

FORMULATION OF SERUM GEL CONTAINING *ANGELICA KEISKEI* LEAF EXTRACT AS AN ANTIOXIDANT AND TYROSINASE ENZYME INHIBITOR

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ABSTRACT

Objective: The aim of this study was to formulate and evaluate the efficacy of *Angelica keiskei* (AK) leaf extract in serum gel as an antioxidant and tyrosinase enzyme inhibitor.

Methods: The leaves of AK were extracted using the maceration method, while the antioxidant and tyrosinase enzyme inhibitory activities were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and L-Tyrosine as assay and substrate, respectively. Subsequently, the extract was formulated into serum gel and evaluated for physical stability, antioxidant and inhibitory activity to tyrosinase enzyme.

Results: The results showed that AK leaf extract has antioxidant activity, with an IC₅₀ value = 7.73 µg/ml, while the tyrosinase enzyme inhibitory power had an IC₅₀ value = 500 µg/ml. Based on physical stability, the serum gel was stable after 28 d of storage. The serum gel of AK leaf extract also showed antioxidant activity and tyrosinase enzyme inhibition with IC₅₀ value of 16.68 µg/ml and 741.62 µg/ml, respectively.

Conclusion: This study demonstrated that the serum gel of AK leaf extract has potential as a skin lightening agent and is safe for use as a topical preparation.

Keywords: *Angelica keiskei* extract, Serum gel, Antioxidant, Tyrosinase enzyme inhibitor

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INTRODUCTION

Generally, the skin as an organ protects the body from harmful effects and damage from the external environment, such as ultraviolet (UV) rays since their effect causes faster skin aging and sunburn [1, 2]. However, the use of *Angelica keiskei* (AK) leaf extracts as antioxidants and photoprotection help in preventing the effects of UV rays. This leaf contains a pigment known as melanin, which acts as a defence mechanism against harmful factors [3]. It also affects abnormal pigmentation and melanoma, such as hyperpigmentation. Also, the overproduction and accumulation of this pigment could be a serious aesthetic problem of the skin resulting in melasma or age spots. To synthesize this substance, the body requires tyrosinase enzyme to mediate the conversion of tyrosine to dihydroxyphenylalanine (DOPA), which converts to dopaquinone and eventually melanin. Inhibiting tyrosinase enzyme activity becomes an important step to controlling its biosynthesis, which helps in preventing hyperpigmentation disorders [4, 5].

In addition, there are various topical products used as antioxidants and tyrosinase enzyme inhibitor, which are either from natural sources or synthesized. Some of these are prepared during cosmetic production, and the use of the synthetic types in high doses causes harmful side effects [1, 2, 4]. However, AK occurs naturally and could serve as an alternative to these synthetic products.

This AK is a native plant from Japan. The dark green leaves of this plant have been recognized as a medicinally important herb in Asia. Previous studies have shown that the ethanol extract of AK is an excellent antioxidant, which also inhibits melanin production with low cytotoxic effects [1, 6]. One of the group of compounds contained in AK which have many biological activities including being an antioxidant and a melanin inhibitor are chalcones. Also, these two activities are attributed to the two aromatic rings with an unsaturated chain contained in these compounds [7].

Furthermore, the serum gel is the popular topical dosage form used to enhance the quality of the skin because it acts as a moisturizer, thereby enhancing the effects of these compounds in the epithelium [8].

Therefore, a serum gel of AK leaf extract was prepared and evaluated for antioxidant and tyrosinase enzyme inhibitory activities.

MATERIALS AND METHODS

Materials

Plant material

The leaf of AK was collected from Lombok, West Nusa Tenggara and was authenticated by School of Life Sciences and Technology, Bandung Institute of Technology (ITB) No. 401/I1.C02.2/PL/2019.

Chemicals

The tyrosinase enzyme, L-Tyrosine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, Quercetin and Vitamin C were purchased from Sigma Aldrich. All other chemicals were of pharmaceutical and technical grades.

Methods

Preparation of plant extracts

The leaves of AK were dried at room temperature and ground in a mortar. Two hundred g of AK leaves powder were extracted in 2000 ml 96% ethanol using maceration method for three x 24 h at room temperature. The ethanol was removed by a rotary evaporator, IKA Company, Germany (IKA RV 10) at 40-50 °C to obtain the crude extract [9].

Phytochemical screening extract

Phytochemical screening was carried out to determine the presence of secondary metabolites in AK leaf extract, including alkaloids, flavonoids, tannins, polyphenols, quinones, steroids/triterpenoids, saponins, monoterpenes and sesquiterpenes [9].

