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Judul Artikel:	Phytochemichal identification and antioxidant activity of Passiflora Foetida fruits and leaves exctracts: a comparative study
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Phytochemical Identification and Antioxidant Activity of *Passiflorafoetida*Fruits and Leaves Extracts: A Comparative Study

Abstract

Objective: The objective of this study was to compare phytochemical composition and antioxidant activity of *Passiflora foetida*fruits and leaves extract.

Method: The parameters observed in this study were phytochemical compounds, total phenols, total flavonoids, free radical DPPH scavenging activity, and ferric reducing power.

Results: *Passiflora* leaves extract has phytochemical compound such as alkaloids, phenolics, flavonoids, saponins,and cardiac glycosides, total phenol was 22,92 ± 0,18mg GAE/g sample dry base, total flavonoid was 7,01 ± 0,10 mg CE/g sample dry base, DPPH scavenging activity was 2.77 ± 0.02 mg GAE/g sample dry base and ferric reducing power was 3,20 ± 0,04 mg GAE/g sample dry base meanwhile *Passiflora*fruits extract had phytochemical compounds such as alkaloid, phenolic, flavonoids, cardiac glycosides, total phenol was 6.53 ± 1.02 mg GAE/g sample *dry base*, total flavonoids was 1.56 ± 0.27 mg CE/g sample *dry base*, DPPH free radical scavenging activity was 1.00 ± 0.15 mg GAE/g sample *dry base*, and ferric reducing power was 1.12±0.17 mg GAE/g sample *dry base*.

Conclusion: *Passiflora* leaves extract has higher total phenol, total flavonoid and antioxidant activity measured by DPPH scavenging activity and ferric reducing power value compared with *Passiflora* fruits extract.

Keywords: *Passiflora*fruits extract, *Passiflora*leaves extract, antioxidant,

Introduction

Passiflorafoetida usually called rambusais a wild plant usually found in the tropical region and found creeping on another plant. *Passiflorafoetida* can be eaten raw as *lalapan* or used as medicine to cure many diseases like fever, headache and asthma (Lim, 2012; Quattrocchi, 2012). *Passiflorafoetida* is grouped in*the Passifloraceae* family and generally grow in humid places like river and swamp (Lim, 2012).

Passiflorafoetida can be used as traditional medicine because it contains phytochemical compound. The phytochemical compound in*Passiflorafoetida*is an alkaloid, phenolic, glycoside, flavonoid (Lim, 2012;Patil*et al.*, 2013) and cyanogenic compound that can be used as an antioxidant (Lim, 2012). *Passiflorafoetida*hasmany biological activities such as anti-inflammation, antitumor, anticancer, antimicrobe and many pharmacological activities.According to Widyawati*et al.*(2014), phytochemical compound has a contribution as an antioxidant. This

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research was conducted to compare phytochemical compound and antioxidant activity of *Passiflorafoetida*leaves and fruits extracts.

Materials and methods

Plant material: Leaves and fruits of *Passiflorafoetida*were collected from Mangrove forest region, Wonorejo, Surabaya with classification for *Passiflorafoetida*leaves were green color, has a length of ± 9 cm and width ± 10 cm, intact, and not perforated while the classification for *Passiflorafoetida* fruits were green color, has a diameter of ± 1.5 cm, intact, and flat skin surface. The plant was authenticated in the Herbarium of Biology and Food Industry Microbiology Laboratory at the Department of Food Technology, Agricultural Technology Faculty, the Widya Mandala Catholic University of Surabaya with voucher specimen no FTP-UKWMS-0002 for future reference.

***Passiflorafoetida*leaves extraction:** *Passiflorafoetida*leaves that have been collected were dried at ambient temperature,groundand sievedwith 28 mesh size. Dried flour of *Passiflorafoetida*leaveswas measured moisture content. For the extraction process, 2 g of *Passiflorafoetida*leaves dried flour was packed in a tea bag and extracted with 100 mL of hot water (95 ^{o}C). Parameters were analyzed including phytochemical content, total phenol, total flavonoid, DPPH free radical scavenging activity,andferric reducing power.

***Passiflorafoetida*fruits extraction:** *Passiflorafoetida*fruits that havebeen collected were weighted 250 g and crushed with the addition of aquadest (fruit:aquadest= 1:3). The mixture of crushed fruit was macerated with a magnetic stirrer at ambient temperature for 3 hours. The mixture was filtered and the filtrate was dried with a freeze dryer for ±72 hours. Dried powder of *Passiflorafoetida*fruits extracts weighted 1 g and dissolved in 50 mL water. Parameters were analyzed including phytochemical content, total phenol, total flavonoid, ferric reducing power, and DPPH free radical scavenging activity.

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Moisture Content: Moisture content of *Passiflorafoetida* dried leaves flour and fruit are determined with thermogravimetric method (Sudarmadji, 2007). One gram of samples is measured with the oven at 105 ^{o}C. The differenceweight between before and after heating was moisture content of the sample.

Yield Analysis: Yield analysis of Passiflorafoetida fruits was measured with the comparison between the weight of dried fruit extract and initial fresh fruit weight (% w/w dry base). The yield of Passiflorafoetida fruits was used to determine the concentration of the antioxidant compound in fresh fruits.

