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RESEARCH ARTICLE

Antioxidant and Anti-tyrosinase Activities of *Aloe vera* Rind and Gel Extracts

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Abstract

Aging is a natural process in human that can be characterized by the appearance of black spot on the skin due to hyperpigmentation. Aging may occur due to an excessive amount of free radicals in the body. Antioxidants possess ability to capture free radicals and inhibit tyrosinase which induces skin aging. *Aloe vera* has been used in traditional medicine because it contains several bioactive compounds that act as antioxidant and prevent aging process. This study aims to determine phytochemical content, antioxidant activity and tyrosinase inhibition activity of *Aloe vera* rind (AVRE) and gel (AVGE) extract. This research was carried out at the laboratorium of Aretha Medika Utama-Biomolecular and Biomedical Research Center in Bandung city in September–November 2018. Phytochemical assay was determined using modified Farnsworth method. Antioxidant assay was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and antiaging assay was obtained using tyrosinase inhibition assay. AVRE contains flavonoid, phenol, steroid, and alkaloid. Meanwhile, AVGE contains steroid and alkaloid. IC_{50} DPPH scavenging activity of AVRE was 113.18 µg/mL followed by AVGE was 291.96 µg/mL. IC_{50} tyrosinase inhibition activity of AVRE was 65.04 µg/mL followed by AVGE was 111.89 µg/mL. AVRE had more active DPPH scavenging activity and tyrosinase inhibition activity than AVGE.

Key words: *Aloe vera*, anti-tyrosinase, antioxidants, DPPH

Aktivitas Antioksidan dan Antitirosinase Kulit dan Daging Lidah Buaya (*Aloe vera*)

Abstrak

Penuaan merupakan proses alamiah pada manusia. Penuaan dapat terjadi akibat kadar radikal bebas yang tinggi di dalam tubuh. Antioksidan memiliki kemampuan memerangkap radikal bebas dan menghambat kerja enzim yang berperan dalam proses penuaan. Lidah buaya (*Aloe vera*) telah digunakan dalam pengobatan tradisional karena diketahui mengandung senyawa bioaktif yang bermanfaat dalam menangkal radikal bebas dan menghambat penuaan. Penelitian ini bertujuan mengetahui kandungan fitokimia dalam ekstrak etanol kulit lidah buaya (EKLB) dan ekstrak etanol daging lidah buaya (EDLB), mengetahui aktivitas antioksidan, pemerangkapan 2,2-difenil-1-pikrilhidrazil (DPPH), serta aktivitas antitirosinase EKLB dan EDLB. Penelitian ini dilakukan pada September–November 2018 di laboratorium Aretha Medika Utama Biomolecular and Biomedical Research Center (BBRC). Analisis fitokimia dilakukan menggunakan metode Farnsworth yang dimodifikasi. Aktivitas antioksidan dianalisis menggunakan metode pemerangkapan DPPH, sementara uji antipenuaan dilakukan menggunakan metode uji aktivitas antitirosinase. Hasil uji fitokimia menunjukkan EKLB mengandung flavonoid, fenol, steroid, dan alkaloid; sedangkan EDLB mengandung senyawa steroid dan alkaloid. EKLB dan EDLB memiliki aktivitas pemerangkapan DPPH dengan nilai IC_{50} secara berurutan 113,18 µg/mL dan 291,96 µg/mL. Penghambatan tirosinase EKLB dan EDLB dengan nilai IC_{50} secara berurutan 65,04 µg/mL dan 111,89 µg/mL. EKLB memiliki aktivitas antioksidan dan penghambatan tirosinase yang lebih baik dibanding dengan EDLB.

Kata kunci: *Aloe vera*, antioksidan, antitirosinase, DPPH

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Introduction

The skin is the outermost part of the body that is most visible making us aware of the aging process every time. Everyone's desire is to live longer, be younger, or at least look younger. According to Mukherjee et al.[1] there are two types of skin aging. The first one is physiological aging, which means chronological aging where aging is due to the passage of time with increasing age and the second is pathological aging, which means photoaging due to environmental factors, namely exposure to ultraviolet (UV) light. This illustrates clinical signs including dry skin, appearance of rough skin, skin pigmentation, deep wrinkles or severe atrophy, telangiectasia, and premalignant lesions.

