_{\text{sample}} - A_{\text{inhibition}}}{A_{\text{sample}}} \times 100 \quad (2)$$

The final results were expressed as IC₅₀ values, which represented the concentration of the sample required to inhibit 50% of the enzyme activity being evaluated.

Statistical Analysis

All measurements were performed in triplicate and are presented as mean $\pm$ standard deviation. For the SLD optimization, model significance and component effects were evaluated by analysis of variance (ANOVA) using Design Expert 12, with $p < 0.05$ considered statistically significant. Model verification was assessed by comparing the experimental values with the 95% prediction interval. No inferential pairwise comparison was performed for the SPF screening because the study was designed as an initial comparative screening; this limitation is considered when interpreting the results.

Results and Discussion

Phytochemical screening analysis

Phytochemical screening was a preliminary procedure used to identify secondary metabolite constituents in plant materials based on characteristic color changes or precipitate formation [43]. **Table 3** shows the results of the phytochemical screening.

Based on the phytochemical screening results in Table 3, the *A. clypearia* leaf contained alkaloids, phenolics, flavonoids, saponins, steroids, and terpenoids. These results were consistent with the report by Risman et al., which confirmed the presence of flavonoids, terpenoids, and steroids in *A. clypearia* leaf extract. The results were further supported by previous studies [44] showing that the leaf of this species contained flavonoid, phenolic, and terpenoid compounds.

The secondary metabolite profile observed in this study exhibited a pattern that was consistent with the phytochemical characteristics of other species within the genus *Archidendron*. For example, earlier investigations of *Archidendron bubalinum* showed the presence of flavonoids, phenolics, saponins, alkaloids, as well as monoterpenes and sesquiterpenes in both leaf and seeds. This result confirmed that the metabolite groups represented common chemotaxonomic signatures of the genus [45, 46].

The similarity of secondary metabolite composition across species supported the chemotaxonomic principle that plants belonging to the same genus typically shared structurally related classes of metabolites, which may consequently exhibit similar biological activities [47]. Therefore, the phytochemical results in this study validated the chemical identity of the tested extract. It also showed consistency with the metabolite profiles reported for other *Archidendron* species, particularly the predominance of flavonoid and phenolic compounds known for the significant biological activities.

Quercetin and gallic acid, which have previously been reported in *A. clypearia* [16], contain conjugated aromatic systems and hydroxyl groups that may support UV absorption and reactive oxygen species scavenging [51]. However, the present phytochemical screening was qualitative, and the individual compounds were not quantified; therefore, their specific contributions require confirmation by chromatographic analysis.

The value of sun protection factor (SPF)

The SPF evaluation results are presented in **Table 4**. The results of the study showed that the SPF increased proportionally with the concentration of the tested samples. At the highest concentration of 400 ppm, the ethyl acetate fraction exhibited the highest SPF value at 38.44. This was followed by the ethanol extract, water, and n-hexane fraction at 33.40, 21.75, and 8.54, respectively. SPF values were generally classified into three categories, namely high (SPF >30), medium (SPF 12–30), and low (SPF 2–12) [48].

The ethyl acetate fraction produced the highest SPF value due to its semipolar nature, which enabled

efficient extraction of active constituents, such as aglycone flavonoids, alkaloids, and certain terpenoids. Flavonoids were the primary contributors to sunscreen activity, as ethyl acetate was among the most effective solvents for extracting these compounds [49, 50].

The SPF activity observed in the fractions of *A. clypearia* leaf was closely related to the presence of phenolic and flavonoid compounds. Both metabolite groups exhibited photoprotective properties through the ability to absorb UVA and UVB radiation. Phenolic compounds protect phospholipid membranes from UV-induced peroxidation by inhibiting lipid peroxidation chain reactions. The photoprotective effects also included the attenuation of oxidative stress, suppression of MMP-1 induction, and reduction of reactive oxygen species (ROS) in human keratinocytes. These effects occurred through the enhancement of antioxidant defense mechanisms, including glutathione (GSH), catalase, and glutathione peroxidase (GPx).

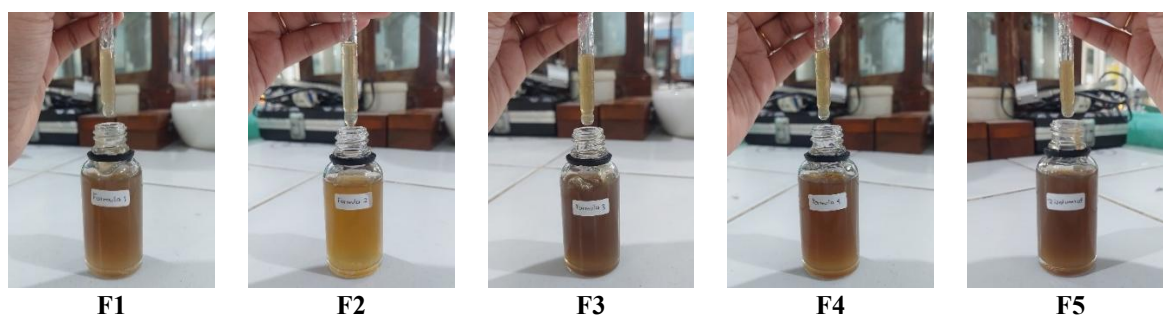
Flavonoids contributed to UV absorption and mitigated oxidative damage triggered by ROS. The UV-absorbing ability was attributed to the conjugated double-bond system within the structure. Meanwhile, ROS-neutralizing activity was associated with hydroxyl groups located on the aromatic rings [51]. The high SPF value of the ethyl acetate fraction at 400 ppm is consistent with the earlier report on *A. clypearia* ethyl acetate fractions [18], although direct numerical comparison is limited by differences in concentration and experimental conditions. The absence of a reference sunscreen and inferential pairwise testing means that the present findings should be interpreted as preliminary evidence of relative photoprotective potential.