Phytochemical identification: Phytochemical identification was done to determine

phytochemical content in samples such as alkaloid, flavonoid, phenolic, sterol, triterpenoid, saponin, tannin, and cardiac glycoside in *Passiflorafoetida*leaves and fruits extracts.

Total phenol analysis: Total phenol analysis was determined by spectrometry method (Muntana and Prasong, 2010). 100 µL sample was added with 1 mL FolinCiocalteu 10% and 2 mL Sodium Carbonate 7,5%. The mixture was added with water in 10 mL volumetric flask and shook. The solution was incubated at ambient temperature for 30 minutes and the absorbance of the sample was measured at λ 760 nm. Total phenolic content of the sample was stated by gallic acid equivalence (GAE)/ g sample dry base.

Total flavonoid analysis: Total flavonoid analysis was determined by $AlCl_3$colorimetry method (Kumar *et al*., 2008). 200 µL sample was added with 0,3 mL $NaNO_2$ 5% (b/v), 0,3 mL $AlCl_3$ 10% (b/v), and 2 mL NaOH 1 M in 10 mL volumetric flask. The mixture shookand diluted with water until volume 10 mL.The absorbance of the sample was measured at λ 510 nm. Total flavonoid content of the sample was stated by catechin equivalence (CE)/ g sample dry base

DPPH radical scavenging activity: *Passiflorafoetida* leaves and fruits extracts antioxidant activity was measured based on the modification of Somponge*t al.* (2011) method. 3 mL of DPPH solution (4 mg/ 100 mL methanol) was added to 100 µL sample in a test tube and diluted until 5 mL. The mixture was incubated in ambient temperature for 30 minutes. The absorbance of the sample was measured at λ517 nm. DPPH radical scavenging activity was stated as % inhibition using the equation:

% inhibition: $\frac{Abs_{t=0}-Abs_{t=30}}{Abs_{t=0}} x\ 100\%$

$Abs_{t=0}$= Control absorbance

$Abs_{t=30}$= Sample absorbance

Ferric reducing power: Ferric reducing power of *Passiflorafoetida* leaves and fruits extracts were measured based on Park *et al.* (2008). 200 µL sample mixed 2,5 mL phosphate buffer (pH 6,6) and 2,5 mL potassium ferricyanide 1% and incubated at 50 ^oC for 20 minutes. 2,5 mL chlorogenic acid 10% was added to the solution and the mixture centrifuged at 3000 rpm for 10 minutes. 2,5 mL of supernatant was added with 2,5 mLaquabidest and 0,5 mL ferric chloride 0,1% and incubated for 10 minutes. The absorbance of the sample was measured at λ 700 nm. High absorbance indicates an increased ferric reducing power. Ferric reducing power of the sample was stated as gallic acid equivalence (GAE)/ g sample dry base.

Result and Discussion

Passiflorafoetida dried leaves flour and fruits had moisture around 13,51 ± 0,34% and 85,36 ± 0,36% respectively. The yield obtained from fruits extraction with aquadestwas 8,81 ± 1,36%.*Passiflorafoetida* leaves and fruits extracts contained phytochemical compound that wasshown at Table 1.

Data-informed that both leaves and fruits extract contained alkaloid, flavonoid, phenolic compound and cardiac glycoside. The difference was saponin content in leaves extract that wasn't detected in fruits extract.

Tannin, terpenoid,and sterol weren't detected in both extracts. Terpenoid and sterol was nonpolar compound (Patterson andNes, 1991) and solvent used to extract was aquadest which is a polar solvent. Tannin is a water-soluble active compound that can be found in the plant. In this study, tannin wasn't detected in both extracts. A different result was obtained from George (2017) who found tannin in both extract and Odewo*et al.* (2014) found tannin in Passiflora leaves. The difference result was caused by a difference place of plant growth that can influence the nutritional value and phytochemical content of plant (Decoteau, 2005).

Total phenol, Flavonoid, DPPH scavenging activity, and ferric reducing power shown in Figure 1, 2, 3 and 4 respectively. Total phenol and flavonoid of Passiflora leaves extract (22,92 ± 0,18 mg GAE/ g Sample dry base and 7,01 ± 0,10 mg CE/ g Sample dry base respectively) was higher compared with Passiflora fruits extract (6,53 ± 1,02 mg GAE/ g Sample dry base and 1,56 ± 0,27 mg CE/ g Sample dry base respectively). Consequently leaves extract of Passiflora leaves extract scavenging activity and reducing power was higher (2,76 ± 0,01 and 3,20 ± 0,04 mg GAE/ g Sample dry base respectively) than Passiflora fruits extract (1,00 ± 0,15 and 1,12 ± 0,17 mg GAE/ g Sample dry base respectively).

