

The Effects of *Passiflora Edulis Sims*'s Seed Extract on Total Cholesterol in Atherogenic Young Rats

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Abstract: Purple passion fruit (*Passiflora edulis Sims*) is one of antioxidant that contain flavonoid, as a protection atherosclerosis process. Its juice has been proof to reduced total cholesterol, but its seed also has been predicted to reduce it. The objective was to find the effects of *Passiflora edulis Sims*'s seed extract in different doses in affecting lipid profile serum level in young rats (*Rattus novergicus*) in fourteen days. There were four groups containing 20 male rats which were devided into: normal diet group (C0); atherogenic diet group (C1); atherogenic diet and 50mg/kg body weight (P1); atherogenic diet and 100mg/kg body weight (P2). Results of this study were there was significant difference of body weight, total cholesterol, and triglyceride, but not for low and high density lipoprotein serum level. The result reported that body weight showed significant difference between C0, C1, P1, and P2. Total cholesterol serum between C0 and C1 group ($p=0.04$), also between C1 and P2 ($p=0.003$). There was also significant difference of triglyceride serum level between C0 and P1 ($p=0.04$) and C0 and P2 ($p= 0.03$). Based on these results it can be concluded that *Passiflora edulis Sims*'s seed extract has potential effect in lowering total cholesterol and triglyceride serum in young rats.

1 INTRODUCTION

The high incidence of atherosclerosis diseases, promotes the research for more effective treatments and looking for better treatment, one of them is with antioxidant treatment. However, improvement of lifestyle especially diet therapy, such as lowering cholesterol food sources is important for the prevention of cardiovascular disease (García, 2016; Larson-Meyer et al., 2006; Matsuzawa et al., 2011).

Mixtures of bioflavonoids in the diet and as supplements have been recognized to play a protective role by reducing the prevalence of cardiovascular diseases, one of them is passion fruit. It has been used widely in folk medicine in tropical countries as a hermal treatment to treat anxiety, insomnia, asthma, bronchitis, and urinary tract infection. The extracts of different parts of the passion fruit plant contain flavonoids compounds,

but lack of research found flavonoid in the seed (Baur et al., 2006).

Passiflora edulis Sims (Passifloraceae) is a woody climber, commonly native of tropical country, chiefly for its edible fruits. The plant is commonly called as yellow passion fruit, maracuja, yellow granadilla, and pomme liane jaune. In traditional system of medicine, *P. edulis* is used as sedative, antiasthmatic and emetic. *P. edulis* leaves are used in the treatment of insomnia and traditionally known to produce a restful sleep without any narcotic hangover (Kawakami et al., 2014; Kershaw & Kim, 2017). All parts of the fruits is edible, including the leaves, that have been reported to contain a bitter principle maracugine, resins, acids and tannin exceptionally rich in ascorbic acid (Kirkland, 2013; Sunitha & Devaki, 2009). The leaves used to treat epilepsy, ulcers and haemorrhoids, but need further research for the seed.

Although the antioxidant activities of some of the purple passion fruit peel extract's compounds have been studied extensively (Lagouge et al., 2006; Matsui et al., 2010), but activities of the seed has not been showed beneficial effects. No data are available with respect to the clinical usefulness of *Passiflora edulis* Sims seed's extract in lowering cholesterol total and triglyceride. This research hypothesized that the *Passiflora edulis* Sims seed's extract, a novel mixture of bioflavonoids, may attenuate the lowering cholesterol total and triglyceride in fourteen days.

2 MATERIAL AND METHOD

2.1 Animal Treatment

The study was conducted in accordance with the ethical principles for animal experimentation and the study was approved by the ethics committee on animal research at the Animal Research in Biology Faculty of Universitas Sumatera Utara, with ethical number 112/KEPH-FMIPA/2018. In total, 25 young male Wistar rats (*Rattus norvegicus*) weighing 180-250 g that were obtained from the animal research were used in this study. The rats were housed in a temperature-controlled room on a 12 h light/dark schedule with food and water available ad libitum.