Human skin colour is one of the most visible phenotypic variations between humans and is mainly determined by the type and amount of melanin synthesized in melanosomes and the pattern of distribution of melanosomes in melanocytes.[2,3] Dark skin occurs because there is a formation of melanin pigment in the skin through tyrosine oxidation by the tyrosinase enzyme to form 3,4-dihydroxy-L-phenylalanine (L-DOPA) which forms the melanin pigment in the final stage.[4] To inhibit the formation of melanin, the tyrosinase enzyme becomes one of the targets of whitening drugs.[5] Melanin production is induced after exposure to UV radiation and plays a major role in protecting skin cells from UV radiation. However, melanin pigmentation in the epidermis can cause skin changes such as darkening of skin color and pigmentation spots.

The need for skin care nowadays is a common thing for everyone, especially for women. Facial skin is a part of the body that describes an overall condition, so it is not surprising that today many kinds of beauty products are sold, especially lightening products that are sold freely from cheap to expensive. Many skin care products have evolved to those which cause mild side effects such as allergies and even those that endanger health to get instant and cheap results. The use of skin lightening products often occurs side effects, such as hydroquinone can cause ochronosis. Excessive use of steroids can cause side effects of skin thinning, hypertrichosis, and hormonal disorders, while mercury is toxic, as well as kidney and nerve damage.[5]

Due to the increasing circulation of various beauty products to watch out for, especially those that use chemicals that ultimately endanger the consumer, the medical world of the present era has studied again the plants that have antioxidant and antiaging effects scientifically. Some examples of plants that have been proven to be antioxidants and antiaging here are roselle flowers (*Hisbiscus sabdarifa*),[6] jasmine flowers (*Jasminum sambac*),[7] ripe sesoot (*Garcinia picrorrhiza* Miq.).[8] The source of antioxidants is mangosteen peel (*Garcinia mangostana* L.),[9] black tea, green tea, oolong tea (*Camellia sinensis* L.),[10] meniran (*Phyllanthus niruri*).[11] In addition to these plants, plants that are efficacious for the health of the body and skin are *Aloe vera*.[12] *Aloe vera* plants can be used for wounds healing and beauty treatments. Originally planted in Indonesia, *Aloe vera* is used for special skin care treatments.[13] One of the plants that is effective for body and skin health is the *Aloe vera* plant.

This is the background of the authors to examine natural ingredients that are safe and affordable, which can be one of the natural choice ingredients in skin care for skin antioxidants and skin lightening. In this case, the authors want to explore and examine the skin and gel of the *Aloe vera* plant in inhibiting the aging process of the skin and brightening the skin.

Methods

Plant determination is carried out to determine the real plant identity that will be used in the study and avoid sampling errors in the phytochemical test. The subjects of this study were the rind and gel extracts from *Aloe vera* (L.) Burm.f. from Semplak Kaum village, Semplak village, Semplak district, Bogor regency. The plants were identified by Herbarium Bogoriense, Botanical Field Research Center for Biology-Indonesian Institute of Sciences Bogor. Each rind and gel of *Aloe vera* (250 g) was mashed and extracted by using 1,000 mL destilled ethanol 70% with a maceration method. In every 24 h the ethanol filtrate was filtered and wastes were remacerated until the filtrate is colorless. Maceration was concentrated using 50°C evaporator to obtain the extract.

Phytochemical screening of *Aloe vera* rind extract (AVRE) and *Aloe vera* gel extract (AVGE) was evaluated with modified Farnsworth method to identify qualitatively presence of flavonoids, saponins, tannins, phenols, terpenoids, steroids/triterpenoids, and alkaloids.[6,14,15]

For flavonoids identification, about 10 mg of each extracts was inserted into the test tube and then Mg and 2N HCl were added. The sample mixture was heated for 5–10 minutes and then cooled and filtered. Then, the amyl alcohol solution was added to the filtrate. The reaction is positive if red/orange colour is formed.[6,14,15]

For tannins identification, about 10 mg of each extracts was added with 2 mL of 2N HCl in a test tube and then heated over a water bath for 30 minutes. The mixture was cooled and filtered. The filtrate was added to amyl alcohol. The reaction is positive if a purple colour is formed.[6,14,15]

For phenols identification, about 10 mg of each extracts was placed on a drip plate and then 1% of FeCl3 (Merck 1.03861.0250, USA) was added into the sample. The presence of phenols was indicated by green/red/purple/blue/black color.[6,14,15]

For steroids/triterpenoids identification, about 10 mg of each extracts was placed on the drip plate and then soaked with acetic acid until the sample was covered. After 10–15 minutes, one drop of absolute sulfuric acid (H_2SO_4) was added to the sample. The formation of green/blue colour showed the presence of steroids while the red/orange sediment showed the triterpenoid.[6,14,15]