Table 1: Phytochemical compound in Passiflorafoetida leaves and fruits extracts

Sample	Alkaloid	Flavonoid	Phenolic	Saponin	Tannin	Cardiac glycoside	Terpenoid	Sterol
Leaf extract	+	+	+	+	-	+	-	-
Fruitextract	+	+	+	-	-	+	-	-

Note: + detected based on color change, - not detected based on color change

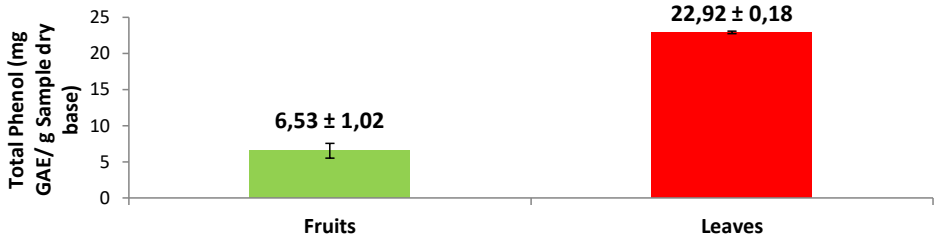

Fig. 1: Total Phenol in Passiflora Leaves and Fruits Extract

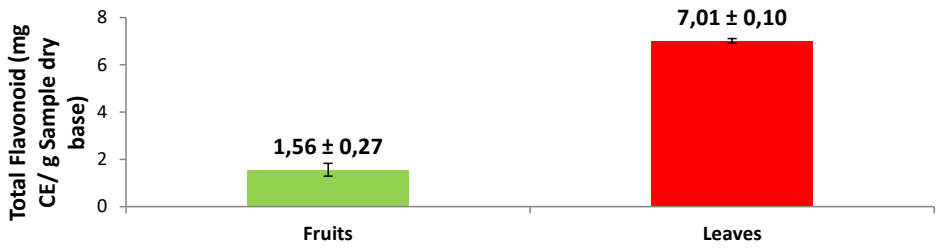

Fig. 2: Total Flavonoid in Passiflora Leaves and Fruits Extract

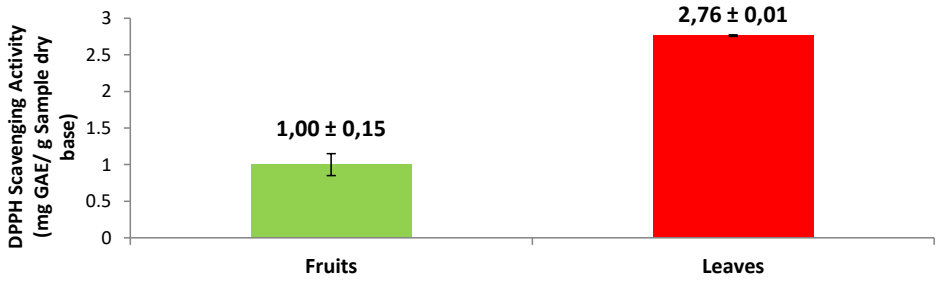

Fig. 3: DPPH Scavenging Activity in Passiflora Leaves and Fruits Extract

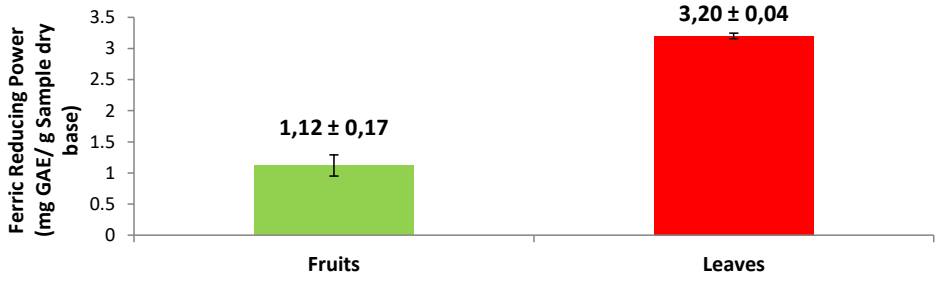

Fig. 4: Ferric Reducing Power in Passiflora Leaves and Fruits Extract

Total phenolic content of determined by some factors like enzyme activity of phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) in plant (Cheynier*et al*.,2013) and the number of free hydroxyl group in sampleDeore*et al*., 2009 inWidyawati*et al*., 2010).Formation of flavonoids is influenced by the action of CHS enzymes that form chalcone compounds which are subsequently isomerized by CHI enzyme (chalcone isomerase) into another flavonol compounds (Ramawat and Merillon, 2013).

Total phenol and flavonoid from Passiflora leaves and fruits extractswere different because a different part of the plant has different function and nutrition content. Leaves have a function for photosynthesis and place to storage nutrition (Decoteau, 2005) meanwhile fruit has the function to protect the seeds

by surrounding it with flesh containing mineral, simple organic compound,and substrate and facilitate its dispersal (Robinson, 2001)

Data in figure 1 and 2 showed that flavonoid content in both extracts was too low compared to total phenolic content. Aberoumand and Deokule (2008) in Widyawati*et al*. (2014) reported that 80% of the phenolic compound in the plant was flavonoid. High result in total phenol assay correlated with the Folin-Ciocalteu method. Ainsworth and Kelly (2007) reported that Folin-Ciocalteu method wasn't a specific method to measure total phenol. Presence of another reducing agent like aromatic amine, reducing sugar and ascorbic acid in a sample can increase the result of total phenol.

