

A Comparative Study of Peel and Seed Extract of Passion Fruit (*Passiflora edulis*) as Antihyaluronidase

Darwis^a, I Nyoman Ehrich Lister^b, Edy Fachrial^{c*}

^aMaster Program of Biomedical Sciences, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

^bFaculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

^cLaboratory of Molecular Biology, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

^cEmail: fachrial_edy@yahoo.co.id

Abstract

The plant produces various metabolites and one among the benefit is to inhibit the aging process. The aim of the study was to compare the antioxidant activity between the peel and the seeds of *P.edulis* and also to compare anti-hyaluronidase activity. The antioxidant activity was determined using the ABTS reduction method. The result showed that the reduction percentage of ABTS of seed and peel of *P.edulis* was $15,58 \pm 1,04$ % and $27,68 \pm 0,09$ % respectively at concentration 50 $\mu\text{g/ml}$. The inhibition activity of hyaluronidase of seed and peel of *P.edulis* was $70,96 \pm 3,55$ % and $61,68 \pm 4,05$ %. The IC_{50} of anti hyaluronidase activity of seed dan peel extract was $122,70 \pm 6,35$ and $67,35 \pm 6,58$ respectively. From the result, it can be concluded that the seeds and peel of *P.edulis* have the potential as a source of anti-aging particularly as anti hyaluronidase.

Keywords: antioxidant; anti hyaluronidase; *P.edulis*; anti-aging; passion fruit.

1. Introduction

Aging is a process that cannot be avoided by all living things. In human, the tissue that the most affected by the aging process is skin [1]. Aging is divided into two parts namely intrinsic aging and extrinsic aging. The extrinsic factor of the skin including UV rays and smoking habit, whereas intrinsic factor due to genetic and epigenetic mechanisms [2]. The aging of the skin will lead to changes in the appearance of the skin. The most common changes are dry and scaly skin. Recovery of damaged barrier function occurs more slowly on aging skin, resulting in dryness of the skin [3].

* Corresponding author.

Although extrinsic and intrinsic skin aging is different, there are similarities in molecular mechanisms, for example, reactive oxygen species (ROS), which arise from oxidative cell metabolism. ROS in extrinsic or intrinsic skin aging induce the transcription factor c-Jun via mitogen-activated protein kinase (MAPK), leading to overexpression of matrix metalloproteinase (MMP)-1, MMP-3 and MMP-9 and prevention of the expression of procollagen-1. The degraded collagen and reduced collagen synthesis are pathologies occurring in intrinsically aged as well as photoaged skin [4]. On aging skin, the collagen has been shown irregular, the ratio of Col-3 to Col-1 appears to be increased, due to significant loss of Col-1. Glycosaminoglycan (GAG's) particularly hyaluronic acid (HA) is a matrix dermis constituent that helps in binding water to keep the skin soft and moist. Total HA levels in aging skin of the dermis intrinsically remain stable, but epidermal HA significantly loss along with the aging process [5]. Plants are one source of antioxidant that has potential as an anti-aging agent, including inhibiting hyaluronidase activity. Plants have been extensively used as ingredients in cosmetics and therapeutics, as beautifying agents and also remedy for the dermatological disorder. These provide largely unexplored sources for the potential development of active ingredients for cosmetic formulations, and also driven by the rising desire of people to maintain healthy skin without using chemicals [6]. *P.edulis* is a plant that is widespread in the world, particularly in the tropical region. This plant has several health benefits including anti-inflammation, anti-hypertension, antifungal, antitumor and also has high antioxidant levels [7]. Only the fruit is consumed, while the peel and seeds are removed. In this study, the peel and the seed of *P.edulis* will be investigated for the antioxidant activity using ABTS reduction method, and also its ability to inhibit hyaluronidase enzyme.

2. Experimental Section

Materials

Materials used in this study are peel and seed of *P.edulis*, distilled water, ethanol 70%, ABTS, potassium persulfate, phosphate buffer saline, dimethylsulfoxide, sodium phosphate monobasic, hyaluronic acid, hyaluronidase, sodium chloride, bovine serum albumin, sodium acetate, acetic acid, chloride acid

Instrumentation

Instruments used in this study are macerator, rotary evaporator, analytical balance, multiScan Go reader, micropipette, 96 well plate, falcon tube, vortex, pH meter, incubator, and other glassware.

