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

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Abstract

Natural plant compound is known to have various effects that are beneficial to human health including as antioxidant and anti-aging. The aim of this study was to investigate the antioxidant activity of passion fruit peel and seeds an the activity in inhibiting collagenase. The ethanolic extract of seed and peel of *P.edulis* was determined by antioxidant activity using DPPH scavenging method and continued with the investigation of anticollagenase activity. The result showed that the IC_{50} of peel extract and seed extract of *P.edulis* were 56.11 ± 2.26 µg/mL and 88.25 ± 14.31 µg/mL respectively. The IC_{50} of anti-collagenase activity of peel and seed extract of *P.edulis* were 82.53 ± 1.42 µg/mL and 225.40 ± 9.12 µg/mL respectively. From the result, it concluded that peel and seed extract of *P.edulis* possesses remarkable potency as anti-collagenase activity and have the potential to be developed as anti-aging skin nutraceutical.

Keywords: *Passiflora edulis*; antioxidant; anti collagenase; DPPH.

1. Introduction

The aging process has occurred since we were born. The skin is the outermost organ and directly exposed to environmental changes, making us aware that skin aging occurs in every minute. The desire of humans all over the world for a long time is to live longer and stay young, or at least look younger, so the study in related with anti-aging has become a special concern by a researcher [1]. Aging is defined as a physiological genetic process that associated with changes in function and morphology of cellular and extracellular components aggravated by injury throughout life and resulting in a progressive imbalance of the control regulatory systems of the organism, including hormonal, autocrine, neuroendocrine and immune homeostatic mechanisms [2].

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There are various ways to inhibit the skin aging process, one of them by inhibiting the free radical activity. materials that can be used to inhibit free radical activity are called antioxidant [3]. Many plants can be used as treatment and prevent diseases. Natural plant compound is showing a wide range of activities including anti-aging [4]. Tight and firmness of the human skin is due to elastin and collagenous present in its dermis. Collagen is synthesized and secreted by the fibroblast cells of the dermis and its rate of synthesis decreases with aging. With the increase in age and particularly due to overexpression to sunlight, collagen deteriorates resulting in skin wrinkling [5]. *Passiflora* spp, popularly known as passion fruit, is a medicinal plant and is recognized by several associations including Brazilian Pharmacopoeia and British Herbal. This plant is rich in flavonoid, phenols, and alkaloids which acts as an antioxidant [6]. This fruit is usually consumed freshly, the skin and seeds are removed. The aim of this study was to investigate the antioxidant activity of passion fruit peel and seeds and the activity in inhibiting collagenase.

2. Experimental Section

2.1 Samples Preparation

P.edulis obtained from Sampali village, Percut Sei Tuan sub-district, the regency of Deli Serdang. *P.edulis* is peeled and separated between the peel and the seeds and then weighed. The wet weight of the peel was 1500 gram and the wet weight of the seeds was 650 gram. The peel and the seeds the milled and wind dried for 14 days in room temperature. The drying losses are calculated by the formula below :

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

2.2 Sample Extraction

as much as 240 gram of peel and 200 gram of seeds that have dried, mashed into powder. Each *P.edulis*'s peel and seeds are then macerated to obtain an extract. The peel and the seeds powder are macerated using 70% ethanol. Every 24 hours the filtrate was collected. Maceration is repeated until the ethanol filtrate became colorless. The filtrate was evaporated using a rotary evaporator

2.3 Determination of antioxidant activity

The concentration of each extract of *P.edulis* peel and seed were 200 µg/mL; 100 µg/mL ; 50 µg/mL ; 25 µg/mL µg/mL; 12,5 µg/mL ; and 6,25 µg/mL. 50 µL samples (peel and seed extract) were pipetted into 96-well plates. As much as 0.077 mmoles DPPH was added as 200 µL well plate containing the samples. DMSO was used as a blank, and DPPH alone was used as a control. The well plate was incubated for 30 minutes in dark condition. Absorbance was measured using microplate reader at λ = 517 nm.

$$\% \text{ scavenging activity} = \frac{control\ absorbance - samples\ absorbance}{control\ absorbance} \text{ x } 100\%$$