Antioxidant activity related to total phenol and flavonoid in Passiflora leaves and fruits extract that can be measured as free radical scavenging activity and ferric reducing power. The measurement was based on donation of a hydrogen atom or electron from antioxidant compound to free radical. Total phenol and flavonoid were correlated with DPPH scavenging activity and ferric reducing power (Anesini*et al*., 2008; Rorong and Suryanto, 2010; Widyawati*et al.,* 2014; Widyawati*et al.,* 2017). DPPH assay was based on decolorization of DPPH free radical from purple into yellowish color in the presence of antioxidant compound (Aksoy*et al.*, 2013; Widyawati, 2016). DPPH scavenging activity stated as inhibition rate that calculated from the difference of control and sample absorbance divided with control absorbance. Ferric reducing power was measured as secondary antioxidant activity (Pokorny*et al.,* 2001). Ferric reducing power was measured based on the ability of antioxidant compound to reduced ferric (III) iron ion to ferrous (II) iron ion that can be seen from change of color from yellow to green-prussian blue color (Liu *et al.*, 2011) Data showed that Passiflora leaves extract has higher DPPH free radical scavenging activity and ferric reducing power compared with fruits extract. This caused by a higher concentration of phenolic and flavonoid content in leaves extract. The higher concentration of the phenolic compound in the sample, the scavenging activity and ferric reducing power value will be increased (Rorong and Suryanto, 2010).

Conclusion

The result obtained in this study showed that *Passiflora* leaves extract has higher total phenol, total flavonoid and antioxidant activity measured by DPPH scavenging activity and ferric reducing power value compared with *Passiflora* fruits extract.

Acknowledgment

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CONFLICTS OF INTERESTS

Authors declare no conflicts of interest

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proportions. Int J Pharm Pharm Sci 2017;9(8):203-208

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3.	Bukti Hasil Review dan Revisi : Provisionally Accepted (Minor Revision)	8 April 2020

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[IJPPS] Your manuscript IJPPS31505: Provisionally Accepted (Minor Revision)

Kotak Masuk ×

Editor IJPPS <editor@ijppsjournal.com>
kepada saya, Paini, Tarsisius, Grace

Rab, 8 Apr 2020 18.25

Dear Yohanes Tandoro, Paini Sri Widyawati, Tarsisius Dwi Wibawa Budianta, Grace Sumargo,

We have reached a decision regarding your submission to International Journal of Pharmacy and Pharmaceutical Sciences, "Phytochemical Identification and Antioxidant Activity of Passiflora foetida Fruits and Leaves Extracts: A Comparative Study" with reference no. IJPPS 31505

Your manuscript has been recommended (Provisionally Accepted) for publication after peer review.

Acceptance will be sent on receipt of the registration fee and adequate revision (Revised article).

For publication, all the authors are required to send registration charges (US$ 50.00 per author). You are requested to inform regarding your acceptance for registration within 3-4 days.

11 dari 23

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- **Include the names of all the authors along with affiliations carefully in the main body of the manuscript to avoid any error.**
- Punctuation errors should be rectified. Deal it very critically to avoid any such error.
- Authors are advised to read the manuscript cautiously to make all corrections (mentioned previously by reviewers/editors) as per instructions to put high standards of writing and quality
- It is imperative that you ensure that the text adheres to the rules of English grammar and usage. For the most part, the text is clear, but it require improvements

(All the changes made must be highlighted with RED coloured fonts or it should be done in track change mode).

Phytochemical Identification and Antioxidant Activity of Passiflora foetida Fruits and Leaves Extracts: A Comparative Study

Abstract

Objective: The objective of this study was to compare phytochemical composition and antioxidant activity of *Passiflora foetida* fruits and leaves extract.

Method: The parameters observed in this study were phytochemical compounds including alkaloid, flavonoid, phenolic, sterol, triterpenoid, saponin, tannin, and cardiac glycoside, total phenolic content Folin Ciocalteu method; total flavonoids content with $AlCl_3$ Colorimetric method; free radical DPPH scavenging activity; and ferric reducing power using ferric chloride method .

Results: *Passiflora* leaves extract has phytochemical compound such as alkaloids, phenolics, flavonoids, saponins, and cardiac glycosides, total phenol was 22.92 ± 0.18 mg GAE/g sample dry base, total flavonoid was 7.01 ± 0.10 mg CE/g sample dry base, DPPH scavenging activity was 2.77 ± 0.02 mg GAE/g sample dry base and ferric reducing power was 3.20 ± 0.04 mg GAE/g sample dry base meanwhile *Passiflora* fruits extract had phytochemical compounds such as alkaloid, phenolic, flavonoids, cardiac glycosides, total phenol was 6.53 ± 1.02 mg GAE/g sample *dry base*, total flavonoids was 1.56 ± 0.27 mg CE/g sample *dry base*, DPPH free radical scavenging activity was 1.00 ± 0.15 mg GAE/g sample *dry base*, and ferric reducing power was 1.12 ± 0.17 mg GAE/g sample *dry base*.

Conclusion: *Passiflora* leaves extract has higher total phenol, total flavonoid and antioxidant activity measured by DPPH scavenging activity and ferric reducing power value compared with *Passiflora* fruits extract.