Table 3. Phytochemical screening results of *Archidendron clypearia* extract

Secondary Metabolite	Reagent	Results	Observations
Alkaloid	Mayer Dragendorff Bouchardat	Positive	Formation of yellow precipitate Formation of orange to reddish-brown precipitate Formation of brown precipitate
Phenolic	FeCl ₃ 5%	Positive	Formation of dark purplish-black coloration
Flavonoid	Mg powder, concentrated HCl, and amyl alcohol	Positive	Yellow precipitate observed in the amyl alcohol layer
Saponin	Hot water, and HCl 2N	Positive	Stable foam persisted for ≥10 min with a height of ~1 cm. Foam remained after adding 2 N HCl
Steroid-Terpenoid	Chloroform, acetic acid anhydride, and concentrated H ₂ SO ₄	Positive	Green coloration in upper layer (steroids) and red coloration in lower layer (terpenoids)

Table 4. Sun protection factor (SPF) test results of *Archidendron clypearia* extract

Sample	Concentration (ppm)	Mean $\pm$ SD	Category SPF
Ethanol extract	100	9.3353 $\pm$ 0.23	Low
	200	18.5381 $\pm$ 0.19	Medium
	300	27.7830 $\pm$ 0.35	Medium
	400	33.4093 $\pm$ 2.28	High
n-Hexane fraction	100	2.2670 $\pm$ 0.03	Low
	200	4.3763 $\pm$ 0.06	Low
	300	6.5566 $\pm$ 0.04	Low
	400	8.5419 $\pm$ 0.07	Low
Ethyl acetate fraction	100	12.3211 $\pm$ 0.71	Medium
	200	23.9611 $\pm$ 0.19	Medium
	300	33.6006 $\pm$ 0.17	High
	400	38.4467 $\pm$ 0.05	High
Water fraction	100	5.4795 $\pm$ 0.12	Low
	200	10.8108 $\pm$ 0.08	Low
	300	16.5691 $\pm$ 0.04	Medium
	400	21.7556 $\pm$ 0.23	Medium

**Figure 1.** *Archidendron clypearia* Extract Serum: (a) Formula 1, (b) Formula 2, (c) Formula 3, (d) Formula 4, and (e) Formula 5**Evaluation of serum formulation**

The serum formulations containing *A. clypearia* leaf extract were developed using the SLD method in Design Expert software version 12. The upper and lower limits for the variables xanthan gum and triethanolamine were entered into the software, leading to five experimental runs generated through SLD recommendations. These consisted of three simplex points and two augmented design points without replication. The resulting formulations included Formula 1 (1% : 0.5%), 2 (0.5% : 1%), 3 (0.75% : 0.75%), 4 (0.625% : 0.875%), and Formula 5 (0.875% : 0.625%).

Organoleptic test

Organoleptic evaluation was conducted to examine the physical characteristics of the serum formulation through visual observation of its consistency, color, and odor [36]. Variations in the color and consistency among the formulations were influenced primarily by differences in the concentration of the gelling agent xanthan gum. Higher concentrations of xanthan gum

tended to produce a more turbid appearance due to increased opacity, thereby decreasing color intensity and resulting in a cloudier serum. Differences in consistency were also attributed to variation in xanthan gum concentration, as reported previously [20].

Homogeneity test

The homogeneity test was carried out to determine the uniform distribution of the active substances and other components within the serum, shown by the absence of coarse particles in the formulation [52]. The results showed that all serum formulations were homogeneous and no visible particles were detected. This homogeneity was achieved due to the use of a magnetic stirrer during preparation, which facilitated continuous and uniform mixing at controlled speeds. The mixing process ensured proper dispersion of all ingredients, particularly the xanthan gum gelling agent, thereby preventing lump formation within the serum [53].

Table 5. Evaluation of serum formula *Archidendron clypearia* extract

Parameter	F1	F2	F3	F4	F5
Organoleptic (Consistency, Color, Odor)	Moderately viscous, not too fluid; pale yellow, slightly turbid; characteristic odor	Slightly fluid; pale yellow, semi-transparent; characteristic odor	Slightly viscous; light brown-yellowish, semi-transparent; characteristic odor	Slightly fluid; light brown-yellowish, semi-transparent; characteristic odor	Moderately viscous; light brown, semi-transparent; characteristic odor
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Viscosity (cPs)	2016.66±28.86	200±0	1.300±0	500±0	1583.33±28.86
Spreadability (cm)	6.3±0.34	10.7±0.26	7.7±0.30	10±0	6.63±0.23
pH	7.82±0.07	8.51±0.03	8.44±0.02	8.48±0.03	8.28±0.03

Viscosity test

The viscosity test was used to determine the thickness of the serum. This was because viscosity directly influenced formulation consistency [54]. Differences in viscosity values among the formulations were attributed to variations in gelling agent concentration. A greater xanthan gum concentration translated to a higher viscosity [55]. Furthermore, viscosity had a significant effect on the mobility of active compounds across the skin surface during topical application [21]. A formulation with excessively high viscosity hindered spreading, while a lower value facilitated easier application and better distribution of the serum on the skin [56].

