American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Antioxidant and Anti-elastase Activity of Seed and Peel Extract of *P.edulis*

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Abstract

Passiflora edulis is a woody climber, native in Brazil and now cultivated in all parts of the world, chiefly for its edible fruits and for ornamental flowers. The seed and the peel of *P.edulis* always removed and do not have economical values. The aim of the study was to determine the antioxidant activity of the seed and peel of *P.edulis* and its potential as an elastase inhibitor. The seed and the peel were extracted using ethanol 70%. The extracts were evaporated and the antioxidant activity was determined using H_2O_2 scavenging method. The IC_{50} values of H_2O_2 scavenging activity of peel and seed extract were 626.31 ± 2.10 µg/mL and 2106.46 ±33.21 µg/mL respectively. the effect of variation concentration of seed and peel extract showed that the antioxidant activity was dose-dependent manner. The IC_{50} value of elastase inhibitor of peel and seed extract was 62.82 ± 1.50 µg/mL and 41.06 ± 0.31 µg/mL respectively. This result gave the promising effect of peel and seed extract of *P.edulis* as an anti-aging cosmetic ingredient.

Keywords: *Passiflora edulis*; antioxidant; elastase inhibitor; anti-aging.

1. Introduction

Passiflora edulis, or commonly called as passion fruit is a plant species native from Brazil and now has been cultivated in all parts of the world. It is cultivated for its edible fruit, as an ornamental plant and also for pharmaceutical interest. *P.edulis* is known to posses several pharmacological properties including as sedative, anti depressant, anti hypertensive, anti tumor and antioxidant [1].

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The antioxidant activity of the leaves of *P.edulis* was reported by [2], and found that the leaves extract of *P.edulis* possesses strong antioxidant activity. Antioxidants are the molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Free radicals are known to cause cellular damage and consequent age-related medical disorder. Supplementing the skin with additional antioxidants has been demonstrated to give additional protection from sun-induced damage, slow down skin aging and ultimately improve skin appearance [3]. Aging is accelerated in areas exposed to sunlight, a process known as photoaging. It is called photoaging because of a combination of short wavelength (UVB) injury to the outer layers of the skin (epidermis) and long wavelength (UVA) injury to the middle layers (dermis). Clinical presentation of aging includes dryness of the skin, irregular pigmentation-freckles, hyperpigmentation, wrinkling and inelasticity [4]. Elastin is an extracellular matrix protein providing elasticity to the connective tissue. It forms elastic fiver in the skin dermis and has an influence on skin elasticity. Elastase is the proteinase enzyme capable of degrading elastin, therefore inhibition of elastase activity could be used as a method to protect against skin aging [5]. In this study, we examined the antioxidant and antielastase activity of peel and seed extract of *P.edulis*. Both activities were employed as a preliminary test for skin anti-aging. Antielastase was assayed using the spectrophotometric method with slight modification.

2. Experimental Section

2.1 Reagent and Material

P.edulis were collected from Sampali village, Percut Sei Tuan regency. The reagents used in this study including ferrous ammonium sulfate (Sigma 7783859), hydrogen peroxide (Merck 1.08597.1000), sulphuric acid (Merck 109981), 1,10-phenanthroline (Sigma 131377), dimethylsulfoxide (Merck 1.02952 lot K46505352) distilled water, N-succinyl-Ala-Ala-Ala-P-nitroanilide, elastase substrate (Sigma 54760), elastase from porcine pancreas (Sigma 45124), Tris (Pharmacia biotech 17-1321-01), sodium chloride (Merck 1023821000), hydrochloric acid solution (Merck 109057)

2.2 Preparation of plant extracts

Dried seed and peel of *P.edulis* were extracted with ethanol 70% by maceration. The extract was filtered through Whatmann paper and concentrated using a vacuum rotary evaporator.

2.3 Antioxidant activity test

The seed and peel of *P.edulis* extract were added into 96-well plate. Ferrous ammonium sulfate 1 mM was added into control well plate and samples well plate. DMSO was used as a blank. 3µL H2O2 was added into the well plate containing the samples. 1,10-phenanthroline 1mM was added into the well plate containing the samples, incubated for 10 minutes in a dark condition and room temperature. Absorbance was measured at λ=510 nm. Percentage of scavenging activity was determined using the formula: % scavenging = $\frac{\lambda samples}{\lambda control}$ x 100%.

2.4 Anti-elastase assay

The anti-elastase assay was carried out using a spectrophotometric method with slight modification was using porcine pancreatic elastase (PPE) with the substrate N-Succ-(Ala)3-p- nitroanilide [6]. PPE and tris buffer were mixed and incubated at 25°C. the control consisted of an enzyme and tris buffer while the blank consisted of tris buffer and sample. The substrate was added to the solution and incubated at 25 °C for 15 minutes. Absorbance is measured at λ=410 nm.

3. Result and Discussion

3.1 Antioxidant activity

In this study, the seed and peel of *P.edulis* were tested for its antioxidant activity and antielastase activity for the development of anti-wrinkle skin material in cosmetic. The yield of extraction showed that the ethanol soluble of seed and peel was similar. The result of extraction was shown in table 1

Table 1: Extract Yield of seed and peel of P.edulis

extract	Yield (%)
Seed	11,68
peel	11,75

The free radical scavenging was measured using H_2O_2 scavenging activity. The result was showed in table 2

Table 2: H_2O_2 scavenging activity of seed and peel extract of *P.edulis*

Final Concentration (µg/mL)	**Average value of H_2O_2 scavenging activity (%)**	
	Seed extract	**Peel extract**
500	37.83 ± 0.17^{d}	13.21 ± 0.19^{f}
250	28.24 ± 0.4^{c}	7.18 ± 0.03^{e}
125	13.29 ± 0.18^{b}	2.51 ± 0.01^{d}
63	8.45 ± 0.51^{a}	3.26 ± 0.10^{c}
31	4.85 ± 0.16^{a}	2.18 ± 0.16^{b}
16	3.27 ± 0.19^{a}	2.48 ± 0.05^{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).