Keywords: *Passiflora* fruits extract, *Passiflora* leaves extract, antioxidant,

Introduction

Passiflora foetida usually called rambusa is a wild plant usually found in the tropical region and found creeping on another plant. *Passiflora foetida* can be eaten raw as *lalapan* or used as medicine to cure many diseases like fever, headache and asthma [1,2]. *Passiflora foetida* is grouped in *the Passifloraceae* family and generally grow in humid places like river and swamp [1].

Passiflora foetida can be used as traditional medicine because it contains phytochemical compound. The phytochemical compound in *Passiflora foetida is* an alkaloid, phenolic, glycoside, flavonoid and cyanogenic compound that can be used as an antioxidant [1]. *Passiflora foetida* has many biological activities such as anti-inflammation, antitumor, anticancer, antimicrobe and many pharmacological activities. [3]. This research was conducted to compare phytochemical compound and antioxidant activity of *Passiflora foetida* leaves and fruits extracts.

Telah Diformat: Judul 2, Kiri

Dikomentari [S Smith1]: Rectify or improve the language and sentence construction

Dikomentari [S Smith2]: Introduction should be elaborated. It should summarize the rationale, provides a concise research background (not an exhaustive review) and states in single sentence the objective of the study.

Materials and methods

Plant material: Leaves and fruits of *Passiflora foetida* were collected from Mangrove forest region, Wonorejo, Surabaya. Leaves and fruits of *Passiflora foetida* used for this study has different classification for *Passiflora foetida* leaves were green color, has a length of ± 9 cm and width ± 10 cm, intact, and not perforated while the classification for *Passiflora foetida* fruits were green color, has a diameter of ± 1.5 cm, intact, and flat skin surface.The plant was authenticated in the Herbarium of Biology and Food Industry Microbiology Laboratory at the Department of Food Technology, Agricultural Technology Faculty, the Widya Mandala Catholic University of Surabaya with voucher specimen no FTP-UKWMS-0002 for future reference.

Chemical reagent: aquabidest, aquadest, sodium hydroxide, chloroform, ammonia, sulfuric acid, mercury chloride, potassium iodide, iodine, methanol, ethanol, ether, acetic acid, magnesium powder, hydrochloric acid, n-amyl alcohol, ferric chloride, copper (II) sulfate, potassium sodium tartrate, gallic acid, Folin Ciocalteu, sodium carbonate, (+)-catechins, sodium nitrite, aluminium chloride, DPPH, sodium phosphate monobasic, sodium phosphate dibasic, potassium ferricyanide, chloroacetic acid.

***Passiflora foetida* leaves extraction:** *Passiflora foetida* leaves that have been collected were dried at ambient temperature, ground and sieved with 28 mesh size. Dried flour of *Passiflora foetida* leaves was measured moisture content. For the extraction process, 2 g of *Passiflora foetida* leaves dried flour was packed in a tea bag and extracted with 100 mL of hot water (95 ^{o}C). Parameters were analyzed including phytochemical content, total phenol, total flavonoid, DPPH free radical scavenging activity, and ferric reducing power.

***Passiflora foetida* fruits extraction:** *Passiflora foetida* fruits that have been collected were weighted 250 g and crushed with the addition of aquadest (fruit:aquadest= 1:3). The mixture of crushed fruit was macerated with a magnetic stirrer at ambient temperature for 3 hours. The mixture was filtered and the filtrate was dried with a freeze dryer for ±72 hours. Dried powder of *Passiflora foetida* fruits extracts weighted 1 g and dissolved in 50 mL water. Parameters were analyzed including phytochemical content, total phenol, total flavonoid, ferric reducing power, and DPPH free radical scavenging activity.

Moisture Content: Moisture content of *Passiflora foetida* dried leaves flour and fruit are determined with thermogravimetric method [4]. One gram of samples is measured with the oven at 105 ^{o}C. The difference weight between before and after heating was moisture content of the sample.

Yield Analysis: Yield analysis of *Passiflora foetida* fruits was measured with the comparison between the weight of dried fruit extract and initial fresh fruit weight (% w/w dry base). The yield of *Passiflora foetida* fruits was used to determine the concentration of the antioxidant compound in fresh fruits.

Phytochemical identification: Phytochemical identification was done to determine phytochemical content in samples such as alkaloid, flavonoid, phenolic, sterol, triterpenoid, saponin, tannin, and cardiac glycoside in *Passiflora foetida* leaves and fruits extracts.[5]

Total phenol analysis: Total phenol analysis was determined by spectrometry method [6]. 100 µL sample was added with 1 mL Folin Ciocalteu 10% and 2 mL Sodium Carbonate 7.5%. The mixture was added with water in 10 mL volumetric flask and shook. The solution was incubated at ambient temperature for 30 minutes and the absorbance of the sample was measured at λ 760 nm. Total phenolic content of the sample was stated by gallic acid equivalence (GAE)/ g sample dry base.