Spreadability test

The spreadability test aimed to assess the ability of the serum to spread upon application, which was essential for ensuring adequate delivery of the active compound and enhancing user acceptability [37]. Formulations 2, 3, and 4 exhibited spreadability diameters exceeding 7 cm, surpassing the recommended range for optimal topical formulations. Larger spreadability diameters showed that the serum spreads more easily with minimal application force, suggesting enhanced distribution of the active ingredient across the skin surface. Increased spreadability was attributed to a lower formulation viscosity, enabling for more extensive spreading. In general, spreadability was inversely related to viscosity. According a previous study, formulation with lower viscosity produced greater spreadability [57, 58].

pH test

The pH evaluation was used to determine the safety of the serum upon topical application, ensuring that the

formulation did not cause skin irritation [34]. Variations in the pH values among the formulations were influenced by the intrinsic pH characteristics of the gelling agent used [59]. Consistent with a previous study, xanthan gum exhibited stability over a broad pH range (3–12) [33]. However, increasing its concentration tended to decrease the pH of the formulation. This reduction was attributed to the higher amount of CO₂ dissolved in the aqueous phase during mixing, which promoted the formation of acidic species and subsequently lowered the pH [60]. The addition of an alkalizing agent was required in topical formulations to adjust the pH to a physiologically acceptable range [23]. Triethanolamine, with a basic pH of approximately 10.5, was used as the alkalizing agent in combination with xanthan gum [33].

Human skin naturally exhibited a mildly acidic pH, generally ranging from 4.1 to 5.8. This acidic environment played a critical role in maintaining the integrity of the skin barrier, supporting the balance of the skin microbiome, and facilitating the activity of epidermal enzymes in lipid synthesis and desquamation. Consequently, the acidic, neutral, or alkaline differences in formulation pH had direct implications for skin health. Acidic formulations were generally compatible with skin physiology because the conditions helped preserve the acid mantle, increased barrier recovery, and stabilized the native microbial community. Several studies have shown that formulations with a pH around 4 promote superior barrier repair compared to those with near-neutral pH, largely because many skin enzymes function optimally under mildly acidic conditions. However, neutral to alkaline formulations increase the skin

surface pH, disrupt the lipid organization within the stratum corneum, and raise transepidermal water loss (TEWL), leading to dryness, irritation, and greater susceptibility to colonization by pathogenic microorganisms. For example, alkaline cleansers have been reported to delay the recovery of normal skin pH following application.

The selection of an appropriate pH was a critical aspect of cosmetic formulation, particularly for products such as anti-aging serums, sunscreens, and other bioactive topical preparations. Ideally, these formulations should maintain a pH close to the physiological range to ensure optimal biological activity while preserving the skin's natural barrier.

Understanding the implications of acidic, neutral, and alkaline pH was essential for formulation stability and also for ensuring the safety, effectiveness, and general acceptability of skincare products [61].

In the present formulations, F2–F5 had pH values above 8.0 and therefore did not meet the cited upper acceptance limit for topical preparations. The optimized formulation had a pH of 7.82, which was within the stated 4.5–8.0 range but remained higher than the physiological skin pH. Consequently, the optimized serum should not be considered ready for human application without further pH adjustment, irritation testing, stability assessment, and skin-barrier compatibility evaluation.

Table 6. Simplex lattice design (SLD) equation for response value

Response	Equation	
Viscosity	$Y = 206333 (A) + 17666 (B)$	(3)
Spreadability	$Y = 5832 (A) + 107 (B)$	(4)
pH	$Y = 7821 (A) + 8511 (B) + 1.12 (AB)$	(5)