Total phenols determination

0.5 ml AK leaf extract was dissolved in methanol (770 PPM) and mixed with aqueous Na₂CO₃ (4 ml, 1 M) and Folin Ciocalteu reagent

(5 ml, 1:10 in distilled water), the mixture was then incubated for 15 min. The total phenols were evaluated by UV/visible rays at 755 nm. The curve of the standard was prepared using gallic acid solutions with various concentrations. The values of total phenol were expressed in terms of *gallic acid* equivalent as the reference compound [10].

Total flavonoids determination

0.5 ml AK leaf extracts (500 mg/ml) in methanol were mixed with 1.5 ml of methanol, 0.1 ml of 1 M potassium acetate, 0.1 ml of 10% aluminium chloride and 2.8 ml of distilled water. This was then incubated for 15 min. The absorbance of the mixture was measured at 410 nm. The curve of the standard was prepared using quercetin solutions with various concentrations in methanol. Total flavonoids values were expressed in terms of *quercetin* equivalent, as the reference compound [11].

Antioxidant activity test of AK leaf extract

The solution of DPPH (50 ppm) was prepared using methanol as a solvent. The sample solution and DPPH (1:1) were mixed and the mixed operating time was determined. The sample solution was prepared with various concentrations and mixed with the solution of DPPH (1:1), stored during an operating time and then measured for absorbance at λ_{max} . The absorbance value was calculated for the value of % inhibition by the following equation:

$$\% \text{ Inhibition} = [1 - (A_{\text{sample}}/A_{\text{DPPH}})] \times 100$$

Where, % Inhibition is the percentage capacity of the free radical inhibition, A_{sample} is absorbance of the sample at 515 nm, and A_{DPPH} is the absorbance of DPPH solution at 515 nm. Linear regression curve

between % inhibition and sample concentration were made, the linear equation and IC_{50} were obtained [2].

In vitro testing of tyrosinase enzyme inhibitory activity

Extracts were dissolved in phosphate buffer (pH 6.5) containing 1 % of dimethyl sulfoxide (DMSO) to obtain solution concentrations of 500 $\mu\text{g}/\text{ml}$. A total of 70 μl of the extract solution of each dilution was added to 30 μl of the enzyme tyrosinase (Sigma, 333 units/ml in phosphate buffer). Plates were incubated for 5 min at room temperature, to which were added 110 μl of substrate 1 mmol L-tyrosine and incubated for 30 min at room temperature. The solution in each well was measured using a microwell plate reader at a wavelength of 492 nm to determine the percent inhibition and the value of 50% inhibitory concentration (IC_{50}). The percentage of tyrosinase activity inhibition was calculated as inhibition % = $[(A - B)/A] \times 100$, where A is the absorbance of the control at 492 nm and B is the absorbance of the samples at 492 nm. Kojic acid was used as a positive control [5].

Formulation of serum gel from AK leaf extract

The serum gel consisting of AK leaf extract and all other ingredients were prepared according to the formula presented in table 1. Hydroxyethylcellulose (HEC) was dispersed into distilled water (50 °C) with constant stirring using a mechanical stirrer. The Extract and all ingredients were dissolved in distilled water before adding to the HEC solution. The mixtures were stirred until homogeneity was achieved. The physical stability of serum gel containing AK leaf extract was evaluated for organoleptic property, homogeneity, pH, viscosity, and spreadability, after 28 d of storage [12].

Table 1: Formulation of serum gel containing AK leaf extract

Ingredients	Composition of serum gel (%)
AK leaf extract	0.5
HEC	0.5
Propylene glycol	10
DMSO	2
Methylparaben	0.3
Distilled water added	100

Statistical analysis

The data of the experiment were analyzed by the one-way analysis of variance (ANOVA) at the level of ($P < 0.05$) and all data results were measured as a mean of samples \pm standard deviation (SD).

RESULTS AND DISCUSSION

The results obtained at School of Life Sciences and Technology, Bandung Institute of Technology (ITB) demonstrated *Angelica keiskei* as the specie used in this study. 96 % ethanol was used as a solvent due to its universal dissolution characteristics for both polar and non-polar compounds, producing a percent recovery of 21.83% [13]. In the extraction process, maceration method was used to protect compounds contained in AK leaf, which are responsible for the inhibition of tyrosinase enzyme and for the antioxidant activity [4].

The phytochemical screening was carried out to evaluate the presence of secondary metabolites in the AK leaf extract. Alkaloids, flavonoids, polyphenols, quinones, tannins, steroids and triterpenoids were

detected. This result supports the assumption that tyrosinase enzyme inhibitory compounds and antioxidant compounds exist in AK leaf extracts, as flavonoids and phenols [14, 15].

