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Temperature-Dependent Variations in Antioxidant Activities and Phytochemical Profiles of *Passiflora edulis* Leaf Extracts

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Abstract

Passiflora edulis leaves contain bioactive compounds and have beneficial and important biological activities. The focus of this research is to pinpoint the optimum water extraction temperature and methods for the greatest antioxidant activity and phytochemical content. Dry powder of *P. edulis* leaves was extracted using aqueous solvents with temperature variations i.e., maceration (TR), 40 °C (T40), 60 °C (T60), decoction (TD), and ultrasonic-assisted extraction (UAE). All extracts were compared for antioxidant activity (DPPH, FRAP, ABTS), compound content (total polyphenolic and flavonoid content), marker assay (saponin assay using Liebermann-Burchard reagent, and quercetin assay using TLC-densitometry), and determination of the fingerprint pattern of compounds through Fourier Transform Infrared (FTIR) spectra. The antioxidant capacity demonstrated that the extract activity was dose-dependent at all procedure temperatures. Our data showed that decoction (TD) enhanced the antioxidant capacity and also levels of water-soluble flavonoids, polyphenolic, and quercetin compounds. However, the best saponin content was obtained at T60. The FTIR spectra showed the same pattern of all extract functional groups. Therefore, the increasing extraction temperature is directly related to the increase of flavonoid and polyphenol content and their antioxidant activities.

Keywords: Antioxidant activity, TPC, TFC, Saponin assay, Quercetin assay

Introduction

Passiflora edulis is a part of the Passifloraceae that is popular not only for its edible fruit but also for its leaves, which are commonly made into an infusion or decoction for medicinal purposes [1]. The plant originated in Brazil but nowadays is farmed globally. *P. edulis* is easy to plant, can grow well in the Indonesian climate, and is widely cultivated as an Australian native spread. *P. edulis* leaves have historically been utilized as a traditional medicine in many countries, including Indonesia. *P. edulis* leaves contain important secondary metabolite substances such as polyphenolics, saponins, alkaloids, tannins, flavonoids, and glycosides. The flavonoid contents reported are luteolin 7-O-(2-rhamnosylglucoside), orientin 2"-O-rhamnoside, apigenin derivatives, quercetin, and other luteolin derivatives [2-4]. Polyphenol and flavonoid compounds that are detected in *P. edulis* leaves have antioxidant properties that function to inhibit cancer invasion, and metastasis and can help prevent or counteract free radical attacks. *P. edulis* leaves are also reported to have several properties including anti-inflammatory, which can reduce histamine, serotonin, and prostaglandins; antinociceptive; antidiabetic; treating anxiety; anti-hypertension; increasing the body's immunity; protection of liver health by reducing AST and ALT; maintaining heart health; inhibition of matrix-metalloprotease-2 and 9 enzymes; and as an antibacterial [3-8]. Some studies also show these leaves contain a number of cyclopassiflosides saponins that have anti-inflammatory bioactivity [9,10].

The content of phytochemical constituents and radical scavenging capacity of extracts depends on the

solvent and extraction conditions used. It is also important to study this before utilizing it commercially. *P. edulis* has enormous development potential and wide application in the future for this economically significant plant globally, and it is in high demand as herbal tea, nutraceutical, skin care products, healthcare items, and ingredients for food, and pharmaceuticals [11-14].

Based on the description above, this paper objectives to compare the phytoconstituent content and antioxidant activity of aqueous extract of *P. edulis* leaf prepared by various temperatures. In this context, the research can demonstrate the optimal water extraction temperature to produce the highest antioxidant activity and the best phytochemical content of *P. edulis* leaves. Understanding the relationship between extraction temperature, phytochemical content, and antioxidant activity, is expected to provide useful information for the utilization of *P. edulis* leaves in pharmaceutical and functional food applications.