For saponins identification, about 10 mg of each extracts was put into a test tube with water, boiled for 5 minutes and then shaken vigorously. The saponin content was indicated by the presence of foam on the surface of the solution.[6,14,15]

For terpenoids identification, about 10 mg of each extracts was placed on a drip plate and then vanillin and H_2SO_4 solution were added. The reaction is positive if the colour changes to purple.[6,14,15]

For alkaloids identification, about 10 mg of each extracts was put into the test tube and 10% of ammonia was added to the sample. Then, chloroform was added to the mixture of sample and two layers of liquid were formed. The lower layer was collected and then transferred to the test tube and inserted into 1N HCl until it formed two layers. The top layer was collected and then transferred to a new test tube. Then 1–2 drops of Draggendorf solution was added. The reaction is positive when yellow colour is formed.[6,14,15]

A total of 200 µL 2,2-difenil-1-pikrilhidrazil (DPPH) (Sigma Aldrich D9132, USA) 0.077 mmol in methanol was added with 50 µL of *Aloe vera* ethanol extract on the 96-well microplate. The mixture was incubated at room temperature for 30 minutes and then the absorbance value was read at 517 nm wavelength using a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, USA). For the negative control, 250 µL of DPPH was used, while for blanks, 250 µL of an absolute DMSO was used.[6,7,10] The DPPH extracts and compounds generally fade purple colour into colourless when antioxidant molecules quench DPPH free radicals. The radical scavenging activity was measured using the following formula.

$$\text{Scavenging (\%)} = (Ac - As)/Ac \times 100$$

Description: Ac: negative control absorbance (without sample); As: sample absorbance

The median inhibitory concentration (IC_{50}) value of DPPH activity was calculated.

The inhibition of tyrosinase enzyme activity was measured based on the method described by Sigma Aldrich, Tocco et al.,[16] as well as Tu and Tawata,[17] and Widowati et al.[6,7,10] with minor modifications. The solution mixture consisted of 20 µL samples, 20 µL tyrosinase enzymes from the mushroom (125 U/mL, Sigma T3824), and 140 µL potassium phosphate buffers (20 mm, pH 6.8, Merck 104873, Merck 105104) incubated at room temperature for 15 minutes. In addition, it was also prepared for controls containing only 20 µL enzymes and 160 µL phosphate buffers and blanks containing only 160 µL phosphate buffers and 20 µL samples. Furthermore, a mixture of 20 µL of the L-DOPA substrate (1.5 mm, Sigma D9628) was added and incubated again at room temperature for 10 minutes. The absorbance was measured at 470 nm wavelength. The percentage of inhibitory activity was calculated using the formula.

$$\text{Tyrosinase inhibitory activity (\%)} = \frac{A - B}{A} \times 100$$

Description: A: control absorbance; B: sample absorbance

Statistical analysis was conducted using SPSS software (version 20.0). Value was presented as mean±standard deviation. Significant differences between the groups were determined using the analysis of variance (one-way ANOVA) followed by Tukey's HSD post-hoc test. The results of DPPH and anti-tyrosinase activity tests were continued by linear regression analysis. Then the value of Inhibitory Concentration 50 (IC_{50}) was determined.

Results

The percentage of AVRE yield was 2.71% and AVGE was 9.54%. These results indicate that AVRE extract is lower than AVGE. The quality of extract produced is usually inversely proportional to the amount of yield produced.

Phytochemical screening is a method to determine the class of chemical compounds contained in a plant qualitatively. The selection of solvents and extraction methods is the most important factor in conducting phytochemical screening. The compounds tested were flavonoids, saponins, phenols, tannins, terpenoids, alkaloids, and steroids/triterpenoids.

Based on Table 1, it is known that the levels of alkaloid content in both AVRE and AVGE were high. The content of flavonoids and phenols in the AVRE was low while in AVGE was undetected. Both extracts contain steroids, and there was no saponin, tannin, and terpenoids.

DPPH is a free radical that belongs to the hydrogen radical group. DPPH is sensitive to light, oxygen, and pH. However, it is stable in a radical form so it may be quite an accurate measurement of antioxidant activity. DPPH free radicals can capture hydrogen atoms from extract components which are mixed and then reacted to their reduced form and are characterized by reduced intensity of purple DPPH solution with maximum uptake at 517 nm.[10,18]

In the DPPH scavenging activity test, the final concentration of the sample used was 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL. The DPPH scavenging activities by AVRE and AVGE samples can be seen in Figure 1. The DPPH assay is a colorimetric method to determine anti-free radical activity.[10] To find out whether there are differences in the various concentrations of AVRE and AVGE, statistical analysis was performed using one-way analysis of variance (ANOVA) with a degree of significance $p<0.05$. In Figure 1, it can be seen at all of concentration between AVRE and AVGE that the activities were different which AVGE was lower than AVRE. The IC_{50} value in the DPPH scavenging activity assay can be seen in Table 2.