Procedures

2.1 Samples preparation

P.edulis were obtained from Sampali village, Percut Sei Tuan Subdistrict, Deli Serdang regency, Medan. The peel of *P.edulis* was washed and the seeds are taken. These two materials are air dried for 5 days. The peel and the seeds then milled. Drying losses are calculated by the following formula :

$$\% \text{ drying loss} = \frac{\text{dry simplicia weight (g)}}{\text{fresh simplicia weight (g)}} \times 100\%$$

2.2 Sample Extraction

1500 g of peel and 650 g of dried *P.edulis* seeds are mashed into powder. Each *P.edulis*'s peel and seeds are then macerated to obtain an extract. Maceration was conducted by soaking the peel and seeds powder with ethanol 70% and then filtered. The filtrate was evaporated using rotary evaporator.

2.3 Antioxidant Activity test with ABTS Method

The concentration of extract used in the antioxidant test was 6 concentration variations, namely 500 µg/mL ; 250 µg/mL ; 125 µg/mL ; 31,25 µg/mL ; 15,625 µg/mL. as 2 µL sample was added into the 96-well plate, then added with 198 µL ABTS reagents. At well blank, 200 µL of DMSO was added. In well control, 200 µL reagent was added and then incubated for 6 minutes at 37°C. the absorbance is measured using a microplate reader at $\lambda = 745$ nm.

2.4 Anti hyaluronidase activity test

The variation of concentration used in this test were 166,67 µg/mL ; 83,33 µg/mL ; 41,67 µg/mL ; 20,83 µg/mL ; 10,42 µg/mL ; 5,21 µg/mL. The inhibition of hyaluronidase enzyme activity was measured based on the method used by Widowati and his colleagues (2016) with slight modification [8]. A mixture of a solution consisting of 25µL samples, 3 µL enzymes hyaluronidase from bovine testes and 12 µL phosphate buffer, incubated at 37°C for 10 minutes. In addition, the mixture of the solution was added as much as 10 µL of the hyaluronic acid substrate and re-incubated at 37 C for 45 minutes. The STOP solution in the form acid albumin was added as much as 100 µL into the solution and left at room temperature for 10 minutes. Absorbance is measured using microplate reader at 600 λ nm.

3. Result and Discussion

3.1 Reduction activity of ABTS

Antioxidant test using method 2'-Azino-Bis-3-Ethylbenzothialine-6-Sulfonic Acid (ABTS) was determined based on loss of blue color due to the reduction of ABTS by antioxidants. This blue intensity is measured at a wavelength 745 nm.

Reduction reaction of ABTS by antioxidant :



The reduction of ABTS by peel and seed extract of *P.edulis* was shown at table 1.

Table 1: Reduction activity of ABTS by peel and seed extract of passion fruit (*Passiflora edulis*)

Concentration (µg/mL)	Mean of reduction activity of ABTS (%) by samples	
	Peel extract	Seed extract
50.00	27.68 ±0.09 ^d	15.58 ±1.04 ^d
25.00	18.35 ±1.35 ^c	10.65 ±0.35 ^c
12.50	11.74 ±0.67 ^b	9.81 ±0.83 ^{a,b}
6.25	10.61 ±0.66 ^{a,b}	7.66 ±0.28 ^{a,b}
3.13	9.43 ±0.34 ^a	8.52 ±0.67 ^{a,b}
1.56	7.32 ±0.69 ^a	7.50 ±0.75 ^a

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at P < 0.05 (Tukey HSD post hoc test).

Table 1 showed that the reduction of ABTS by peel extract was higher than seed extract. The similar report was reported by Orak and his colleagues (2012) which studied the comparison of antioxidant activities of juice, peel, and the seed of Pomegranate (*Punica granatum* L) using DPPH scavenging activity.

It is reported that. The EC₅₀ values of DPPH scavenging activities in peel extracts (PE) had 23,4-fold higher than the juice extracts (JE). The reducing power in peel extracts was found to be 4.7-fold higher than SE and 10.5-fold higher than the JE [9]. Ang and his colleagues (2012) reported papaya peel and seed extracts has potency as an antioxidant source. The antioxidant activities of papaya peel were determined using the ABTS method and the reduction activity was 28.30%, while the antioxidant activity of papaya seed was 11.19% [10].

Antioxidants are compounds that inhibit the oxidation process and protect cells from the harmful effects of free radicals. Previous research reported that phytochemical and antioxidant activity screening has been done to 31 types of fruit peel extract, including *P.edulis*.