Materials and methods

Materials and tools

P. edulis leaves obtained from Bogor Regency, West Java, Indonesia, 2,2-Diphenyl-1-picrylhydrazyl/DPPH (SIGMA-ALDRICH, 1898-664), 2,4,6-Tris(2-pyridyl)-s-triazine/TPTZ (SIGMA-ALDRICH 3682-357), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)/ABTS (SIGMA-ALDRICH 30931-67-0), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard (Calbiochem-53188-07-1), quercetin standard (SIGMA-ALDRICH, 117-39-5), sodium carbonate, gallic acid standard (MarkHerb PHE-1-1000), Folin-Ciocalteu reagent (Sigma-Aldrich F9252), and saponin standard (Sigma-Aldrich, 8047-15-2).

The tools include ultrasonicator bath (GT Sonic-P13), microplate reader (Thermo Scientific), micropipette (Thermo Scientific), 96-well Nunc microplate, microbalance (Mettler MT5), FTIR (Shimadzu type IRSprit 206-31001-42), UV light cabinet (Camag), Food dehydrator (Irastar), spectrophotometer Uv-Vis (Shimadzu 1900), Chamber TLC Camag, CATS 4 *Software*, and Camag TLC Scanner 3.

Methods

Collection and preparation of P. edulis leaf aqueous extract (PLAE)

Two-year-old leaves of *P. edulis* were obtained from Ciapus plantation, Bogor, West Java, Indonesia. The plant was confirmed at Herbarium Depokensis (DEP), Biota Collection Room, Universitas Indonesia (No. 992/UN2.F3.11/PDP.02.00/2023). The leaves were air-dried and ground into a fine powder by a laboratory mill. For all extraction temperatures, using 200 g of dried powder of *P. edulis* leaves and water solvent was used. Each extract was dried using a dehydrator at 45 °C for ± 5×24 h.

Kinetic maceration at room temperature (TR)

Kinetic maceration at room temperature (22 - 27 °C) was carried out with a shaker at 450 rpm for 3 h, filtered and the residue was re-macerated again.

Extraction at 40 °C (T40), 60 °C (T60), and boiling point of water (TD)

The extraction was performed in a water bath with temperatures set at 40 °C (T40), 60 °C (T60), and the boiling point of water (TD). The extraction was carried out twice.

Ultrasound-assisted extraction (UAE)

The dried powder of *P. edulis* leaves was extracted in an ultrasonic bath at 30 °C, 40 kHz frequency, and 100 % power. It was then filtered and re-extracted.

Radical scavenging capacity determination of PLAE

Initial antioxidative activity using DPPH reagent

A methanol solution containing 1,500 µg/mL extract was produced. Then a concentration series of 300 - 1,500 µg/mL was made so that the final concentration of 30 - 150 µg/mL was obtained. The 20 µL of test solution was pipetted and mixed with 180 µL 0.2 mM DPPH solution. The mixture was then incubated for 30 min in the dark, and the absorbance was measured using a microplate reader with I_{max} 515 nm. Percent inhibition and IC50 values were calculated using a linear regression equation of sample concentration vs % inhibition. Trolox (concentration series 20, 40, 60, 80 and 100 µg/mL) was used as a standard [15].

Initial antioxidative activity using ABTS reagent

The 7 mM ABTS was prepared by dissolving 19.2038 mg in 5 mL of distilled water, and 2.45 mM $K_2S_2O_8$ was prepared by dissolving 3.3 mg in 5 mL of distilled water. Then mixed in a dark glass bottle, and allowed to stand for 12 - 16 h at room temperature. The 1 mL of ABTS solution that has been incubated was pipetted and mixed with 14 mL distilled water into a volumetric flask, obtaining ABTS reagent solution. Each extract was weighed 10 mg and then dissolved in methanol (10.0 mL). A series of 100 - 500 µg/mL was made in distilled water and stored in the dark. 20 µL of test solution was pipetted and mixed with 180 µL of ABTS reagent. The mixture was incubated for 30 min (according to the results of optimization of incubation time), and the absorbance was measured using a 96-well microplate reader at I_{max} 734 nm. Percent inhibition and IC_{50} values were calculated (using a linear regression equation of sample concentration vs % inhibition). Trolox (serial concentrations of 50, 60, 70, 80 and 90 µg/mL) was used as a standard [16].