Total flavonoid analysis: Total flavonoid analysis was determined by $AlCl_3$ colorimetry method [7]. 200 µL sample was added with 0.3 mL $NaNO_2$ 5% (b/v), 0.3 mL $AlCl_3$ 10% (b/v), and 2 mL NaOH 1 M in 10 mL volumetric flask. The mixture shook and diluted with water until volume 10 mL. The absorbance of the sample was measured at λ 510 nm. Total flavonoid content of the sample was stated by catechin equivalence (CE)/ g sample dry base

DPPH radical scavenging activity: *Passiflora foetida* leaves and fruits extracts antioxidant activity were measured by spectrophotometer [8]. 3 mL of DPPH solution (4 mg/ 100 mL methanol) was added to 100 µL sample in a test tube and diluted until 5 mL. The mixture was incubated in ambient temperature for 30 minutes. The absorbance of the sample was measured at λ 517 nm. DPPH radical scavenging activity was stated as % inhibition using the equation:

% inhibition: $\frac{Abs_{t=0} - Abs_{t=30}}{Abs_{t=0}} x\ 100\%$

$Abs_{t=0}$= Control absorbance
$Abs_{t=30}$= Sample absorbance

Ferric reducing power: Ferric reducing power of *Passiflora foetida* leaves and fruits extracts were measured by spectrophotometer [9]. 200 µL sample mixed 2.5 mL phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide 1% and incubated at 50 ^oC for 20 minutes. 2.5 mL chlorogenic acid 10% was added to the solution and the mixture centrifuged at 3000 rpm for 10 minutes. 2.5 mL of supernatant was added with

2.5 mL aquabidest and 0.5 mL ferric chloride 0.1% and incubated for 10 minutes. The absorbance of the sample was measured at λ 700 nm. High absorbance indicates an increased ferric reducing power. Ferric reducing power of the sample was stated as gallic acid equivalence (GAE)/ g sample dry base.

Result and Discussion

Passiflora foetida dried leaves flour and fruits had moisture around 13.51 ± 0.34% and 85.36 ± 0.36% respectively. The yield obtained from fruits extraction with aquadest was 8.81 ± 1.36%. *Passiflora foetida* leaves and fruits extracts contained phytochemical compound that was shown at Table 1. Data-informed that both leaves and fruits extract contained alkaloid, flavonoid, phenolic compound and cardiac glycoside. The difference was saponin content in leaves extract that wasn't detected in fruits extract.

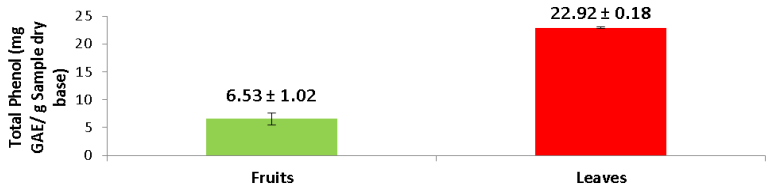
Tannin, terpenoid, and sterol weren't detected in both extracts. Terpenoid and sterol was nonpolar compound [10] and solvent used to extract was aquadest which is a polar solvent. Tannin is a water-soluble active compound that can be found in the plant. In this study, tannin wasn't detected in both extracts. A different result was obtained from [11] who found tannin in both extract and [12] found tannin in *Passiflora* leaves. The difference result was caused by a difference place of plant growth that can influence the nutritional value and phytochemical content of plant [13].

Total phenol, Flavonoid, DPPH scavenging activity, and ferric reducing power shown in Figure 1, 2, 3 and 4 respectively. Total phenol and flavonoid of Passiflora leaves extract (22.92 ± 0.18 mg GAE/ g Sample dry base and 7.01 ± 0.10 mg CE/ g Sample dry base respectively) was higher compared with *Passiflora* fruits extract (6.53 ± 1.02 mg GAE/ g Sample dry base and 1.56 ± 0.27 mg CE/ g Sample dry base respectively). Consequently leaves extract of *Passiflora* leaves extract scavenging activity and reducing power was higher (2.76 ± 0.01 and 3.20 ± 0.04 mg GAE/ g Sample dry base respectively) than *Passiflora* fruits extract (1.00 ± 0.15 and 1.12 ± 0.17 mg GAE/ g Sample dry base respectively).

Table 1: Phytochemical compound in Passiflora foetida leaves and fruits extracts

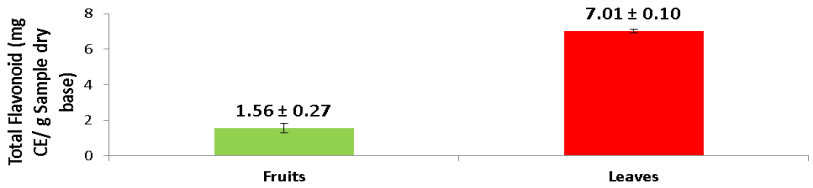
Sample	Alkaloid	Flavonoid	Phenolic	Saponin	Tannin	Cardiac glycoside	Terpenoid	Sterol
Leaf extract	+	+	+	+	-	+	-	-
Fruit extract	+	+	+	-	-	+	-	-

Note: + detected based on color change, - not detected based on color change



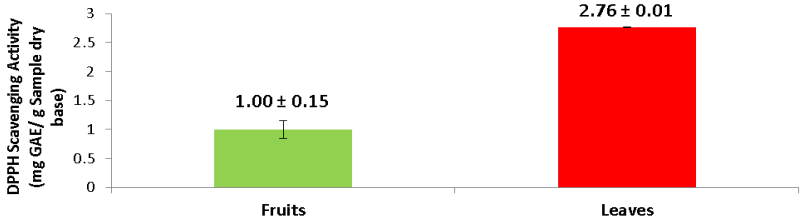
Note: each sample was replicated 5 times with results for fruits and leaves respectively was 6.53 ± 1.02 and 22.92 ± 0.18

