Aging effect of *Aloe vera* (L.) Burm. f. on tyrosinase inhibitory and antioxidant activities

Benjamat Chailap and Thanesuan Nuanyai*

Department of General Education, Faculty of Liberal Arts, Rajamangala University of Technology Rattanakosin Wang Klai Kangwon Campus, Prachuap Khiri Khan 77110

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Abstract

Aloe vera (L.) Burm. f. (*Aloe barbadensis* Mill.) extract is an essential ingredient in a wide variety of cosmetic products. This research compares the antioxidant activity and tyrosinase inhibitors of *A. vera* extracted from leaves at different stages. The results could be applied in arranging *A. vera* leaf into grades for the cosmetic field. In this study, three stages of *A. vera* leaf: A1 (4-6 months), A2 (7-9 months), and A3 (10-12 months) were divided. In extraction processes, *A. vera* leaf samples were peeled and rinsed with deionized water. After *A. vera* gel was blended thoroughly and freeze-dried, *A. vera* powder was obtained. The results showed that the water content of *A. vera* leaf at A1, A2, and A3 were 98.00 ± 0.02%, 97.10 ± 0.02%, and 96.00 ± 0.04%, respectively. *A. vera* powder was extracted with 80% v/v methanol and dried by vacuum rotary evaporator. The extract yields of all three stages (A1-A3) were 24.97 ± 0.28%, 35.27 ± 0.70%, and 32.20 ± 0.29%, respectively. The antioxidant activity of methanol crude extracts was examined by using DPPH and ABTS techniques with Trolox as the standard. The samples (A1-A3) and standard Trolox showed the antioxidant activity against the DPPH radical in terms of percent inhibition at 41.76 ± 0.57%, 40.72 ± 2.31%, 37.01 ± 2.22%, and 49.90 ± 1.74%, respectively. The samples (A1-A3) and standard Trolox showed the antioxidant activity against the ABTS•+ radical in term of percent inhibition at 13.65 ± 3.44%, 53.02 ± 1.16%, 13.85 ± 1.08%, and 74.88 ± 2.15%, respectively. Among *A. vera* extracts from all three stages, the A2 showed the highest antioxidant activity derived from ABTS technique. Tyrosinase inhibition of *A. vera* extracts (A1-A3) was investigated and compared with standardized kojic acid at the concentration of 50 ug/mL. We found that *A. vera* extracted from A2 showed the highest tyrosinase inhibition at 18.68 ± 0.99%, while *A. vera* A1, A2 stages, and the kojic acid showed tyrosinase inhibition at 17.93 ± 0.80%, 15.74 ± 1.17%, and 13.03 ± 0.96%, respectively. The results showed that gel extract from *A. vera* at age 7-9 months had an antioxidant activity and tyrosinase inhibition suitable for cosmetic application.

Keyword: *Aloe vera* (L.) Burm. f., Tyrosinase inhibitory, Antioxidant activities

Corresponding author: thanesuan.nua@rmutr.ac.th

Introduction

Natural products were compounds from plants having various chemical structures such as xanthone, terpenes, polyphenol, anthraquinone, and so on [1]. These biologically active compounds showed antioxidant properties, which can be applied to cosmetic ingredients. For example, *A. vera* (*Aloe barbadensis*) is one of the plants widely used in the food and cosmetic industries because its gel can be soluble in water. Soluble phenolic compounds contained in *A. vera* play an important role in antioxidant capacity [2]. In addition, bioactive compounds isolated from *A. vera* gel have shown effective inhibition of tyrosinase. Tyrosinase is an enzyme that creates melanin or pigments, causing dark spots on the face skin. Many studies have attempted to find natural substances that can inhibit tyrosinase enzymes and apply them as cosmetic ingredients [3-6].

A. vera is a plant belonging to the family Asphodelaceae. It grows in the dry regions of Africa, Asia, Europe, and America [7]. The biological activities of this plant were wound healing, antifungal activities, antidiabetic, anti-inflammatory, anticancer, laxative, and gastroprotective properties [8]. In addition, the leaf gel is used externally to treat skin burn, eczema, reduce inflammation, and so on [9]. Chemical constituents consisting of leaf gel are vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids [7]. The *A. vera* metabolites; glucose, fructose, alanine, valine, aspartic acid, 7-hydroxy-8-*O*-methylaloin, 7-hydroxyaloin A, 6′-malonylataloins A, and B changed to increase in different growth stages [10]. In agriculture, *A. vera* was harvested for use in the food industry. It was preferable to harvest large lower leaves and a maturity of 8-10 months because of the largest leaf size. Besides the food industry, *A. vera* is also used in the cosmetic industry because many bioactive ingredients from *A. vera* extract are suitable for applying to cosmetic products assisting in cosmetic efficiency.