Initial antioxidative activity using FRAP reagent

A 2.5 mL of TPTZ (10 in 40 mM of HCl) was mixed with acetate buffer (25 mL, pH 3.6, 300 mM), and $FeCl_3 \cdot 6H_2O$ (2.5 mL, 20 mM in water) in the ratio (10:1:1), then distilled water was added to raise the volume to 100 mL. The mixture was stood for 10 min and called FRAP reagent. The working reagent was made by pipetting 50 mL of extract solution (1,000 ppm in distilled water), adding 150 mL of FRAP reagent, incubating for 25 min, and measuring the absorbance using a 96-well microplate reader at I_{max} 593 nm. The absorbance data was compared with the absorbance curve of the Trolox solution (20 - 180 ppm) [16].

Determination of compound content of PLAE

Total flavonoid content (TFC)

The extract solution in ethanol (3,000 ppm) was pipetted as 50 µL and added to 50 µL of 2 % $AlCl_3$ reagent in a 96-well microplate. The mixture was then incubated (15 min) and kept in the dark, and the absorbance was read at I_{max} 435 nm. The absorbance data was compared with the absorbance curve of quercetin (12.5 - 200 ppm). TFC is expressed as quercetin equivalent (mg QE/g extract) [17].

Total polyphenolic content (TPC)

Extract solution in ethanol (3,000 ppm) was pipetted 2.5 mL, and added to 100 µL of Folin Ciocalteau solution (25 %), incubated for 4 min, and then 75 µL of Na-carbonate (100 g/L) was added in a 96-well microplate. The solution was kept for 120 min in the dark. The absorbance was read at 750 nm using a microplate reader. The absorbance data were compared with the gallic acid absorbance curve. TPC was expressed as gallic acid equivalent (mgGAE/g extract) [15].

Total saponin content (TSC)

One mL of the extract solution in methanol: water 1:1 (3,000 ppm) of *P. edulis* leaves was pipetted and 3.5 mL Liebermann Burchard reagent (acetic anhydride 16.7 % in concentrated H_2SO_4) was added. The solution was stood for 30 min at room temperature, and the absorbance was read at I_{max} 438 nm using a UV-Vis spectrophotometer. The absorbance data was compared with the absorbance curve of saponin (110 - 190 ppm) tested in the same way [18].

Quercetin content densitometrically

P. edulis leaf extract (3,000 ppm in methanol) was photographed for as much as 10 µL on a silica gel F254 TLC plate. A concentration series of quercetin standard solution (20 - 100 ppm in methanol) was also photographed. Furthermore, the plate of TLC was eluted in a CAMAG chamber with mobile phase Chloroform: Ethyl ethanoate: Formic acid (5:4:1). The area of each spot was measured with Camag TLC Scanner 3 and CATS 4 Software at a 350 nm, then the data were entered into the equation of the standard curve of quercetin [19].

Fourier transform infrared spectroscopic (FTIR) study

FTIR spectra were introduced to investigate any differences in the compound content of each extract. A total of 5 mg samples were introduced into the crystal plate. The infrared spectrometer at wave number 3,500 - 300 cm^{-1} and resolution 4 cm^{-1} was utilized to scan the mixture. The obtained spectra were saved in IR solution format and the spectrum data of each extract was captured in notepad form.

Statistical analysis

All studies were presented as mean ± standard error (SEM) and conducted in triplicates. Data were processed and performed in Graph Pad Prism 9 software. Evaluation of mean differences in each test was processed using ANOVA 1-way, followed by Tukey's multiple comparisons test, with p-value < 0.05. Data correlations were assessed by Pearson's correlation.

Results and discussion

It has been reported that the chemical compounds isolated from the leaves of *P. edulis* were flavonoids, triterpenoids, alkaloids, sulforaphane, carotenoids, and other compounds. The first 2 groups of compounds are reported to have activities as anti-inflammatory, neuroprotective, anxiolytic-like, sedative-like, melanin inhibition and collagen synthesis promotion, antidiabetic, and antioxidant. Several studies have shown that aqueous extracts of *P. edulis* leaves have good bioactivities such as anxiolytic effect [1]. The aqueous extracts mainly contain vicenin-2, spinosin, 6,8-di-C-glycosylchrysin, and C-glycosylflavonoids isoorientin [7,20,21].