Note: Y = response; A = xanthan gum; B = triethanolamine

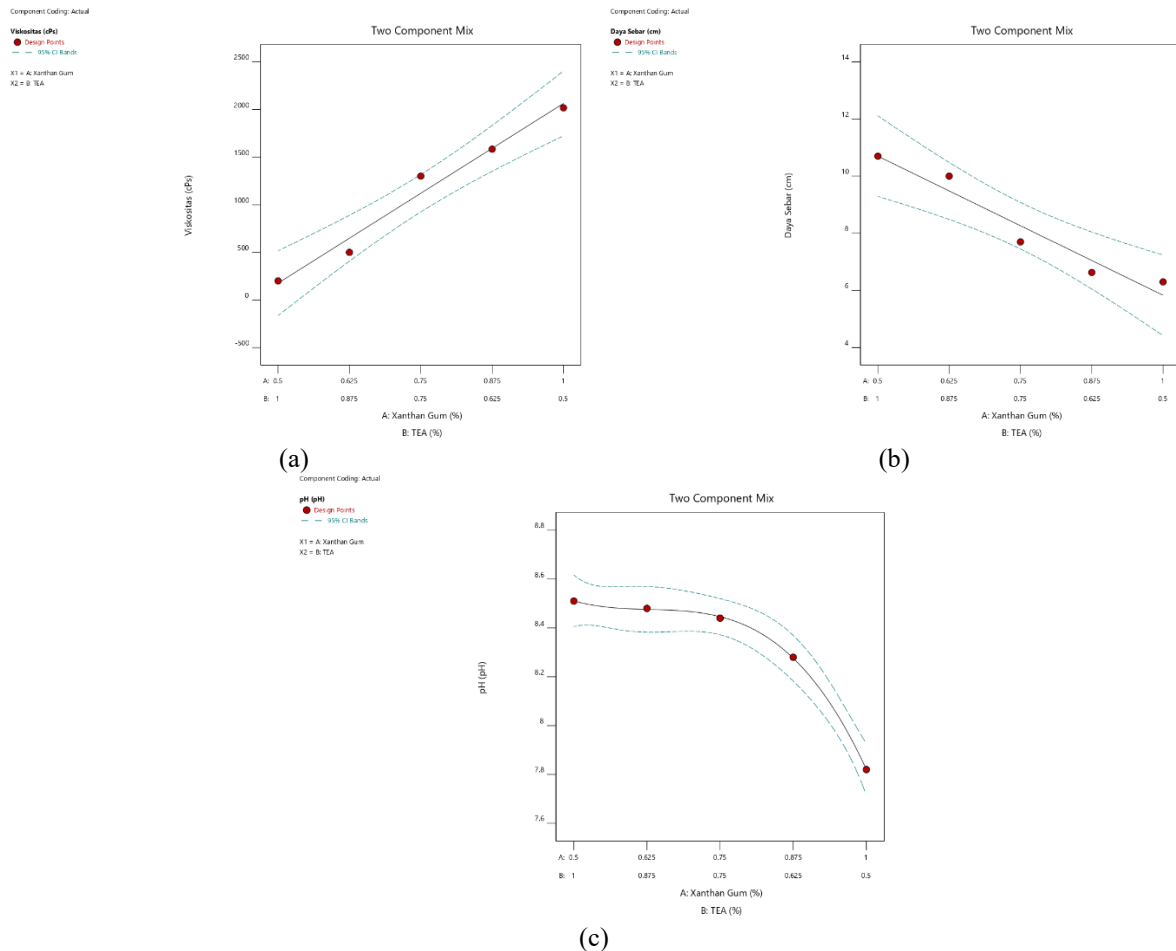


Figure 2. Mixture-model plots showing the effects of xanthan gum and triethanolamine concentrations on (a) viscosity, (b) spreadability, and (c) pH. Solid lines represent model predictions and dashed lines represent the 95% confidence limits.

Analysis of simplex lattice design (SLD) responses

The statistical analysis of variance (ANOVA) from the SLD showed that the viscosity response was best fitted by a linear model, with a p-value of 0.0017. The model equation also suggested that both xanthan gum and triethanolamine contributed positively to the viscosity values. In this case, xanthan gum showed a larger coefficient and exerted a greater influence on viscosity than triethanolamine. This result showed that a higher concentration of xanthan gum could lead to an increase in serum viscosity. **Figure 2** shows the relationship between xanthan gum, triethanolamine, and viscosity. There was a progressive increase in viscosity with rising concentrations of xanthan gum [62]. For the spreadability response, the SLD analysis recommended a linear model with a p-value of 0.0017. The model suggested that xanthan gum and triethanolamine had a positive combined effect on spreadability but were inversely related to viscosity. A lower concentration of xanthan gum resulted in higher spreadability values. This relationship was shown in **Figure 2(b)**, where serum spreadability increased as xanthan gum concentration decreased. The trend was consistent with the inverse correlation between viscosity and spreadability [63].

In the case of pH response, the SLD recommended a cubic model, with a p-value of 0.0107. The model equation showed that both xanthan gum and triethanolamine individually had positive effects on the pH, with triethanolamine exhibiting a stronger influence due to its higher coefficient. The interactive effect of both components on pH was relatively smaller. **Figure 2** shows the relationship between the two variables, where pH increased with higher concentrations of triethanolamine and decreased with increasing concentrations of xanthan gum. However, reducing triethanolamine while increasing xanthan gum lowered the pH of the serum [64].

Xanthan gum exerted the greatest influence on viscosity because its model coefficient was substantially larger than that of triethanolamine, reflecting its primary function as a polymeric thickener. The desirability value of 1.0 indicates complete satisfaction of the predefined criteria only within the relatively narrow experimental design

space and should not be interpreted as a universal optimum outside the evaluated concentration ranges.

Serum formula optimization using the simplex lattice design method

The optimum serum formulation was determined using the SLD method in Design Expert software, based on multilayer response optimization for viscosity, spreadability, and pH. **Table 7** shows the optimization criteria applied for each response variable.

The optimization of the serum formulation was assessed based on the desirability value, with higher values (close to 1) showing better conformity between the predicted responses and the targeted criteria. The desirability metric reflected the degree to which the formulation responses satisfied the specified optimization goals and was influenced by the number of variables in the model as well as the complexity of the interactions among parameters. The optimization process aimed to achieve the highest possible desirability value. In this case, values close to 1 denoted a formulation that was consistent with the defined response specifications.

In this study, the optimum formulation was identified based on the combination of components producing the highest desirability value, and getting closer to the maximum threshold. A desirability value close to 1 showed that the optimization model accurately predicted the characteristics of the formulation (**Figure 3**). Therefore, the selected formula could be considered to represent the optimal conditions among all evaluated variables [25, 65].

Based on the predicted response values in **Table 8**, the optimum formulation of the *A. clypearia* leaf extract serum was obtained at a xanthan gum-triethanolamine ratio of 1:0.5. The optimal formula identified through the SLD model was subsequently subjected to a confirmation test by reformulating the serum and evaluating the corresponding responses experimentally. The experimental and predicted values generated by the SLD models were then compared, as shown in **Table 9**.

Table 7. Simplex Lattice Design (SLD) equation for response value

Response	Goal	Lower Limit	Upper Limit	Importance
Viscosity	In Range	230	3000	+++
Spreadability	In Range	5	7	+++
pH	In Range	4.5	8	+++