From the previous report, total phenolics, flavonoids, flavonols, and antioxidant activities of gel extracts were influenced by the growth periods of *A. vera* [11]. Moreover, the chemometric analyses of freeze-dried *A. vera* gel on the UV adsorption potential and concentration of aloin depended on the growth stages of *A. vera* [12].

From the above data, studies on the biological activities of *A. vera* extracts have been extensively reported [1-12]. Usually, harvesting the lower leaf of *A. vera* is commonly used for cosmetic purposes because of the large size and high yielding of gel. However, the aging effect of *A. vera* on antioxidant activity and tyrosinase inhibition has not been widely studied. Therefore, this research aims to study the effect of *A. Vera* age on tyrosinase inhibitory and antioxidant activities. The results of this study could reveal suitable harvesting period of *A. vera* for applying in cosmetic ingredients.

Materials and Methods

Chemicals and Reagents

Methanol was purchased from RCI Labscan (Bangkok, Thailand). The 2,2'-azinobis (3-ethylbenzothaizoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), L-3,4-dihydroxyphenylalanine (L-DOPA), mushroom tyrosinase, kojic acid, and Phosphate buffer were purchased from Sigma (Singapore). All other chemicals used were of analytical grade.

Plant materials

The whole leaves of *A. vera (A. barbadensis)* were collected in January 2019 from Aloe vera Rai Mae Mali Kui Buri Community enterprise, Hat Kham Subdistrict, Kui Buri District, Prachuap Khiri Khan Province, Thailand. The voucher specimen of *A. vera* (Plant-006) was deposited at General Education Department (Science), Faculty of Liberal Arts, Rajamangala University of Technology Rattanakosin, Wang Klai Kangwon Campus, Nongkae Subdistrict, Huahin District, Prachuap Khiri Khan Province, Thailand. Samples were randomly sorted by weight and age, as shown in table 1.

Table 1 Difference in the sizes and ages of the *A. vera* samples

Sample	Age (month)	Weight (gram) per leaf
A1	4-6	200-350
A2	7-9	350-500
A3	10-12	> 500

Sample Preparation

The whole fresh leaves of *A. vera* were washed with deionized water three times. The leaves were cut into pieces, and the *A. vera* gel was collected after removing the outer rind. All *A. vera* gels were homogenized with a homogenizer. All samples were freeze-dried over two days and kept at -20°C before extraction.

Determination of water content

The water content of *A. vera* gel was investigated for comparison to the bioactive ingredients of extract. Fresh *A. vera* weighing approximately 10.0 grams, weighed before baking, bake at 80 °C, until the weight is stable. Using the formula to calculate as follows.

$$\text{Water Content (\%)} = (W_{dry}/W_{fresh}) \text{ X } 100\% \quad (1)$$

where W_{dry} = Weight of *A. vera* after oven

W_{fresh} = Weight of *A. vera* before oven

Extraction

Methanolic crude extract from *A. vera* gel powder was carried out by a method described previously, with slight modifications [3]. First, the freeze-dried *A. vera* gel powder (1.0 g) was extracted with 80 mL methanol (80% v/v) using a magnetic stirrer at 200 rpm for 4 hours. After extraction, the extract was centrifuged at 3000 rpm for 10 min, and the supernatant was concentrated by vacuum rotary evaporator at 40°C. Methanolic crude extract yields of the samples A1, A2, and A3 were 24.97 ± 0.28%, 35.27 ± 0.70%, and 32.20 ± 0.29%, respectively.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The antioxidant activity by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay was evaluated as previously reported, with a slight modification [13]. A reaction mixture containing 50 uL of *A. vera* crude

extract (2.0 mg/mL in methanol) or Trolox (10 ug/mL in methanol) with 150 uL of DPPH solution (0.1 mM in methanol) were added to a 96-well microplate. The reaction mixture was incubated in the dark at room temperature for 30 minutes and read at 517 nm. The results were revealed as a percentage of the DPPH as the following equation:

$$\text{Inhibition (\%)} = \{[A_{control} - (A_{test\ sample} - A_{blank})]/A_{control}\} \times 100\% \quad (2)$$

where $A_{control}$ = 150 uL DPPH + 50 uL Methanol

$A_{test\ samplel}$ = 150 uL DPPH + 50 uL Sample/standard

A_{Blank} = 150 uL Methanol + 50 uL Sample/standard

2,2'-azinobis (3-ethylbenzothaizoline-6-sulfonic acid) diammonium salt (ABTS) assay