Extraction is an essential technique in most herbal or food processing industries, to acquire desired components from natural product samples [22]. Extraction methods play a crucial role in determining the phytochemical content and antioxidant activity. Temperature variation in water-based extraction can affect the amount and type of compounds extracted. Some studies have shown that increasing the extraction temperature can increase the content of polyphenols and flavonoids which is directly related to increased antioxidant activity. However, too high an extraction temperature can cause degradation of heat-sensitive compounds [23,24], therefore, it is important to determine the optimal temperature for extraction.

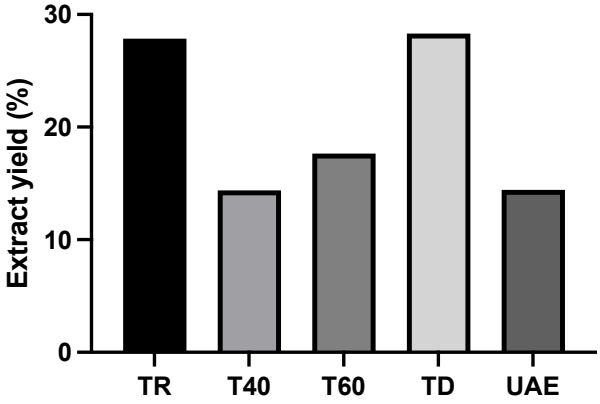


Figure 1 Extract yield of *P. edulis* leaf aqueous extract.

The amount of the extract yield obtained based on different extraction temperatures follows the order TD > TR > T60 > UAE > T40 (**Figure 1**). This test shows that extraction at the boiling point of water produces the best yield and is also in accordance with traditional use. Temperature affects the yield and the bioactive compounds contained therein. Increasing the temperature will provide effectiveness in extraction. However, the bioactive compounds contained may degrade, thus reducing the yield of the extraction [23].

Recently, there has been a fascinating search for natural substances with antioxidant activity. The antioxidant activity of natural ingredients is not only beneficial to be developed into herbal and nutraceutical preparations but also can be utilized in skin care products [24].

In the antioxidant activity test, Trolox was used as a reference according to the suggestion of Abramovič *et al.* [25] who had compared several reference compounds in the chromogenic radical test such as Trolox, ascorbic acid, chlorogenic acid, gallic acid, catechin, epigallocatechin gallate, and caffeic acid. This test shows that Trolox was more suited as a reference substance than other compounds. Trolox is a derivative of α-tocopherol, working to prevent oxidation of the membrane by inhibiting the polyunsaturated fats. Trolox

has a strong ability to remove peroxyl and alkoxyl radicals and can be soluble in water but insoluble in lipids so that it can penetrate the water and lipid compartments [26].

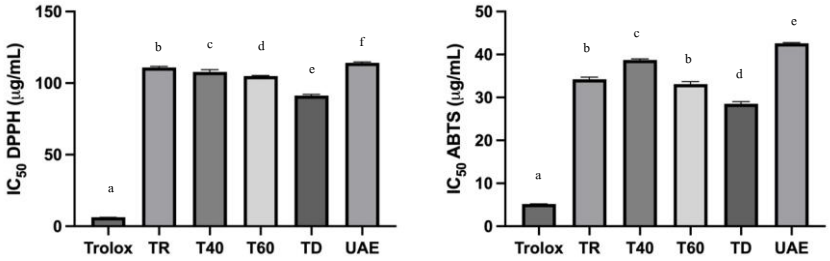
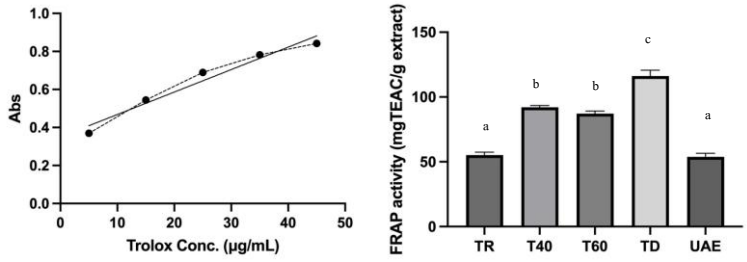


Figure 2 Initial antioxidative activity of PLAE with DPPH and ABTS reagent. Data are shown as mean ± SD, with n = 3. Analysis using 1-way ANOVA-Tukey's multiple comparisons test. Different letters (a) - (f) significantly differ at $p < 0.05$.