Fig. 1: Total Phenol in Passiflora Leaves and Fruits Extract



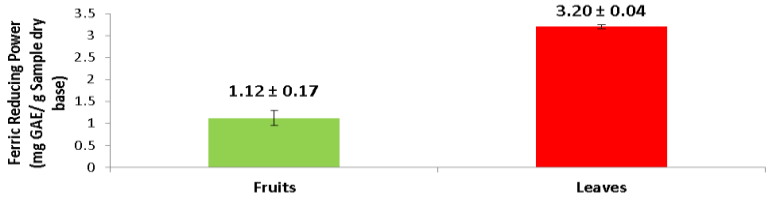
Note: each sample was replicated 5 times with results for fruits and leaves respectively was 1.56 ± 0.27 and 7.01 ± 0.10

Fig. 2: Total Flavonoid in Passiflora Leaves and Fruits Extract



Note: each sample was replicated 5 times with results for fruits and leaves respectively was 1.00 ± 0.15 and 2.76 ± 0.01

Fig. 3: DPPH Scavenging Activity in Passiflora Leaves and Fruits Extract



Note: each sample was replicated 5 times with results for fruits and leaves respectively was 1.12 ± 0.17 and 3.20 ± 0.04

Fig. 4: Ferric Reducing Power in Passiflora Leaves and Fruits Extract

Total phenolic content of determined by some factors like enzyme activity of phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) in plant [14] and the number of free hydroxyl group in sample [15]. Formation of flavonoids is influenced by the action of CHS enzymes that form chalcone compounds which are subsequently isomerized by CHI enzyme (chalcone isomerase) into another flavonol compounds [16].
Total phenol and flavonoid from *Passiflora* leaves and fruits extracts were different because a different part of the plant has different function and nutrition content. Leaves have a function for photosynthesis and place to storage nutrition [5] meanwhile fruit has the function to protect the seeds by surrounding it with flesh containing mineral, simple organic compound, and substrate and facilitate its dispersal [17]
Data in figure 1 and 2 showed that flavonoid content in both extracts was too low compared to total phenolic content. [3] reported that 80% of the phenolic compound in the plant was flavonoid. High result in total phenol assay correlated with the Folin-Ciocalteu method. [18] reported that Folin-Ciocalteu method wasn't a specific method to measure total phenol. Presence of another reducing agent like aromatic amine, reducing sugar and ascorbic acid in a sample can increase the result of total phenol.
Antioxidant activity related to total phenol and flavonoid in *Passiflora* leaves and fruits extract that can be measured as free radical scavenging activity and ferric reducing power. The measurement was based on donation of a hydrogen atom or electron from antioxidant compound to free radical. Total phenol and flavonoid were correlated with DPPH scavenging activity and ferric reducing power [3,19, 20, 21]. DPPH assay was based on decolorization of DPPH free radical from purple into yellowish color in the presence of antioxidant compound [22, 23]. DPPH scavenging activity stated as inhibition rate that calculated from the difference of control and sample absorbance divided with control absorbance. Ferric reducing power was measured as secondary antioxidant activity [24]. Ferric reducing power was measured based on the ability of antioxidant compound to reduced ferric (III) iron ion to ferrous (II) iron ion that can be seen from change of color from yellow to green-prussian blue color [25]. Data showed that *Passiflora* leaves extract has higher DPPH free radical scavenging activity and ferric reducing power compared with fruits extract. This caused by a higher concentration of phenolic and flavonoid content in leaves extract. The higher concentration of the phenolic compound in the sample, the scavenging activity and ferric reducing power value will be increased [20].

Conclusion

The result obtained in this study showed that *Passiflora* leaves extract has higher total phenol, total flavonoid and antioxidant activity measured by DPPH scavenging activity and ferric reducing power value compared with *Passiflora* fruits extract.

Acknowledgment
The authors would like to thank the PPPG (Food and Nutrition Center) of Widya Mandala Catholic University in Surabaya for the financial support for this research.

Funding
Nil

Conflicts of Interests
Authors declare no conflicts of interest

Authors Contributions
All authors have equal contributions.