Tyrosinase is an enzyme that plays a role in the formation of pigments such as melanin and other polyphenols. Tyrosinase inhibition activity from AVRE and AVGE is measured using L-DOPA as a substrate.[16] Tyrosinase catalyzes tyrosine oxidation reactions that produce chromophore and can be detected at wavelengths up to 470 nm. The following is the scheme of the reactions that occur (Figure 2).

This enzyme uses molecular oxygen to catalyze monophenol oxidation to the appropriate o-phenol and subsequent oxidation becomes o-quinone. The active center of tyrosinase, which is composed of dinuclear copper, is flexible during catalysis transfer.

In the tyrosinase inhibition activity test, the sample concentration used was 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.13 µg/mL. The results of the activity test can be seen in Figure 3.

In Figure 3, it can be observed that the higher the concentration of extract used, the higher the tyrosinase inhibition activity that occurs. There was a significant difference in tyrosinase inhibition activity with a concentration of 100 µg/mL between *Aloe vera* rind and gel extracts. IC_{50} values in tyrosinase inhibition activity test

Table 1 Qualitative Phytochemical Screening Results of Aloe vera Rind and Gel Extracts

Contents	AVRE	AVGE
Flavonoids	+	–
Saponins	–	–
Phenols	+	–
Tannins	–	–
Steroids/triterpenoids	+/–	+/–
Terpenoids	–	–
Alkaloids	+++	+++

++++: very high content, +++: high content, ++: medium content, +: low content, –: no content

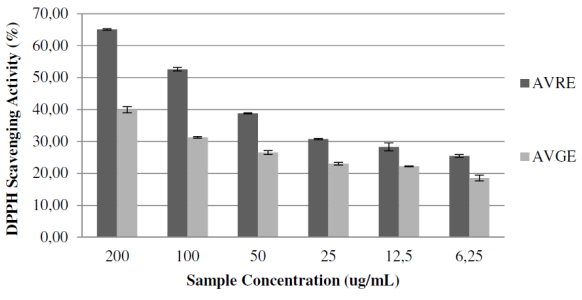

Figure 1 Comparison of DPPH Scavenging Activities by AVRE and AVGE

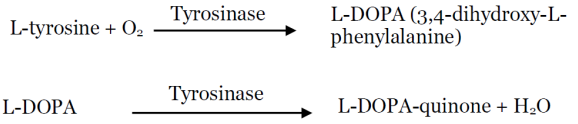

Figure 2 Role of Tyrosinase in Pigment Formation

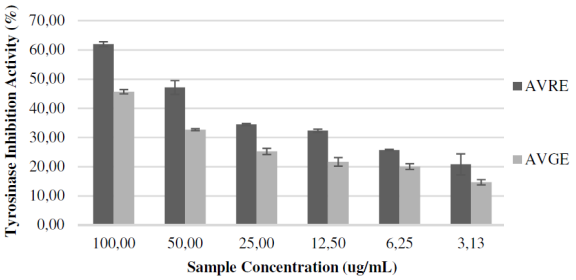

Figure 3 Comparison of Tyrosinase Inhibition Activities by AVRE and AVGE

can be seen in Table 3.

Discussion

Aloe vera contains enzymes, vitamins and minerals, natural sugars, amino acids, and antimicrobial, anti-inflammatory, and antioxidant agents. These plants are often used in beauty products for antiaging, anti-wrinkle and moisturizing creams. *Aloe vera* has the potential to cure skin diseases. *Aloe vera* is also commonly used to treat zits, black or white spots, stretch marks, and wrinkles.[19]

AVRE and AVGE were then tested for their phytochemical content qualitatively using the Farnsworth method with modification.[6,7,14,15] Phytochemical test results showed that AVRE contained a small amount of flavonoids, phenols, and steroids and a large number of alkaloids. Meanwhile, AVGE has only a small amount of steroids and a large number of alkaloids.