The peel extract of *P.edulis* was shown contained triterpenoid, saponin, and phenolics [11]. The IC₅₀ ABTS reduction of peel and seed extract of *P.edulis* was shown in table 2

Table 2: The IC₅₀ value of ABTS reduction by peel and seed extract of *P.edulis*

Samples (average)	equation	R2	IC50 (µg/mL)
Peel extract	Y = 0.4046x + 7.7976	0,99	104.30 ± 0.68
Seed extract	Y = 0.1611x + 7.3042	0,96	268.26 ± 34.65

Based on data in table 2, it showed that the antioxidant activity of peel extract was higher than seed extract. IC_{50} of ABTS scavenging activity is the concentration of sample or standard that can inhibit 50% of ABTS scavenging activity.

The lowest IC_{50} means had the highest antioxidant activity. The IC_{50} were used to categorize antioxidant activity of a sample that compared to standard. The sample that has IC_{50} less than 50 $\mu\text{g/mL}$ is a very strong antioxidant, 50-100 $\mu\text{g/mL}$ is a strong antioxidant, 101-150 $\mu\text{g/mL}$ is a medium antioxidant, while IC_{50} greater than 150 $\mu\text{g/mL}$ is a weak antioxidant [12].

3.2 Anti hyaluronidase activity of *P.edulis*

The analysis data hyaluronidase inhibition activity of peel and seed extract of *P.edulis* was shown in table 3 and 4.

From the tables above it can be seen that the inhibition of hyaluronidase activity of *P.edulis* seed extract was almost twice higher than *P.edulis* peel extract.

It means that the hyaluronidase inhibition activity in peel extract was stronger than seed extract. The similar report was reported by Tu and Tawata (2015) which investigated the anti-oxidant and anti-aging effects of essential oils (Eos) from the leaves of *Alpinia zerumber* (tairin and shima).

The result revealed that tairin and shima Eos showed strong anti-oxidant activities against DPPH and nitric oxide. Tairin EO also exhibited strong antiaging activity by inhibiting hyaluronidase ($IC_{50}= 83\pm 1,6$) [13].

In other study reported that ethyl acetate fraction of *Garcinia indica* at a concentration as low as 25 $\mu\text{g/mL}$ showed significant hyaluronidase inhibition. Garcino and cambogiol present in fruit rinds of *Garcinia indica* were reported to be good antioxidant ability [14].

The exact mechanism of hyaluronidase inhibition is still obscure, but in general, polyphenols such as tannins, are known to form complexes with a variety of proteins, including hyaluronidase, which may reduce the activity [15].

Table 3: hyaluronidase inhibition activity of seed extract

Sample	Final concentration (ug/mL)	Hyaluronidase inhibition activity(%)			Average	SD	RSD	IC 50 average R ²	IC 50			Average of IC50
		1	2	3					1	2	3	
Peel extract	166.67	66.35	59.39	59.29	61.68	4.05	6.56	122.37	115.81	123.96	128.33	122.70
	83.33	40.61	41.94	41.18	41.25	0.67	1.62					
	41.67	28.31	32.51	22.97	27.93	4.78	17.11					122.70 ±
	20.83	12.77	16.97	17.92	15.89	2.74	17.24					6.35
	10.42	12.87	12.20	13.35	12.81	0.57	4.49					
	5.21	6.48	7.05	7.44	6.99	0.48	6.86	0.97	0.977	0.927	0.971	6.35

Table 4: hyaluronidase inhibition activity of peel extract

Sample	Final Conc. (ug/mL)	Inhibition Activity (%)			Average	SD	RSD	Average of IC50 - R ²	IC50 1 st experiment (R2)	IC50 2 nd experiment (R2)	IC50 3 rd experiment (R2)	IC50 (STDV)
		1	2	3								
Seed extract	166.67	68.45	69.40	75.02	70.96	3.55	5.01	67.10	71.93	70.30	59.81	67.35
	83.33	49.48	55.00	51.86	52.11	2.77	5.32					
	41.67	46.90	43.47	44.61	45.00	1.75	3.88					67.35 ± 6.58
	20.83	47.00	42.99	42.33	44.11	2.53	5.73					
	10.42	36.03	36.51	40.32	37.62	2.35	6.25					
	5.21	32.13	32.70	39.18	34.67	3.92	11.30	0.97	0.818	0.9656	0.976	6.58

4. Conclusion

From the study, it can be concluded that the antioxidant activity of *P.edulis* peel is higher than the seeds with percentage ABTS reduction is was $15,58 \pm 1,04$ % and $27,68 \pm 0,09$ % respectively at concentration 50 $\mu\text{g/ml}$. The IC_{50} of antihyaluronidase activity of seed dan peel extract was $122,70 \pm 6,35$ and $67,35 \pm 6,58$ respectively. From the result, it can be revealed that the peel of *P.edulis* has potency as an anti-aging agent.

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