The antioxidant capacity of 2,2'-azinobis (3-ethylbenzothaizoline-6-sulfonic acid) diammonium salt (ABTS) assay was evaluated as previously reported [13]. The stock solution of 7 mM ABTS (5 mL) was reacted with 2.45 mM potassium persulfate solution (88 uL). The reaction mixture was incubated at room temperature in the dark for 16 hours. Next, the solution of ABTS was diluted with ethanol until the absorbance reached 0.7 ± 0.02 at 734 nm. The 50 uL of *A. vera* crude extract (2.0 mg/mL) or Trolox (10 ug/mL) and 150 uL of ABTS diluted solution were added to a 96-well microplate. The reaction mixture was incubated in the dark at room temperature for 10 minutes. Then, the absorbance was measured at 734 nm using a microplate reader. The results were revealed as a percentage of the ABTS as the following equation:

$$\text{Inhibition (\%)} = \{[A_{control} - (A_{test\ sample} - A_{blank})]/A_{control}\} \times 100\% \quad (3)$$

where $A_{control}$ = 150 uL ABTS + 50 uL Ethanol

$A_{test\ samplel}$ = 150 uL ABTS + 50 uL Sample/standard

A_{Blank} = 150 uL Water + 50 uL Sample/standard

Tyrosinase inhibitor assay

Tyrosinase inhibitor assay of the extract on mushroom tyrosinase enzyme was analyzed by a method described previously, with some modification [14]. First, a 40 uL of *A. vera* crude extract (2.0 mg/mL in 50% DMSO), 80 uL phosphate buffer (pH 6.8), and 40 uL of 2.5 mM L-DOPA, were added to a 96-well microplate, and then 40 uL of mushroom tyrosinase enzyme (150 U/mL) was added. The enzymatic reaction was incubated at room temperature for 10 min. The absorbance of the reaction was determined at 490 nm in a microplate reader (EZ2000, biochrome, UK). Kojic acid (50 ug/mL) was used as a standard. The following formula calculated the percentage of inhibition:

$$\text{Inhibition (\%)} = \{[A_{control} - (A_{test\ sample} - A_{blank})]/A_{control}\} \times 100\% \quad (4)$$

where $A_{control}$ = 80 uL phosphate buffer +40 uL 2.5 mM L-DOPA + 40 uL Tyrosinase + 40 uL 50% DMSO

$A_{test\ samplel}$ = 80 uL phosphate buffer +40 uL 2.5 mM L-DOPA + 40 uL Tyrosinase + 40 uL Sample

A_{Blank} = 120 uL phosphate buffer +40 uL 2.5 mM L-DOPA + 40 uL Sample

Statistical analysis

Water content, antioxidant activities, tyrosinase inhibition, and data points for different antioxidation assays were analyzed in triplicate and expressed as mean ± SD.

Results and Discussion

Generally, *A. vera* harvested for industrial delivery or consumption was in the A3 (10-12 months), showing the giant leaves of *A. vera*. In Table 2, it was found that the oldest *A. vera* leaf had the highest solid contents. In the samples of A1-A3, the solid contents were 2.00, 2.90, and 4.00%, respectively. Plant biosynthesis processes in which the internal chemicals were converted into more rigid plant structures such as cellulose, hemicellulose, lignin, and so on. These secondary substances had low water solubility, resulting in a more robust plant structure. Therefore, the older *A. vera* leaf had a higher solid content.

Table 2 Water content analysis of the *A. vera*

Sample	% Solid content	%Water content
A1	2.00 ± 0.02	98.00 ± 0.02
A2	2.90 ± 0.02	97.10 ± 0.02
A3	4.00 ± 0.04	96.00 ± 0.04

The DPPH antioxidant efficacy of all three *A. vera* extracts (A1-A3; 2.0 mg/mL) and Trolox as a standard (10 ug/mL) were compared (Figure 1). The samples (A1-A3) and Trolox showed the antioxidant activity of DPPH in term of percent inhibition at 41.76 ± 0.57%, 40.72 ± 2.31%, 37.01 ± 2.22%, and 49.90 ± 1.74%, respectively. Three aloe extracts did not exhibit the different antioxidant effects using the DPPH technique.

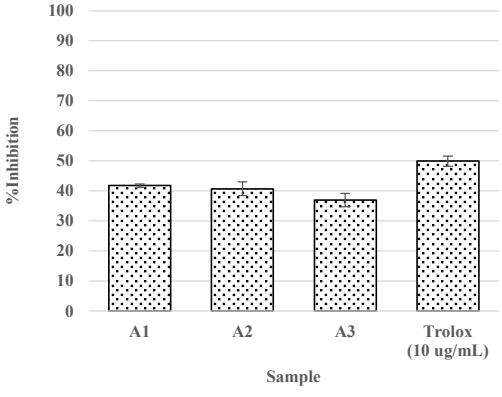

Figure 1 Antioxidant efficacy from DPPH technique of different stages of *A. vera* extract at the concentration of 2.0 mg/mL