According to Furnawanthi,[20] one of the secondary metabolites contained in *Aloe vera* is saponin. However, in this study, no saponin

Table 2 IC_{50} Values of DPPH Scavenging Activities of AVRE and AVGE

Samples	Equation	R^2	IC_{50} (µg/mL)	
AVRE (replication 1)	y=0.2082x+26.331	0.97	113.68	113.18±0.49
AVRE (replication 2)	y=0.2077x+26.498	0.96	113.15	
AVRE (replication 3)	y=0.2043x+26.973	0.96	112.71	
AVRE (average)	y=0.2067x+26.601	0.96	113.20	
AVGE (replication 1)	y=0.1008x+20.634	0.98	291.33	291.96±11.85
AVGE (replication 2)	y=0.098x+20.197	0.96	304.11	
AVGE (replication 3)	y=0.1077x+19.797	0.97	280.44	
AVGE (average)	y=0.1022x+20.21	0.97	291.49	

Linear equations, coefficient of regression (R^2) and IC_{50} of each sample were calculated

Table 3 IC_{50} Values of Tyrosinase Inhibition Activities of AVRE and AVGE

Samples	Equation	R^2	IC_{50} (µg/mL)	
AVRE (replication 1)	y=0.3826x+25.274	0.96	64.63	65.04±1.63
AVRE (replication 2)	y=0.4111x+22.523	0.94	66.84	
AVRE (replication 3)	y=0.4069x+24.095	0.97	63.66	
AVRE (average)	y=0.4002x+23.964	0.96	65.06	
AVGE (replication 1)	y=0.2888x+17.269	0.95	113.33	111.89±1.25
AVGE (replication 2)	y=0.2948x+17.246	0.98	111.11	
AVGE (replication 3)	y=0.3012x+16.495	0.99	111.24	
AVGE (average)	y=0.2949x+17.003	0.98	111.89	

Linear equations, coefficient of regression (R^2) and IC_{50} of each sample were calculated

content was found in the AVRE or in the AVGE. This can be caused by various factors from environmental condition in which the *Aloe vera* was grown to the different solvents used in the maceration process.[20,21]

The antioxidant activity test by AVRE and AVGE in this study was carried out through DPPH scavenging test. The DPPH scavenging test is a fast and easy method. The DPPH free radical method is an antioxidant test based on electron transfer which produces a purple ethanol solution. These free radicals are stable at room temperature and will decrease in color intensity with the presence of antioxidant molecules.[22]

In the DPPH scavenging test, AVRE has better DPPH scavenging activity compared to AVGE (Figure 1). At the highest concentration (200 µg/mL) AVRE has a scavenging activity of 65.07±0.24% followed by AVGE of 39.93±1.00%. In addition, the IC_{50} value of AVRE (110.36 µg/mL) is smaller than the IC_{50} value of AVGE (303.06 µg/mL). This result is better compared to IC_{50} value of *Aloe vera* extract obtained by Prahesti et al.,[23] which is equal to 519.2 mg/L or equivalent to 519.2 µg/mL. According to Widowati et al.,[6] the smaller the IC_{50} value of a sample, the better the ability of the sample to trap free radicals. Meanwhile, according to Molyneux,[24] a substance has antioxidant activity if the IC_{50} obtained ranges from 200–1,000 µg/mL, including substances that are less active but are still considered as potential.

AVRE has a DPPH scavenging activity that is better than AVGE because of the content of flavonoids, phenols, and alkaloids. Phenols act as an antioxidant because their structure contains a hydroxyl (-OH) group which can donate hydrogen atoms (H^+) to free radicals. In addition, according to Gan et al.,[25] alkaloids have an antioxidant role even stronger than phenol

Antioxidant activity of AVRE and AVGE is also estimated to have a correlation with antiaging activity. In the tyrosinase inhibition test, AVRE has more tyrosinase inhibitory activity compared to AVGE (Figure 3). The highest activity was at the concentration of 100 µg/mL, AVRE was 62.02±0.79% and AVGE was 45.70±0.71%. IC_{50} values of AVRE 60.02 µg/mL and AVGE were 111.89 µg/mL.

Conclusions

Based on the research, it is shown that AVRE contains flavonoids, phenols, steroids and alkaloids. AVGE contains steroid compounds and alkaloids. Antioxidant activities in DPPH scavenging of AVRE and AVGE based on IC50 values are 113.18 µg/mL and 291.96 µg/mL. Meanwhile the antiaging activity in tyrosinase inhibition of AVRE and AVGE based on IC50 values are 65.04 µg/mL and 111.89 µg/mL.

Conflict of Interest

All authors state there was no conflict of interest.

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