The results of the antioxidant activity test of PLAE with DPPH reagent showed IC_{50} values in the following order: TD < T60 < T40 < TR < UAE (**Figure 2**). This assay detected a shift in the purple color of the DPPH free radical, which transformed into a stable yellow. One antioxidant electron was given to the DPPH free radical so that the electrons become paired and a stable DPPH was formed. The concentration of the extract to reduce the DPPH radical by 50 % was assessed as lC_{50} and parameterized the antioxidant efficiency of the extract [27]. Comparative analysis indicated that all extraction methods gave significantly different DPPH radical scavenging results.

Test with ABTS reagent showed the IC_{50} value in the following order TD < T60 < TR < T40 < UAE (**Figure 2**). The principle of ABTS antioxidant testing is to capture ABTS free radicals by antioxidant compounds which will be indicated by the removal of blue color in ABTS reagent. ABTS has the advantages of a simple method, the reaction is fast and can be used to measure the activity of antioxidants that are hydrophilic or lipophilic [28]. Comparative statistical tests showed that all extraction methods gave significantly different antioxidant activity results against ABTS, except for the TR and T60 methods.

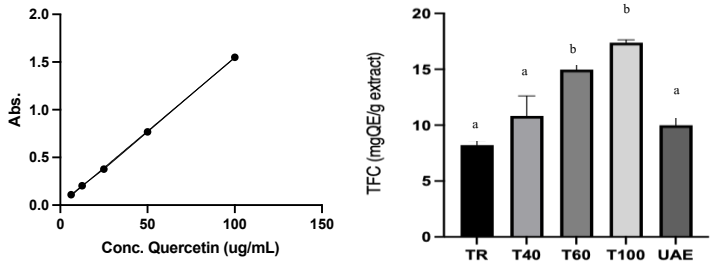


(A) (B)

Figure 3 Initial antioxidative activity of PLAE with FRAP reagent. (A) Calibration curve of Trolox with regression line $y = 0.01182x + 0.3501$ and $R^2 = 0.9610$; (B) FRAP antioxidant capacity of PLAE. Data are shown as mean ± SD, with n = 3. Analysis using 1-way ANOVA-Tukey's multiple comparisons test. Different letters (a) - (f) significantly differ at $p < 0.05$.

Activity test with FRAP reagent showed equivalence value with Trolox activity as follows: TD > T40 > T60 > TR > UAE (**Figure 3**). FRAP assay shows the ability of antioxidant compounds from the extract to reduce Fe^{3+} TPTZ ions to Fe^{2+} TPTZ ions characterized by a color change from yellow to an intensive blue color in an acidic atmosphere [29]. The test of comparison gave the results of antioxidant activity against FRAP not significantly different in T40 and T60, and also between TR and UAE. Meanwhile, the TD against other methods showed a significant difference.

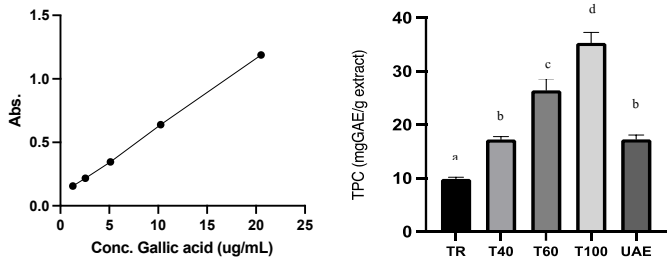
In the antioxidant test with DPPH and ABTS reagents, the smaller the IC_{50} value, the greater the antioxidant activity. While in the FRAP test, the greater the trolox equivalence, the greater the antioxidant ability. It can be seen from the 3 assays that the decoction extraction method (TD) has the greatest antioxidant capacity. This shows that the decoction method can extract the active antioxidant compounds in the PLAE more optimally and the compound content was fairly stable at boiling water temperature, this is in accordance with other studies that have shown that the decoction extracts compounds have more antioxidant activity [30].