2.4 Anti collagenase activity test

The concentration of samples used in this test varied between 250 µg/mL ; 125 µg/mL ; 31.25 µg/mL ; 15.625 µg/mL ; and 7.81 µg/mL. 10µL collagenase enzyme was pipetted into each control-well, samples-well and blank-well. Respectively, 90 µL; 60 µL; and 80 µL was added into control-well, samples-well, and blank-well. 30 µL samples were added into samples-well and control well. The 20 µL FALGPA (N-[3-(2-furyl)acryloyl]-leu-gly-Pro-Ala) was added into each sample well and control well. Absorbance was measured using microplate reader at λ = 335 nm

3. Result and Discussion

3.1 DPPH Scavenging Activity

The result of DPPH scavenging activity was shown in table.1. the

Table 1: DPPH scavenging activity of peel and seed of *P.edulis* extract

Concentration (µg/mL)	**Average of DPP scavenging activity (%)**	
	Peel extract of *P.edulis*	**Seed extract of *P.edulis***
200	95.61 ±0.62^{f}	71.55 ±0.20^{c}
100	71.06 ±0.56^{e}	51.50 ±3.50^{b}
50	52.57 ±1.18^{d}	42.83±3.79^{a,b}
25	40.58 ±1.02^{c}	38.00 ±3.94^{a}
12.5	32.73 ±0.42^{b}	35.00 ±3.09^{a}
6.25	30.07 ±1.54^{a}	33.96 ±3.75^{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 DPPH scavenging activity of *P.edulis* peel extract was higher than *P.edulis* seed extract. The DPP scavenging activity also increased with the increase in concentration. Irawan and his colleagues (2017) reported that the DPPH free radical scavenging activity of *Pometia pinnata* extract varied widely increased with the increase of concentration. Among parts of *Pometia pinnata* fruit, the peels had higher antioxidant activity compared to the seed extract [7]. Our result is in agreement with the findings of Jalal and his colleagues [8] who reported the antioxidant activity of pomegranate peel and seed powder extracts, and in their reported, it mentioned that the DPPH scavenging activity of pomegranate peel powder was significantly higher (P<0,05) than the pomegranate seed powder. DPPH test which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron which is responsible for the absorbance at 515-517 nm. when DPPH accepts and electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance [9].

The IC_{50} value of DPPH scavenging activity of *P.edulis* seed extract and *P.edulis* peel extract was shown in table 2. The test was carried out in triple

Table 2: The IC_{50} value of DPPH scavenging activity od *P.edulis* seed extract and peel extract

samples	IC_{50} (µg/mL)	Average IC_{50} (µg/mL)
P.edulis peel extract (1st)	56,34	
P.edulis peel extract (2nd)	58,25	
P.edulis peel extract (3rd)	53,75	56,11 ±2.26
average	56,15	
P.edulis seed extract (1st)	71,73	
P.edulis seed extract (2nd)	96,49	
P.edulis seed extract (3rd)	96,53	88,25 ± 14,31
average	89,12	

From the table above it showed that the value of IC_{50} in *P.edulis* peel extract is lower than *P.edulis* seed extract. The similar result reported by Kanatt [10] which reported peel extract of pomegranate showed a concentration-dependent DPPH radical scavenging activity with an IC_{50} of 4.9 µg/mL. peel extract scavenged the DPPH radical more efficiently than seed extract, indicating seed extract was lacked hydrogen donating capacity. Benites and his colleagues [11] reported the pulp of A.sylvatica showed a lower value of IC_{50} as 695.61±6.67 µg/mL than the seeds as 724±17.79 µg/mL. *P.edulis* is known to have a strong antioxidant activity. In the study reported by Ramaiya [12] the highest phenolic content was observed in vine-ripened purple and yellow *P.edulis* at 362.00±4.68 mg GAE/L and 361.73±3.99 mg GAE/L respectively. Similarly, the strongest antioxidant activity was observed in vine-ripened *P.edulis* (purple) at 547 ± 3.08 µmol Trolox/L and *P.edulis* (yellow) at 524 ± 1.96 µmol Trolox/L. in general, a higher TPC value gave a stronger antioxidant activity. the antioxidant activity exhibited by plant extracts obtained from the activity is suspected of secondary metabolites present in the plant. Secondary metabolites are produced or synthesized compounds on cells and certain taxonomic groups in the rate of growth or particular stress [13].