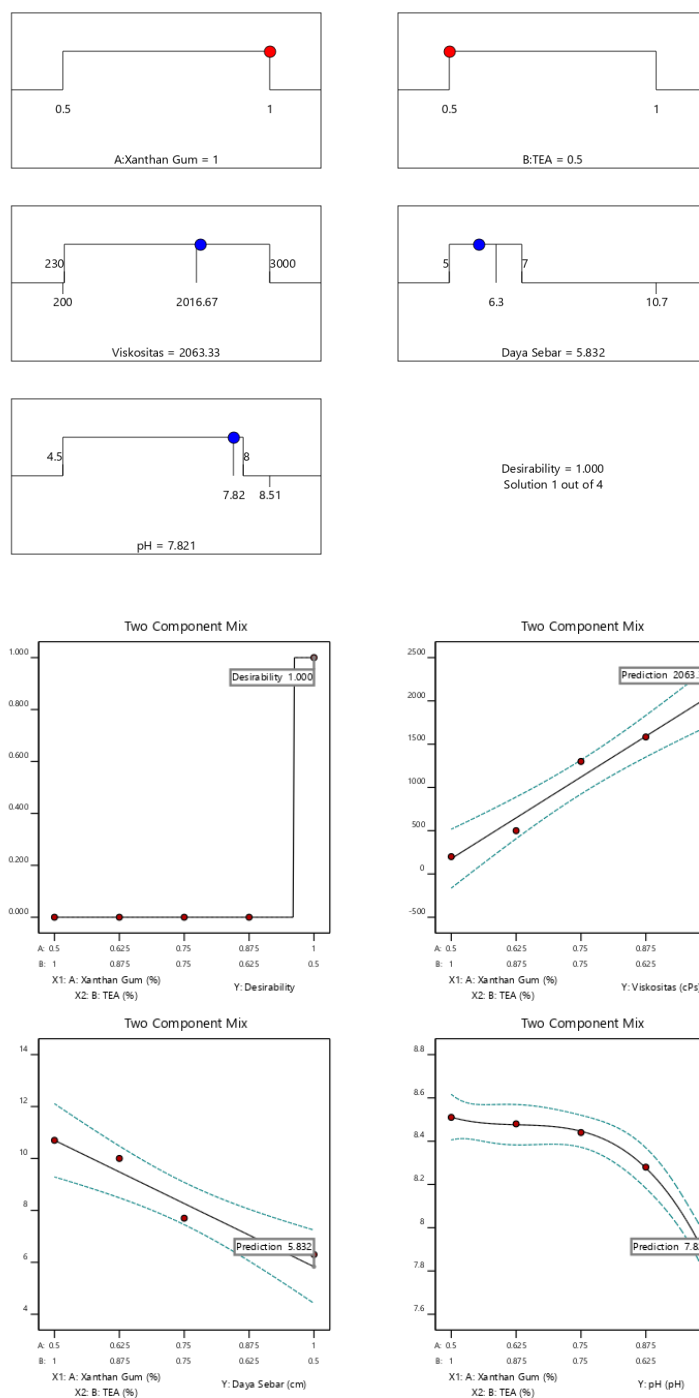


Figure 3. Predicted optimum for serum formulation

Table 8. Predicted response values of the optimum serum formula generated by the SLD

Composition		Response			Desirability
Xanthan Gum (%)	Triethanolamine (%)	Viscosity (cPs)	Spreadability (cm)	pH	
1	0.5	2063.33	5.83	7.82	1

Table 9. Optimum formula verification test

Response	Prediction Value	95% PI Low	Experimental Value	95% PI High
Viscosity	2063.33	1507.51	1816.67±28.86	2619.16
Spreadability	5.83	3.52	5.8±0.35	8.13
pH	7.82	7.67	7.82±0.07	7.97

Table 10. Results of collagenase enzyme activity inhibition test

Readings (minutes)	Concentration	Average % Inhibition	Linear Regression Equation	IC ₅₀ (ppm)
5	2.00	89.17	y = 0.0219x + 48.391	73.47
	1.00	73.20		
	500	63.83		
	250	58.62		
	125	52.64		
10	62.50	38.96	y = 0.0217x + 48.475	70.27
	2.00	88.83		
	1.00	73.05		
	500	64.91		
	250	57.96		
15	125	52.31	y = 0.0217x + 48.523	68.06
	62.50	39.27		
	2.00	88.26		
	1.00	73.77		
	500	65.64		
	250	60.07		
	125	52.06		
	62.50	36.90		

Verification of the optimum formulation was carried out by comparing the experimental response values with the 95% Prediction Interval (PI) generated by the SLD model. The SLD method provided predictive values for each formulation response. Therefore, confirmation testing was carried out by reformulating the optimum composition and re-evaluating the viscosity, spreadability, and pH parameters. The experimental results showed that all response values were within the PI range and were consistent with the model predictions. The only minor deviations observed were within statistically acceptable limits.

The agreement between the predicted and experimental values showed that the SLD model had strong predictive accuracy. Similarly, the optimum formulation met the established physical criteria for each parameter [66]. Based on these confirmation results, the optimization process was considered valid, and the SLD model was shown to be effective in reliably predicting the performance of the serum formulation.

Anti-collagenase activity

The inhibitory activity of the serum formulation containing 0.025% *A. clypearia* leaf extract against

collagenase was evaluated. The IC₅₀ values obtained at 5, 10, and 15 minutes were 73.47 ppm, 70.27 ppm, and 68.06 ppm, respectively. These values indicate a modest time-dependent increase in inhibitory potency and remained within the “strong” inhibition category. According to the classification described by [67], collagenase inhibitory activity was considered very strong, strong, moderate, and weak at IC₅₀ <50 µg/mL, 50–100 µg/mL, 101–150 µg/mL, and >150 µg/mL, respectively.