From the table, it showed that the antioxidant activity of peel and seed extract of *P.edulis* was concentration-dependent. From the result, the antioxidant activity of peel extract was higher than seed extract in all concentration. The H_2O_2 scavenging activity of peel extract was found as 3.27 ±0.19%, 4.85 ±0.16%, 8.45 ±0.5%1, 13.29 ±0.18%, 28.24 ±0.4%, and 37.83 ±0.17% for 16, 31, 63, 125, 250 and 500 µg/mL respectively.

The more concentration of the extract, the antioxidant activity also increased. The similar result reported by [7], which reported the antioxidant activity of Phloretin was found as 22.13%, 42.56%, 69.65%, 89.32% for 20, 40, 60 and 80 µg/mL. Huyut and his colleagues[8] reported the antioxidant and antiradical properties of selected flavonoids and a phenolic compound including malvi, oenin ID-8, silychristin, callistephin, and pelargenin. The antioxidant properties of these compound at different concentration (10-30µg/mL) were compared with those of reference antioxidant such as BHA, BHT, α-tocopherol and trolox. Each substance showed dose-dependent antioxidant activity. the IC_{50} of H_2O_2 scavenging activity of peel extract of *P.edulis* was lower than seed extract. The IC_{50} of peel extract and seed extract was 616.31 ± 2.10 and 2106 ± 33.21 µg/mL respectively. Another research showed the lower IC_{50} of seed and peel extract was reported by Irawan and his colleagues [9], which reported the IC_{50} peel and seed of ethyl acetate of *Pometia pinnata* was 917 and 2688 µg/mL respectively. Another study reported that the *D.longan* peels methanolic extracts posses high antioxidant properties. The antioxidant activity of *D.longan* peel extract was higher than D.longan seed extract. The IC_{50} value of *D.longan* peel extract and seed extract was 23.50 and 32.13 µg/mL respectively. Wong and his colleagues [10] reported the best extraction condition (40% ethanol, 60 min extraction time and 30°C extraction temperature, had yielded at an optimal level of antioxidant properties for the passion fruit level. Moderate level of antioxidant activity was determined for passion fruit peel. Assessed using FRAP and BCB assays, the extract of passion fruit peel had 30.94 µg TE/g sample and 68.54% inhibition. The lowest IC50 means the highest antioxidant activities. 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 µg/mL is a very strong antioxidant, 50-100 µg/mL is a strong antioxidant, 101-150 µg/mL is a medium antioxidant, while IC_{50} greater than 150 µg/mL is a weak antioxidant [11].

3.2 Anti elastase activity

The result of the anti-elastase activity of seed and peel extract of *P.edulis* was shown in table 3.

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

Sample	**Equation**	**R^2**	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**
Peel Extract (1st repetition)	Y = 0.4526x+22.334	0.96	61.13	62.82±1.50
Peel Extract (2nd repetition)	Y = 0.4258x+23.019	0.96	63.37	
Peel Extract (3rd repetition)	Y = 0.4280x+22.617	0.97	63.98	
Peel Extract (average)	Y = 0.4355x+22.657	0.97	62.79	
Seed Extract (1st repetition)	Y = 0.7032x+21.264	0.95	40.86	41.06 ± 0.31
Seed Extract (2nd repetition)	Y = 0.6577x+22.756	0.95	41.42	
Seed Extract (3rd repetition)	Y = 0.7529x+19.205	0.98	40.90	
seed Extract (average)	Y = 0.7046x+21.075	0.96	41.05	

The anti-elastase activity test was carried out in 3 repetitions. Table 4 showed IC_{50} of seed extract of seed extract

of *P.edulis* was lower than peel extract as 41.06 ± 0.31 µg/mL and 62.82±1.50 µg/mL respectively. The protein found in connective tissue which responsible for the elasticity of the skin was lastin. The catalase enzyme was catalyzed this protein. The increasing of the age and the exposure the skin to UV radiation will accelerate the degradation of elastin by *intracellular elastase, which leading ti skin aging. From the result, it showed that the skin seed extract of P.*edulis showed the elastase inhibition activity, which approximately similar to the result reported by Kadum and his colleagues [12], which stated that the date variety Piyarom demonstrated the strong anti elastase activity (61.2 ± 4,9%). The elastase inhibition of P.edulis might be due to phenols and flavonoid content. Hyaluronidase and elastase could inhibited by certain phenols and flavonoid in dose dependent manner [13].

4. Conclusion

From the result it concluded that the IC_{50} values of H_2O_2 scavenging activity of peel and seed extract were 626.31 ± 2.10 µg/mL and 2106.46 ±33.21 µg/mL respectively. The effect of variation concentration of seed and peel extract showed that the antioxidant activity was dose-dependent manner. The IC_{50} value of elastase inhibitor of peel and seed extract was 62.82 ± 1.50 µg/mL and 41.06 ± 0.31 µg/mL respectively.

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