3.2 Anticollagenase activity of peel and seed extract of P.edulis

The collagenase inhibition activity of peel and seed extract of *P.edulis* was shown in table 3

Table 3: Anti-collagenase activity of peel and seed extract *P.edulis*

Concentration (µg/mL)	**Average of Anticollagenase Activity (%)**	
	Peel extract	**Seed extract**
250	76.18 ± 1.40^{e}	51.84 ± 1.72^{e}
125	60.74 ± 0.39^{d}	37.99 ± 0.27^{d}
62.5	50.07 ± 1.27^{c}	31.86 ± 0.97^{c}
31.25	41.09 ± 0.87^{b}	28.10 ± 2.04^{b}
15.625	36.64 ± 0.81^{a}	21.46 ± 3.13^{ab}
7.8125	34.78 ± 1.30^{a}	17.60 ± 2.25^{a}

(*data are presented in the form of averages ± standard deviations. Differences in lowercase letters in the same column show the significance of data $P < 0.05$ (Tukey HSD Post Hoc test)

From the data above it can be seen that the higher the extract concentration, the higher the percentage of collagenase inhibition. Mansauda and his colleagues [3] reported that 50 µg/ mL *S.plagyophyllum* extract inhibited 54.46±0.37 % activity of collagenase enzyme. The higher concentration of *S.plagyophyllum*, the higher activity of anti-collagenase.

Table 4: IC_{50} of anti-collagenase activity of *P.edulis* peel and seed extract

Sample	**Equation**	R^2	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**
P.edulis peel extract (1st)	Y = 0.1612x+36.709	0.97	82.45	82.53±1.42
P.edulis peel extract (2nd)	Y = 0.1778x+35.563	0.97	81.15	
P.edulis peel extract (3rd)	Y = 0.1728x+35.486	0.96	83.99	
Average	Y = 0.1706x+35.919	0.97	82.54	
P.edulis seed extract (1st)	Y = 0.1418x+19.455	0.97	215.41	225.40 ±9.12
P.edulis seed extract (2nd)	Y = 0.1303x+19.605	0.92	234.51	
P.edulis seed extract (3rd)	Y = 0.1269x+21.126	0.92	227.53	
Average	Y = 0.1330x+20.062	0.95	225.63	

The analysis of IC_{50} of anti-collagenase activity using *P.edulis* peel and seed extract was conducted in the triple. Based on the data, it showed that the peel extract of *P.edulis* has lower IC_{50}, this means the anti-collagenase activity of *P.edulis* peel extract is higher than *P.edulis* seed extract. MMPs or collagenase is a group of zinc-containing proteinases. MMP-1 or interstitial collagenase initiates the breakdown mostly type I, II and III collagens which are the abundant interstitial collagens in the dermis, while MMP-2 is responsible for breakdown of type I-III, IV and VII collagens in which the latter two are most abundant in the dermal-epidermal junction. The inhibition effect of peel and seed extract of *P.edulis* might involve several mechanisms. Hydroxyl groups of polyphenols could interact with the backbone or other functional groups side chain of collagenase. In addition, hydrophobic interaction between the benzene ring of polyphenols and collagenase could also result in the conformational changes leading to unfunctioned enzyme. Another mechanism involves the Zn ion active site on collagenase. Collagenase contains structural Zn ions at its active site which plays a major role in facilitating interaction with an inhibitor [14].

4. Conclusion

that the IC_{50} of peel extract and seed extract of *P.edulis* were 56.11 ± 2.26 µg/mL and 88.25 ± 14.31 µg/mL respectively. The IC_{50} of anti-collagenase activity of peel and seed extract of *P.edulis* were 82.53 ± 1.42 µg/mL and 225.40 ± 9.12 µg/mL respectively. From the result, it concluded that peel and seed extract of *P.edulis*

possesses remarkable potency as anti-collagenase activity and have potential to be developed as anti-aging skin nutraceutical

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