Collagenase was a member of the matrix metalloproteinase (MMP) family, enzymes responsible for collagen degradation and the breakdown of extracellular matrix components. Elevated collagenase activity was strongly associated with skin aging, particularly following UV exposure, making it a key biomarker in anti-aging studies [68]. The collagenase inhibitory activity observed in the *A. clypearia* serum formulation was attributed to the presence of secondary metabolites (phenolic and flavonoid compounds), which exerted antioxidant effects and provided synergistic inhibition of collagenase. These compounds contained phenolic rings with hydroxyl substituents capable of scavenging ROS, reducing metal ions, modulating

protein phosphorylation pathways associated with enzyme inhibition, and preventing lipid peroxidation [67]. Excessive collagen hydrolysis led to structural deterioration of the skin and the formation of wrinkles. Therefore, collagenase inhibitors played an important role in maintaining skin integrity and reducing visible signs of aging, serving as valuable components in anti-aging formulations [69].

The inhibitor control, 1,10-phenanthroline, was included to verify assay performance; however, the study did not report a direct statistical potency comparison between the serum and the inhibitor control or other plant extracts. Phenolic and flavonoid compounds are plausible contributors, while possible contributions from alkaloids and terpenoids cannot be excluded because the phytochemical screening was qualitative. The time-dependent change may reflect progressive interaction with the enzyme–substrate system, but enzyme-binding kinetics were not directly investigated.

Study limitations

The SPF assay did not include a standard sunscreen comparator and did not apply inferential pairwise comparisons among the extract and fractions. Although the anti-collagenase assay included 1,10-phenanthroline as an inhibitor control, direct comparative potency was not statistically evaluated. In addition, the optimized pH remained above physiological skin pH, and the study was limited to in vitro activity and physical evaluation without long-term stability, irritation, toxicity, skin-penetration, or in vivo validation.

Conclusion

In conclusion, this study indicates that *A. clypearia* has photoprotective and anti-aging potential. The ethyl acetate fraction produced the highest in vitro SPF value among the tested samples, while SLD identified 1% xanthan gum and 0.5% triethanolamine as the optimum composition within the evaluated design space. The optimized serum showed acceptable viscosity and spreadability and strong collagenase inhibitory activity, but its pH of 7.82 remained above physiological skin pH. Therefore, the findings support *A. clypearia* as a candidate natural cosmetic active ingredient rather than a formulation ready for direct consumer use. Further research should include a standard sunscreen comparator, expanded statistical validation, pH adjustment, stability testing, quantitative phytochemical analysis, in vivo SPF assessment, skin-irritation and toxicity studies, and ultimately well-designed human studies before cosmetic or dermatological application.

Acknowledgement

The authors are grateful to the Directorate of Research

and Community Service, Ministry of Education, Culture, Research and Technology of Indonesia, for funding this study through the Regular Fundamental Research Grant under contract number 113/E5/PG.02.00.PL/2024 dated 11 June 2024.

References

1. Sulistiyowati, A., Yushardi, Y. and Sudarti, S. (2022). Variation in sunscreen sun protection factor (SPF) effectiveness against ultraviolet exposure based on Indonesia's climatic conditions. *Journal of Health Sciences*, 12(3): 261–269.
2. Elsi, Y., Satriadi, T. and Istikowati, T.W. (2020). Ethnobotany of medicinal plants used by the Dayak Meratus indigenous community in Ulang Village, South Hulu Sungai, Kalimantan. *Journal of Sylva Science*, 03(1): 193–201.
3. Partomihardjo, T., Hermawan, E. and Pradana, E.W. (2020). *Peat Swamp Forest Plants of Merang Kepayang*. ZSL Indonesia.
4. Noviantina, E., Linda, R. and Wardoyo, E.R.P. (2018). Ethnobotanical study of natural cosmetic plants of the Dayak Kanayatn community in Sebatih Village. *Protobiont*, 7(1): 61–68.
5. Yuniarsih, N., Indriyati, A. and Munjiani, A. (2021). Review: Herbal face masks in Indonesia. *Jurnal Buana Farma*, 1(1): 17–21.
6. Pratiwi, M.W., Wijaya, T.H., Sumayyah, S. and Kurniawan, D.W. (2023). Narrative review: Herbal nanospray as anti-aging. *Majalah Farmasetika*, 8(3): 267.
7. Rafique, M. and Hussain Shah, S.N. (2019). Anti-ageing potential of a cream (W/O emulsion) containing grape seed extract: formulation and in vivo evaluation. *Journal of Clinical & Experimental Dermatology Research*, 10(4): 1–9.
8. Zofia, N.Ł., Martyna, Z.D., Aleksandra, Z. and Tomasz, B. (2020). Comparison of the antiaging and protective properties of Apiaceae plants. *Oxidative Medicine and Cellular Longevity*, 2020: 1–16.
9. Zorina, A., Zorin, V., Kudlay, D. and Kopnin, P. (2022). Molecular mechanisms of dermal extracellular matrix homeostasis alterations induced by ultraviolet radiation and aging. *International Journal of Molecular Sciences*, 23(12): 1–16.
10. Carrillo-Norte, J.A. et al. (2024). Anti-aging effects of low-molecular-weight collagen peptide supplementation. *Cosmetics*, 11(4): 137.
11. Marbun, F.K., Tarigan, S.B. and Sudarti, S. (2023). Analysis of benefits and impacts of UV radiation on human health. *Jurnal Penelitian Inovatif*, 3(3): 605–612.
12. Adzhani, A., Darusman, F. and Aryani, R. (2022). Study on ultraviolet radiation effects on