Flavonoids have been recognized as an antioxidant agent and their effects are considerable on human nutrition and health. Flavonoids are known to exert their action by a scavenging or chelating process. Phenolic compounds also are antioxidant agents that act as free radical terminators [16]. The total contents of flavonoids from AK leaf extract in terms of quercetin equivalent was 3.532 g QE/100g, while the phenol contents of AK leaf in terms of gallic acid equivalent was 2.367 g GAE/100 g. According to our study, the high contents of flavonoids and phenol in AK leaf extract is responsible for its high radical scavenging activity [10]. This result further confirms that AK leaf extract has potential activity as a tyrosinase enzyme inhibitor.

DPPH method is one of the common methods used to determine the antioxidant activity for preliminary testing of extracts [2]. The result of antioxidant activity can be seen in table 2.

Table 2: The result of antioxidant activity from AK leaf extract

Sample	IC_{50} ($\mu\text{g}/\text{ml}$)
AK leaf extract	7.73
Kojic Acid	3.97

The antioxidant transfers the hydrogen content to DPPH, which is a free radical. When the free radicals are scavenged by antioxidants, the color of DPPH will be changed from purple to yellow. The observed IC_{50} value showed that AK leaf extract exhibits strong antioxidant activity with IC_{50} value below 10 $\mu\text{g}/\text{ml}$. The IC_{50} of AK leaf extracts is almost similar with kojic

acid as a standard substance with IC_{50} of 3.97 $\mu\text{g}/\text{ml}$. The strong antioxidant activity of the extract could be attributed to its high levels of total flavonoids and polyphenols. Having OH at C-3, C-4' oxo, double bond at C-2 and C-3 and ortho di OH at C-3'-C4', indicated that the flavonoids have very high antioxidant activity [17].

To determine the inhibitory activity of tyrosinase in the extract, the AK leaf extract were tested and compared with kojic acid as a control. The IC₅₀ values were evaluated by the dose-response curves

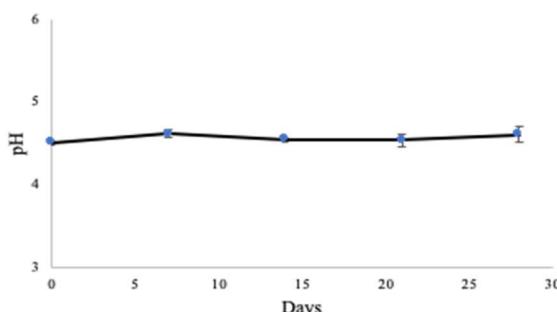
through serial dilution of the AK leaf extract and kojic acid at various concentrations. The inhibition of tyrosinase enzyme by AK leaf extract can be seen in table 3.

Table 2: The results of tyrosinase enzyme inhibitory activity of AK leaf extract

Sample	IC ₅₀ (μg/ml)
AK leaf extract	500
Kojic Acid	573

The results indicated that the ethanolic extract of AK leaf shows weak activity for tyrosinase enzyme inhibition. The previous article reported that the isoprenoid-substituted moiety contained in AK leaf extract can enhance the ability of flavonoids to inhibit melanin biosynthesis with less cytotoxicity [18]. The article also stated that xanthoangelol and 4-hydroxyderricin contained in AK leaf extract show tyrosinase enzyme inhibitory activity with IC₅₀ values of 15.87±1.21 μM and 60.14±2.29 μM, respectively [19]. Though the results in this study show weak activity, the extracts of AK leaf still have potential as tyrosinase inhibitors and the advantage of the low cytotoxicity makes it safer for topical usage in humans.

The gel was prepared with AK leaf extract because of the advantages it profers. This includes being easy to use, having high water content and reduced contact time, all of which contribute to the reduction of the risk of inflammation. HEC is a polymer that has been used in the gel formulation due to its biosafety, efficacy, and stability [20]. Propylene glycol was used as a moisturizer and enhancer of gel, which could improve the solubility and diffusion of the active substance contained in AK through the skin [21]. Methylparaben was used as a preservative to prevent the gel damage caused by bacteria [22]. DMSO has been known as a permeation enhancer of hydrophilic and hydrophobic drugs. The properties of DMSO can make it an interesting excipient in topical dosage form due to its enhancement of drug permeability [23]. The serum gel formulation has a smooth texture that is homogeneous and characteristic of AK leaf extracts. The homogeneity of serum gel characteristics remained similar after 28 d of storage. The pH of serum gel containing AK leaf extract can be seen in the fig. 1.



**Fig. 1: pH of serum gel containing *Angelica keiskei* leaf extract
(The data were calculated as mean±SD; n=3)**

Fig. 1 indicates that the pH of serum gel containing AK leaf extract did not change significantly after 28 d storage and remained within the acceptable pH for topical preparations, which is between 4.5–6.5 [4].