(A) (B)

Figure 4 Total flavonoid content assay of PLAE. (A) Calibration curve of quercetin with regression line y = 0.0154x + 0.0049; R^2 = 0.9998; (B) TFC of *P. edulis* leaf aqueous extract. Data are shown as mean ± SD, with n = 3. Analysis using 1-way ANOVA-Tukey's multiple comparisons test. Different letters (a) - (f) significantly differ at $p < 0.05$.

Flavonoid-rich fraction and flavonoid compounds vicenin-2, 6,8-di-C-glycosylchrysin, and spinosin have been reported to exert anti-inflammatory activity *in vitro* and *in vivo* [5]. Determination of TFC was done using the colorimetric method with $AlCl_3$ reagent and quercetin as standards. The reaction principle is the formation of an aluminum chloride ion complex with a keto group at the C-4 atom and a hydroxyl group at the C-3/C-5 atom of flavone or flavonol compound. This complex will shift the wavelength of maximum absorption (yellow solution) and measure the absorption at 435 nm. The test results showed that TD extract had the highest flavonoid content (16.38 ± 2.00 mg QE/g), and the lowest was TR extract (9.12 ± 1.86 mg QE/g) (**Figure 4)**. This indicates that flavonoids were extracted more at high temperatures in water. Some studies state that the level of flavonoids increases with increasing temperature up to the boiling point of water [31,32]. Previous research by Santos *et al.* [33], shows that the total flavonoid content in *P. edulis* leaves was 41.10 ± 0.01 mg QE/g.

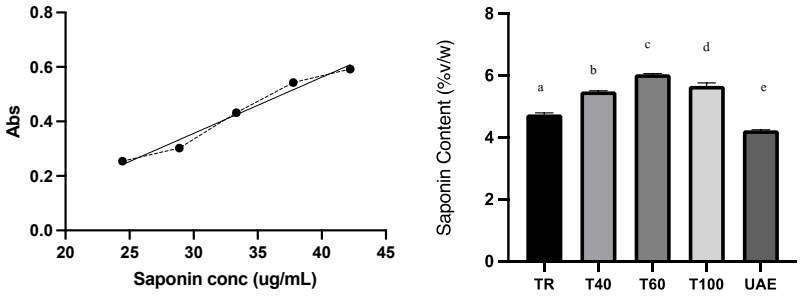


(A) (B)

Figure 5 Total polyphenolic content assay of PLAE. (A) Calibration curve of gallic acid regression line y = 0.0541x + 0.0796; R^2 = 0.9997; (B) TPC assay of *P. edulis* leaf aqueous extract. Data are shown as mean ± SD, with n = 3. Analysis using 1-way ANOVA-Tukey's multiple comparisons test. Different letters (a) - (f) significantly differ at $p < 0.05$.

TPC assay in this study was done using colorimetric method with Folin-Ciocalteu 10 % and 0.1 M sodium carbonate as a reagent, and gallic acid as a reference. The reaction principle is the formation of a Folin Ciocalteu reagent complex consisting of phosphomolybdic acid and phosphotungsten acid which