- skin. In *Bandung Conference Series: Pharmacy*, pp. 106–112.
13. Ayuhecaria, N., Nugroho, W. and Aryzki, S. (2022). SPF determination and physicochemical characterization of Dayak Nanoherbal powder. *Journal of Pharmaceutical Care Science*, 3(1): 140–146.
14. Anggreini, D., Saputri, M. and Sari, N. (2024). Understanding SPF value in cosmetics. *Jurnal Pengabdian Masyarakat Tjut Nyak Dhien*, 3(1): 33–38.
15. He, H. H., Li, A., Li, S., Tang, J., Li, L., and Xiong, L. (2021). Natural components in sunscreens. *Biomedicine & Pharmacotherapy*, 134: 111161.
16. Ji, W., Li, Z., Gu, L., Zou, X., Wu, J., Zhang, S., & Deng, H. (2022). Composition of Archidendron clypearia across harvest periods. *SSRN Electronic Journal*, 418: 6533.
17. Ji, W. (2023). Identification of antibacterial and antidiabetic compounds of Archidendron clypearia. *Molecules*, 28(3): 1–18.
18. Risman, M. (2022). In vitro and in vivo sunscreen activity of A. clypearia ethyl acetate fraction. *Jurnal Penelitian Farmasi Indonesia*, 11(1): 32–38.
19. Hidayah, R. and Nurrosyidah, H.L. (2023). Formulation and stability of grape seed oil serum. *Journal of Islamic Pharmacy*, 8(1): 34–38.
20. Sawiji, R.T. et al. (2024). Effect of gelling agent variation on retinoic acid serum. *Acta Holistica Pharmaceutica*, 6(1): 1–10.
21. Aprilia, C., Faisal, M. and Prasetya, F. (2022). Formulation and optimization of xanthan gum serum. In *Mulawarman Pharmaceuticals Conferences*, pp. 30–34.
22. Suleman, A.W., Wahyuningsih, S., and Puspitasari, Y. (2023). Formulation of antioxidant serum from *Syzygium polyanthum* extract. *Pharmamedica Journal*, 8(2): 235–243.
23. Tsabitah, A. F., Zulkarnain, A. K., Wahyuningsih, M. S. H., and Nugrahaningsih, D. A. A. (2020). Optimization of carbomer, propylene glycol, and TEA in gel formulation. *Majalah Farmaseutik*, 16(2): 111–118.
24. Febriani, Y., Lubis, S. H., and Annisa, F. (2022). Formulation of antioxidant serum from red betel leaf extract. *Journal of Pharmaceutical Science*, 5(1): 120–127.
25. Purwanto, A., Amrina, F. and Riauwati, R. (2024). Optimization of *Euphorbia hirta* cream using SLD. *Jurnal Insan Farmasi Indonesia*, 7(2): 53–65.
26. Ekawati, A.R., Supriningrum, R. and Handayani, F. (2023). Characterization of ethanol extract of *Tabernaemontana macrocarpa* leaf. *Journal of Pharmaceutical Sciences and Clinical Pharmacy*, 20(1): 43–52.
27. Qomaliyah, E.N., Indriani, N., Rohma, A. and Islamiyati, R. (2023). Phytochemical screening, total flavonoid content, and antioxidant activity of *Kalanchoe pinnata* leaf. *Current Biochemistry*, 10(1): 1–10.
28. Nasution, A.W., Nasution, H.M., Lubis, M.S. and Rahayu, Y.P. (2023). Antibacterial activity of *Etlingera elatior* leaf fractions against *Staphylococcus aureus* and *Escherichia coli*. *Journal of Pharmaceutical Science*, 6(4): 1488–1497.
29. Lestari, P., Yusuf, F., Fahdi, F., Tinggi, S. and Kesehatan, I. (2024). Phytochemical screening of *Picria fel-terrae* leaf using graded maceration. *BEST Journal*, 7(1): 2249–2255.
30. Kusumo, D.W., Susanti, Ningrum, E.K. and Makayasa, C.H.A. (2022). Phytochemical screening of secondary metabolites in *Carica papaya* flower ethanol extract. *Journal of Current Pharmaceutical Science*, 5(2): 2598–2095.
31. Aini, S.H., Sabrina, N., Soleha, R. and Lestari, S.M. (2025). Determination of SPF and total phenolic content of *Pometia pinnata* leaf extracts. *Journal of Jamu Indonesia*, 10(2): 55–61.
32. Arifin, B., Nasution, R., Savila, S., Ramadani, R., Helwati, H., Marianne, M., ... and Saidi, N. (2020). Sunscreen activities of *Artocarpus heterophyllus* bark in lotion formula. *Open Access Macedonian Journal of Medical Sciences*, 8(A): 461–467.
33. Rowe, R.C., Sheskey, P.J. and Quinn, M.E. (2009). *Handbook of Pharmaceutical Excipients*. 6th ed. Pharmaceutical Press, American Pharmacists Association.
34. Iskandar, B., Putri, R., Wei, C. P., and Renovita, F. (2021). Formulation and antibacterial evaluation of anti-acne serum containing *Ocimum africanum* leaf extract. *Jurnal Buana Farma*, 1(4): 14–20.
35. Yuliani, F., Alfiah, F.B. and Handayani, K.R. (2024). Formulation and physical evaluation of *Zingiber cassumunar* rhizome emulgel. *FARMASAINKES Journal*, 4(1): 106–115.
36. Fitria, N. and Ratu, A.P. (2022). Characteristics and stability of *Muntingia calabura* fruit extract serum. *Pharmamedica Journal*, 7(1): 17–27.
37. Hikmah, F.N., Malahayati, S. and Nugraha, D.F. (2023). Formulation and evaluation of jasmine flower serum gel. *Journal of Pharmaceutical Care Science*, 3(2): 93–108.
38. Safaruddin et al. (2024). Formulation and stability study of banana peel extract serum. *Inhealth Indonesian Health Journal*, 3(2): 172–182.