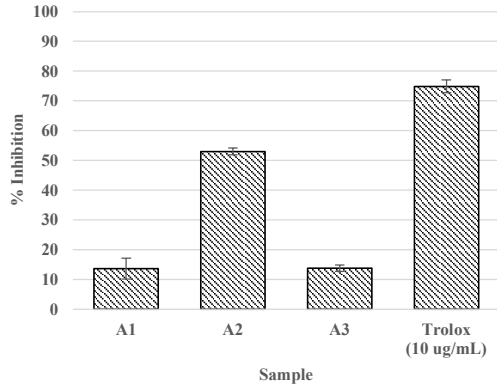

Figure 2 Antioxidant efficacy by ABTS technique of different stages of *A. vera* extracts at the concentration of 2.0 mg/mL

From figure 2, the antioxidant activity using the ABTS technique of *A. vera* extracts (2.0 mg/mL) and standard Trolox (10 ug/mL) was displayed. The samples (A1-A3) and a standard compound showed antioxidant activity against ABTS•+ in term of percent inhibition at 13.65 ± 3.44%, 53.02 ± 1.16%, 13.85 ± 1.08%, and 74.88 ± 2.15%, respectively. Among three *A. vera* extracts, the A2 stage showed the highest antioxidant activity derived from the ABTS technique.

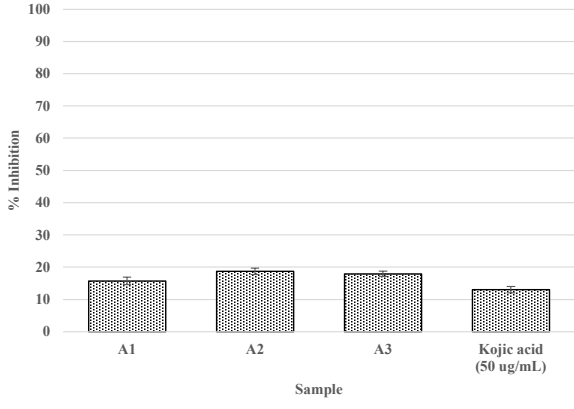

Figure 3 Tyrosinase inhibitor of different stages of *A. vera* extract at an extract concentration of 2 mg/mL

Tyrosinase inhibition of *A. vera* extracts (A1-A3; 2.0 mg/mL) was investigated and compared with standardized kojic acid at the concentration of 50 ug/mL. From figure 3, the tyrosinase inhibition efficacy of *A. vera* extracted at the A2 stage showed the highest tyrosinase inhibitory efficacy at 18.68 ± 0.99%. While *A. vera* extracted at stages, A1 and A3 showed tyrosinase inhibitory efficacy at 15.74 ± 1.17% and 17.93 ± 0.80%, respectively. In contrast, the standard compound (kojic acid) showed tyrosinase inhibitory at 13.03 ± 0.96%.

Conclusion and Suggestions

As a result, the slow reaction between DPPH radical and antioxidant might cause less antioxidant activity. In addition, some substances

with low solubility in ethanol may precipitate after the reaction, especially *A. vera* extract. Consequently, *A. vera* in phases A1 and A2 had similar DPPH antioxidant efficiency, while A3 *A. vera* had lower DPPH antioxidant efficiency. The ABTS antioxidant efficacy test results showed that *A. vera* in phase A2 had the highest percent inhibitory value at 53.02 ± 1.16%. It implied that the older *A. vera* had the lower water solubility because the strong plant structure led to fewer antioxidants in *A. vera* extract in phase A3. On the other hand, the plant biosynthesis processes of *A. vera* in phase A1 might not be as finished well as *A. vera* in phase A2, bringing about low antioxidant activity [15].

The *A. vera* extract demonstrated efficacy in inhibiting tyrosinase. Among all *A. vera* extracts, the *A. vera* in phase A2 showed higher tyrosinase inhibitory efficacy. Nevertheless, their tyrosinase inhibitions did not display the difference significantly.

A. vera has found an extensive application in cosmetic due to its beneficial moisturizing emollient effect and its effect on treating of scar tissue [16]. The development of effective processes in extracting *A. vera* gel leaves that preserve and maintain almost all bioactive chemicals has been investigated. However, the research data about the suitable duration of harvesting *A. vera* leaves to produce the *A. vera* extract that can manifest a better antioxidant effect is still ambiguous. This research found that *A. vera* extracted in stage A2 (7-9 months) displayed better antioxidant effects and a tyrosinase inhibition effect. The results could be applied in arranging the *A. vera* leaves into grades for the cosmetic field and increasing the value of *A. vera*.

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