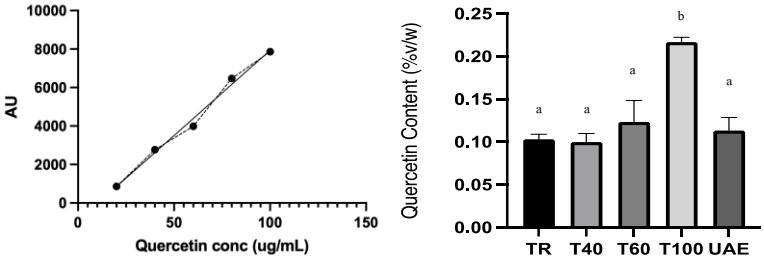
is oxidized by phenol groups then the phenolic compounds are reduced to form a tungsten-molybdenum blue oxide complex that can be measured at I_{max} 750 nm. Similar to the TFC test results, this research showed that the TD extract had the highest polyphenolic compounds and the lowest was the TR extract (**Figure 5**). This indicates that the higher the temperature of heating the water extract, the higher the polyphenol content obtained. Polyphenol extraction increased with elevated temperature and was relatively stable up to water-boiled temperature, but decreased when the temperature was raised again. This is due to various factors such as the use of solvents, variations in concentration, and the presence of enzymes such as polyphenol oxidase (PPO) and peroxidase which are widely distributed in plant tissues. These enzymes can reduce polyphenol levels because they catalyze the oxidation of phenolic compounds. PPO is an enzyme that tends to be unstable to heat and can be inactive at temperatures of 70 - 90 °C so according to the degradation of polyphenolics can be reduced and in line with the results of the study which showed the best results at boiling water temperature heating [30,34]. Research conducted previously by Silva et al showed the TPC contained in the water extract of *P. edulis* leaves was 8.3 ± 0.20 mg GAE/g [35].



(A) (B)

Figure 6 (A) Calibration curve of saponin with regression line y = 0.0206x - 0.2626; R^2 = 0.9755; (B) Saponin content of *P. edulis* leaf aqueous extract. Data are shown as mean ± SD, with n = 3. Analysis using 1-way ANOVA-Tukey's multiple comparisons test. Different letters (a) - (f) significantly differ at $p < 0.05$.

Determination of total saponin content was done by colorimetric method with Liebermann-Burchard 16.7 % as reagent and saponin as standard reference. The test principle of the formation of acetyl derivative forms of acetylation reactions of OH groups forming colored rings in steroid and triterpenoid group compounds by Liebermann-Burchard reagent with marked brownish color changes. The formation of this color changes and the absorbance was read at I_{max} 438 nm using a UV-Vis spectrophotometer [18]. The results showed that the saponin content was T60 > T100 > T40 > TR > UAE (**Figure 6**). This is quite different from TFC, TPC, and antioxidant activity testing. The results of this study are in line with the statement of Cheng *et al.* [36] in their research which states that saponins increase in levels as the temperature increases and tend to be stable in the heat but decrease in levels above 70 ℃. Another study showed that the saponin content in *Passiflora foetida* leaves was 19.6 % [37].



(A) (B)

Figure 7 Representative spectrodensitogram of standard Quercetin for the HPTLC method. Calibration curve of quercetine with regression line y = 88.495x - 919.63; R^2 = 0.991, and assay of quercetine content of *P. edulis* leaf aqueous extract. Data are shown as mean ± SD, with n = 3. Analysis using 1-way ANOVA-Tukey's multiple comparisons test. Different letters (a) - (f) significantly differ at $p < 0.05$.

Determination of quercetin content was done densitometrically by comparing the area of concentration series of quercetin standard and extract at the same Rf. TLC developed using a mobile phase mixture of $CHCl_3$: Ethyl acetate: Formic acid (5:4:1), the single peak detected on the TLC chromatogram of quercetin standard was at max Rf 0.7, while on the TLC chromatogram of the extract, the same peak was seen at Rf 0.66 - 0.71. The spectrodensitograms for the HPTLC assay were recorded and the maximum chromatography response was carried out at 350 nm for the HPTLC assay. Quercetin is one of the flavonol detected in PLAE. Similar to TFC determination, quercetin content showed the same pattern of T100 > T60 > UAE > T40 > TR (**Figure 7**). This shows that there is an effect of temperature on the ability of quercetin in water extracts, which is the higher the heating temperature, the higher the flavonoid content obtained [31,32].

Table 1 Pearson's correlation coefficients of total flavonoid, total phenolics, quercetin content, and saponin content with antioxidant activity (DPPH, ABTS and FRAP).