The viscosity of the serum gel containing AK leaf extract can be seen in the fig. 2.

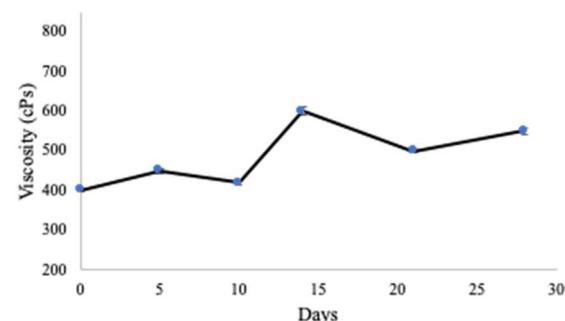


Fig. 2: Viscosity of serum gel containing AK leaf extract (The data were calculated as mean±SD; n=3)

Fig. 2 indicates that the viscosity of serum gel containing AK leaf extract did not change significantly after 28 d storage and remained within the acceptable viscosity for serum gel. The acceptable viscosity value of serum gel is in the range of 230-1150 cPs.

Spreadability is one parameter that can affect the efficacy of serum gel; hence, the evaluation of spreadability is important in topical dosage forms [24-26]. The Spreadability test result of serum gel containing AK leaf extract can be seen in the fig. 3.

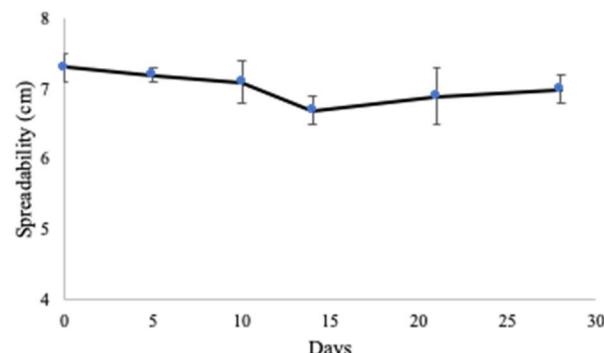


Fig. 3: The result of Spreadability of serum gel containing AK leaf extracts (The data were calculated as mean±SD; n=3)

Fig. 3 indicates that the spreadability of serum gel containing AK leaf extracts did not change significantly after 28 d storage and remained within the acceptable range of 5-7 cm [12, 27].

The result of tyrosinase enzyme inhibitory activity and antioxidant activity of serum gel containing AK leaf extract are shown in table 4.

Table 4: The result of tyrosinase enzyme inhibitory activity and antioxidant activity of serum gel containing AK leaf extract

Sample	IC ₅₀ of tyrosinase enzyme inhibitory activity (μg/ml)	IC ₅₀ of antioxidant activity (μg/ml)
Serum gel containing AK leaf extract	741.62	16.68
Serum gel containing Kojic Acid	689.12	12.20

The serum gel of AK leaf extract also showed antioxidant activity and tyrosinase enzyme inhibition with IC_{50} value = 16.68 $\mu\text{g/ml}$ and 741.62 $\mu\text{g/ml}$, respectively. This result is almost similar with that obtained with the serum gel of kojic acid as a positive control with an IC_{50} value = 12.20 $\mu\text{g/ml}$ for antioxidant activity and IC_{50} value = 689.12 $\mu\text{g/ml}$ for tyrosinase enzyme inhibitory activity. This indicated that the serum gel containing AK leaf extract has high levels of total flavonoids and polyphenols, which have strong antioxidant activity and tyrosinase enzyme inhibitory activity [10].

CONCLUSION

In this research, the AK leaf extract was found to have antioxidant activity with an IC_{50} value = 7.73 $\mu\text{g/ml}$, while the activity of AK leaf extract against the tyrosinase enzyme showed inhibition with an IC_{50} value = 500 $\mu\text{g/ml}$. This result is almost similar with kojic acid as a positive control with an IC_{50} value = 3.97 $\mu\text{g/ml}$ for antioxidant activity and IC_{50} value = 573 $\mu\text{g/ml}$ for tyrosinase enzyme inhibitory activity. The serum gel of AK leaf extract also showed antioxidant activity and tyrosinase enzyme inhibition with IC_{50} value = 16.68 $\mu\text{g/ml}$ and 741.62 $\mu\text{g/ml}$, respectively. This indicated that serum gel containing AK leaf extract still has high levels of total flavonoids and polyphenols with strong antioxidant activity and tyrosinase enzyme inhibitory activity. Based on physical stability, the serum gel of AK leaf extract was stable after 28-day storage. It can be concluded that the serum gel of AK leaf extract has potential as a skin lightening preparation and is safe to be used as a topical preparation.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

None to declare

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