All the young rats divided into four groups, which were normal diet group (C0); atherogenic diet group (C1); atherogenic diet and 50mg/kg body weight (P1); atherogenic diet and 100mg/kg body weight (P2). Normal diet group given normal food pellet and for atherogenic diet gave atherogenic food pellet. The ethanolic seed's extract was diluted in water and administered to the animals by gavage for two weeks, after the induction of atherogenic. The animals were anaesthetised on the 14th day, and blood was collected by cardiac puncture after a 12-hour fast. The animals were euthanised, and collected for blood serum.

2.2 *Passiflora edulis* Sims's Seed Extract

The purple passion fruit (*Passiflora edulis* f *edulis* Sims) belongs to the genus *Passiflora* of the family Passifloraceae. It is grown in Sumatera Island, found in Berastagi Farm, North Sumatera, Indonesia. The *Passiflora edulis* Sims seed were dried at 40 °C, powdered in an industrial blender, extracted by 70%

ethanol maceration, evaporated under reduced pressure and lyophilised.

2.3 Flavonoid Analysis

The *Passiflora edulis* Sims's seed extract was analyzed by high-performance liquid chromatography (HPLC) on a Hewlett-Packard 1100 instrument equipped with a DAD detector and a LiChrospher 100 RP-18 column (5 µm), 125 × 4 mm held at 30°C, as described elsewhere. The solvent program started from 3.6% B (2% HOAc in acetonitrile) in solvent A (2% HOAc in water) up to 12% B in 20 minutes, to 20% in 30 minutes, and to 50% B in 45 minutes. Flow rate was set at 1 mL/min and compounds were monitored by UV absorption set at 280 nm for phenolic acids, 350 nm for flavonoids, and 520 nm for anthocyanins. The identity of the compounds was confirmed by comparison of their retention time and UV/ visible spectra with those of authentic materials (Rodrigues).

2.4 Total Cholesterol Analysis

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H₂O₂ is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration.

2.5 Low Density Lipoprotein and High-density Lipoprotein Analysis

A polyclonal antibody specific for LDL has been pre-coated onto a 96-well microplate with removable strips. LDL in standards and samples is competed with a biotinylated LDL sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme. Incubated in twelve minutes for LDL and fifteen minutes for HDL, absorbance is measured at 450 nm.

2.6 Triglyceride Analysis

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H₂O₂, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm.

2.7 Statistical Analysis

The results were expressed as the mean ± standard deviation; they were also submitted to one-way analysis of variance and compared using the LSD analysis

3 RESULT

The results will be discussed in three subsections, they were flavonoid analysis, total cholesterol, and triglyceride serum level of the young rats. Low and high density lipoprotein did not show significant difference in all groups, but showed lowering (LDL) and increasing (HDL) serum level, probably because of short experimental duration.

3.1 Flavonoid Analysis

In this flavonoid analysis, IC₅₀ value was calculated, ascorbic acid was used as the reference standard. The reducing power of the ethanol extract was carried out (Oyaizu, n.d.). Different concentrations of the extracts (1000, 500, 250, 125, 62.5, 31.25 µg/ml) were prepared. To all the test tubes 2.5 ml of sodium phosphate buffer followed by 2.5 ml of 1% potassium ferrocyanide solution was added. The contents were vortexed well and then incubated at 50° for 20 min. After incubation, 2.5 ml of 10 % trichloroacetic acid was added to all the tubes and centrifugation was carried out at 3000 rpm for 10 min. To 5 ml of the supernatant, 5 ml of distilled water was added. To this about 1 ml of 1% ferric chloride was added to each test tube and incubated at 35° for 10 min. The absorbance was read at 700 nm. The reducing power of the extract was linearly proportional to the concentration of the sample. Ascorbic acid was taken as reference standard (Oyaizu, n.d.).

The result of phytochemical analysis was recorded in Table 1, showed that *Passiflora edulis* Sims’s extract containing flavonoid, Tannin, and Saponin. By comparing with vitamin C standart, *Passiflora edulis* Sims’s extract showed strong antioxidant.