39. Setiawan, P.A., Rahmawanty, D. and Sari, D.I. (2023). Formulation of cassava leaf extract serum. *Jurnal Pharmascience*, 10(2): 394–404.
40. Fauzah, Noval and Rohama. (2024). Formulation of pumpkin extract serum using xanthan gum. *Jurnal Surya Medika*, 10(1): 277–287.
41. Murdiana, H.E., Rahmavika, T. and Rawar, E.A. (2023). Antioxidant serum from lime essential oil using vitamin E. *Pharmamedica Journal*, 8(2): 209–219.
42. Fikry, E. et al. (2023). Dermato-cosmeceutical properties of *Pseudobombax ellipticum*. *Saudi Pharmaceutical Journal*, 31(10): 1778.
43. Ananta, M.N.F. et al. (2024). Phytochemical screening of *Trigona* sp. propolis extracts. *Sasambo Journal of Pharmacy*, 5(1): 38–45.
44. Li, D. et al. (2024). Genotype characterization of *Archidendron clypearia* using microsatellite markers. *Forests*, 15(7): 1168.
45. Riasari, H. et al. (2019). Extraction methods and antioxidant activity of *Archidendron bubalinum* seeds. *Pharmacognosy Journal*, 11(6): 1278–1284.
46. Riasari, H., Wibowo, D.P. and Fauzi, N.I. (2022). Hypoglycemic potential of *A. bubalinum* leaf extract. *International Journal of Applied Pharmaceutics*, 14(5): 50–55.
47. Amin, A. and Park, S. (2025). Chemotaxonomy as an efficient tool for medicinal plant identification. *Plants*, 14(14): 2234.
48. Nigel, E.E.A. et al. (2023). SPF of Malaysian fruit and vegetable extracts. *Malaysian Journal of Chemistry*, 25(2): 87–97.
49. Wardatun, S., Mahyuni, S. and Setiawan, P. (2025). Sunscreen activity of papaya leaf extract fractions. *Indonesian Journal of Pharmacy*, 5(2): 422–431.
50. Triyasmono, L. et al. (2025). UV–Vis and chemometric SPF prediction of *Acacia* leaf extracts. *Tropical Journal of Natural Product Research*, 9(8): 3854–3859.
51. Torres-Contreras, A. M. et al. (2022). Plant secondary metabolites against skin photodamage: Mexican plants, a potential source of UV-radiation protectant molecules. *Plants*, 11(2), 220.
52. Khaira, Z., Monica, E. and Yoedistira, C.D. (2022). Formulation and physical quality of melinjo seed microemulsion serum. *Sainsbertek Journal*, 3(1): 299–309.
53. Ristianti, V., Monica, E. and Aziz, N. (2023). Formulation of an anti-acne serum with citrus essential oil. *Sainsbertek Journal*, 4(1): 58–65.
54. Aprilianti, N., Hajrah, H. and Sastyarina, Y. (2020). Optimization of PVA as a gel base. In *Mulawarman Pharmaceuticals Conferences*, pp. 17–21.
55. Aisyah, M.N., Purnomo, Y. and Widyaningrum, I. (2022). Role of xanthan gum on physicochemical properties of oleanolic acid gel. *Journal of Complementary Medicine Biology*, 9(2): 1–5.
56. Widyartha, G.N.A.Z. et al. (2020). Simplex lattice design for wound dressing gel. *Acta Holistica Pharmaceutica*, 2(2): 28–36.
57. Handayani, R., Qamariah, N. and Maretania, J. (2023). Serum formulation from *Dioscorea bulbifera* tubers. *Jurnal Farmasetis*, 12(2): 227–236.
58. Ratnasari, N., Puspariki, J. and Farhan. (2023). Physical stability of cucumber extract serum. *Journal of Holistic Health Science*, 7(1): 9–16.
59. Agustiani, F.R.T., Sjahid, L.R. and Nursal, F.K. (2022). Literature review: role of polymers as gelling agents. *Majalah Farmasetika*, 7(4): 270–287.
60. Nugrahaeni, F., Srifiana, Y. and Rokhman, A.N. (2021). Effect of xanthan gum concentration on secang hair dye gel. *Indonesian Natural Research Pharmacy Journal*, 6(2): 29–42.
61. Lukić, M., Pantelić, I. and Savić, S.D. (2021). Optimal pH for skin and topical formulations. *Cosmetics*, 8(3): 69.
62. Widyaningrum, N. et al. (2025). Optimization and antioxidant activity of clay masks. *Pharmaceutical Sciences and Research*, 12(1): 33–40.
63. Pratiwi, P.D. and Arnas, D.L. (2024). Simplex lattice optimization of emulsifier in orange peel oil cream. *Journal of Clinical Pharmacy and Natural Products*, 4(2): 85–93.
64. Dewi, I.K. et al. (2021). Optimization of ethyl acetate fraction gel from corn cobs. *Jurnal Jamu Kusuma*, 1(2): 57–66.
65. Bolton, S. and Bon, C. (2003). *Pharmaceutical Statistics: Practical and Clinical Applications*. 4th edition. CRC Press.
66. Wiyono, A.E. et al. (2023). Optimization of CMC-Na and glycerin in tobacco hand sanitizer gel. *International Journal of Food, Agriculture and Natural Resources*, 4(1): 10–17.
67. Tanur, E. et al. (2020). FRAP and collagenase inhibition of pineapple core extract. *American Scientific Research Journal for Engineering, Technology, and Sciences*, 70(1): 99–105.
68. Aji, A.P., Nurulita, N.A. and Diniatik. (2021). Antiaging activity of purified *Azolla microphylla* leaf serum. In *Prosiding SainTeKes*, pp. 159–168.
69. Piyasena, K.G.N.P.P. et al. (2024). Elastase, collagenase, and tyrosinase inhibition in tea cultivars. *Discovery Chemistry*, 1: 36.