	Pearson correlation coefficient (r)			
	TFC	**TPC**	**Quercetin content**	**Saponin content**
DPPH	−0.8616*	−0.8749*	−0.8922*	−0.6664
ABTS	−0.6866	−0.6378	−0.7137	−0.6622
FRAP	0.8356*	0.8378*	0.7003	0.8106*

In addition, we also analyzed the correlation between antioxidant activity and phytochemical constituents TPC, TFC, quercetin content, and saponin content in PLAE (**Table 1**) due to the concept that antioxidant capacity is generally associated with these substances [4,5,38,39]. DPPH showed a strong correlation with TPC, TFC, and quercetin content ($p < 0.01$). On the other hand, ABTS values had moderate correlations with all the phytoconstituents measured. FRAP values were highly correlated with TFC and TPC ($p < 0.01$), and moderately correlated with quercetin and saponin levels. These results suggest that antioxidant test methods with different reaction mechanisms were caused by differences in the content of phytochemical constituents.

Table 2 Interpretation results of FTIR chromatogram patterns of *P. edulis* leaf extracts.

No.	**Wave number (cm^{-1})**					**Range wave number/n (cm^{-1})**	**Functional group**
	TR	**T40**	**T60**	**TD**	**UAE**		
1.	3,352.52	3,352.52	3,352.52	3,350.21	3,352.52	3,200 - 3,600	O-H phenol
2.	2,358.95	-	-	2,358.95	2,361.81	-	-

No.	Wave number (cm^{-1})					Range wave number/n (cm^{-1})	Functional group
	TR	T40	T60	TD	UAE		
3.	1,551.72	1,560.28	1,560.28	1,546.01	1,560.28	1,500 - 1,600	C=C (aromatic)
4.	1,400.54	1,403.39	1,403.39	1,403.39	1,403.39	1,340 - 1,470	C-H
5.	1,118.15	1,118.15	1,118.15	-	1,118.15	1,050 - 1,300	C-O
6.	1,086.77	1,078.22	1,078.22	1,075.36	1,078.22		
7.	1,046.84	1,046.84	1,046.84	1,046.84	1,046.84		

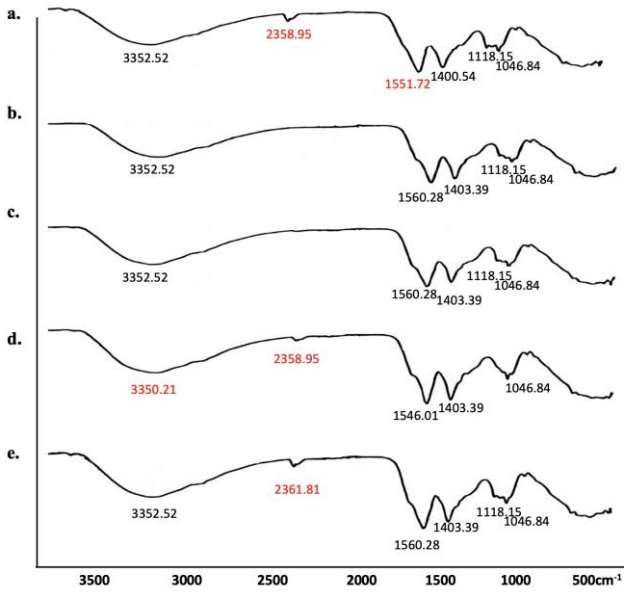


Figure 8 Chromatogram FTIR of *P. edulis* leaf aqueous extract; (a) TR, (b) T40, (c) T60, (d) TD and (e) UAE.

Based on the FTIR chromatograms, the 5 extracts have similar spectrum patterns, although there is a slight difference in T40 and T60 where no absorption peak appears at wave numbers around 2,300 cm^{-1} (**Table 2** and **Figure 8**). This has been investigated by the library which shows that the wave number around 2,300 cm^{-1} is not a specific functional group characteristic, so this can be ignored. This indicates that the water extraction method with temperature variation qualitatively has no different compound content.

Conclusions

The antioxidant capacity was assessed by several different types of assays, showing a dose-dependent effect whatever the temperature of the extraction method. The TD extract had the best antioxidant activity (DPPH, ABTS and FRAP), as well as the highest TPC, TFC, and quercetin content. However, the extract with the highest saponin content was T60. Therefore, an enhancement in antioxidant activity and the concentration of flavonoid and polyphenol substances are highly associated with an increase in extraction temperature. However, saponins will decompose at temperatures > 60 °C.

Acknowledgments

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