Table 1. Analysis of Flavonoid Content

Sample	Identity and sample	Parameter	Results		Analysis Technique		
Passiflora edulis Sims’s seed extract	Density	Phytochemical :				Colour visualisation	
		Flavonoid	Positive	-			
		Alkaloid	Wagner	Negative	-		
			Mayer	Negative	-		
			Dragendrof	Negative	-		
		Tannin	Positive	-			
		Saponin	Positive	-			
		Quinon	Negative	-			
		Steroid	Negative	-			
		Triterpenoid	Positif	-			
Antioxidant IC ₅₀ - DPPH	< 31.25	ppm	Spektrophotometry				
Vitamin C standard	Density	Antioxidant IC ₅₀ - DPPH	6.75	ppm	Spektrophotometry		

3.2 Total Cholesterol Analysis

The cholesterol total serum level showed lower serum level between C0 and C1, this may indicate that atherogenic food pellet for young rats gave atherogenic effect. Atherogenic food pellet were high in isaturated fatty acids, cholesterol, and caloric. There was significant difference between C0 (mean±standart deviation/SD: 84.5±13.7mg/dL) and P2 (mean±SD: 68.1±6.2mg/dL), it showed that 100mg/bodyweight Passiflora edulis Sims's seed extract had lowering effect to total cholesterol serum level.

Previous research showed that Passiflora edulis Sims's seed extract had phytochemical action as antioxidant (Sun et al., 2007; Szkudelski & Szkudelska, 2015; Timmers et al., 2011). The research demonstrated that Passiflora edulis Sims's seed extract could lowered blood glucose levels in mice consuming a high-fat diet. Other previous study showed anti-hypertensi effect and anti-ageing effect in rats (da Silva et al., 2013; Fusco & Pani, 2013; Pearson et al., 2008; Tchkonja et al., 2010).

Table 2. Analysis of Total Cholesterol Serum Level

Group	Groups	Mean difference	Std error	Significance
C0	C1	11.14	5.20	0.44
	P1	10.03	5.20	0.68
	P2	16.49	4.98	0.03
C1	C0	-11.14	5.20	0.44
	P1	-1.11	5.20	0.83
	P2	5.36	4.98	0.29
P1	C0	1.11	5.20	0.83
	C1	-10.03	5.20	0.68
	P2	6.47	4.98	0.21
P2	C0	-5.36	4.98	0.29
	C1	-16.49	4.98	0.03
	P1	-6.47	4.98	0.21

3.3 Triglyceride Analysis

Triglyceride lowering effects showed in group P1 and P2, that would be 50 mg and 100 mg/bodyweight Passiflora edulis Sims's seed extract. There was significant difference between C0 (mean±SD: 1.0±0.3mg/dL) and P1 (mean±SD: 0.77±0.3mg/dL). There was also significant difference between C0 (mean±SD: 1.0±0.3mg/dL) and P2 (mean±SD: 0.74±0.2mg/dL), it showed that 50 and 100mg/bodyweight Passiflora edulis Sims's seed extract had lowering effect to triglyceride serum level, but based on these result showed that

Passiflora edulis Sims's seed extract 50mg/bodyweight already showed lowering effect.

Table 3. Analysis of Triglyceride Serum Level

Group	Groups	Mean difference	Std error	Significance
C0	C1	0.35	0.153	0.04
	P1	0.32	0.153	0.05
	P2	0.35	0.147	0.02
C1	C0	-0.35	0.153	0.04
	P1	-0.02	0.153	0.89
	P2	0.09	0.147	0.95
P1	C0	-0.32	0.153	0.89
	C1	0.02	0.153	0.04
	P2	0.03	0.147	0.83
P2	C0	-0.35	0.15	0.95
	C1	-0.09	0.15	0.02
	P1	-0.03	0.15	0.83

There were several limitations to this study design, this study occurred over a short time period, and the number of rats. However, the most effective dose of Passiflora edulis Sims's seed extract to be investigated in future study.

4 CONCLUSIONS

Passiflora edulis Sims's seed extract has potential effect in lowering total cholesterol and triglyceride serum in young rats. This research also concluded that 100mg/bodyweight Passiflora edulis Sims's seed extract had lowering effect on total cholesterol serum level and 50mg/bodyweight triglyceride serum level in two weeks in young rats.

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