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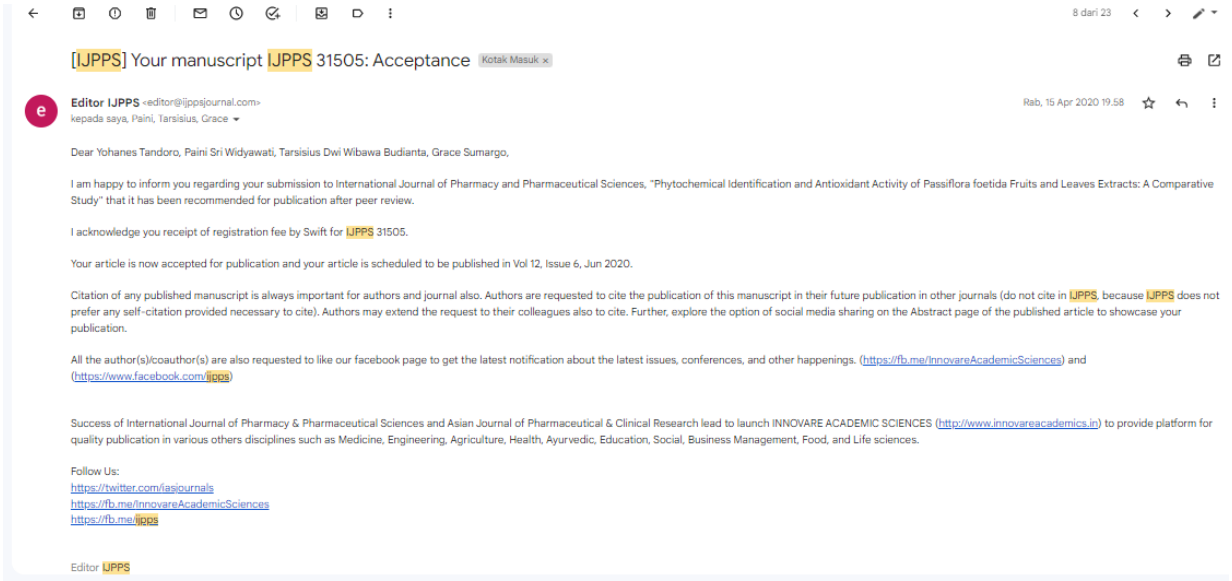
3.	Bukti Penerimaan Artikel	15 April 2020



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I am happy to inform you regarding your submission to International Journal of Pharmacy and Pharmaceutical Sciences, "Phytochemical Identification and Antioxidant Activity of Passiflora foetida Fruits and Leaves Extracts: A Comparative Study" that it has been recommended for publication after peer review.

I acknowledge you receipt of registration fee by Swift for IJPPS 31505.

Your article is now accepted for publication and your article is scheduled to be published in Vol 12, Issue 6, Jun 2020.

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Original Article

PHYTOCHEMICAL IDENTIFICATION AND ANTIOXIDANT ACTIVITY OF *PASSIFLORA FOETIDA* FRUITS AND LEAVES EXTRACTS: A COMPARATIVE STUDY

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ABSTRACT

Objective: The objective of this study was to compare the phytochemical composition and antioxidant activity of *Passiflora foetida* fruits and leaves extract.

Methods: The parameters observed in this study were phytochemical compounds including alkaloid, flavonoid, phenolic, sterol, triterpenoid, saponin, tannin, and cardiac glycoside, total phenolic content *Folin Ciocalteu* method is based on reduction of Folin Ciocalteu reagent in alkaline medium; the metal complex produced measured at λ_{max}: 760 nm; total flavonoids content with $AlCl_3$ Colorimetric method based on complex formation of $AlCl_3$ and flavonoid content in alkaline medium, the $AlCl_3$-flavonoid complex produced measured at λ_{max}: 510 nm; free radical DPPH scavenging activity; and ferric reducing power based on reduction of Fe^{3+}ion into Fe^{2+}ion that reacted with $FeCl_3$ to form a ferric-ferrous complex that measured at λ_{max}: 700 nm.

Results: *Passiflora* leaves extract has phytochemical compound such as alkaloids, phenolics, flavonoids, saponins, and cardiac glycosides, total phenol was 22.92±0.18 mg GAE/g sample dry base, total flavonoid was 7.01±0.10 mg CE/g sample dry base, DPPH scavenging activity was 2.77±0.02 mg GAE/g sample dry base and ferric reducing power was 3.20±0.04 mg GAE/g sample dry base meanwhile *Passiflora* fruits extract had phytochemical compounds such as alkaloid, phenolic, flavonoids, cardiac glycosides, total phenol was 6.53±1.02 mg GAE/g sample *dry base*, total flavonoids were 1.56±0.27 mg CE/g sample *dry base*, DPPH free radical scavenging activity was 1.00±0.15 mg GAE/g sample *dry base*, and ferric reducing power was 1.12±0.17 mg GAE/g sample *dry base*.

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Keywords: *Passiflora* fruits extract, *Passiflora* leaves extract, Antioxidant

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INTRODUCTION

Passiflora foetida, usually called rambusa is a wild plant usually found in the tropical region and found creeping on another plant. *Passiflora foetida* can be eaten raw as *lalapan* or used as medicine to cure many diseases like fever, headache and asthma [1, 2]. *Passiflora foetida* is grouped in *the Passifloraceae* family and generally grows in humid places like river and swamp [1].

Passiflora foetida can be used as a traditional medicine because it contains phytochemical compounds. The phytochemical compound in *Passiflora foetida is* an alkaloid, phenolic, glycoside, flavonoid and cyanogenic compound that can be used as an antioxidant [1]. *Passiflora foetida* has many biological activities such as anti-inflammation, antitumor, anticancer, antimicrobe and many pharmacological activities. [3]. Therefore it is necessary to conduct further research on *Passiflora foetida* leaves and fruits as a source of antioxidants. This research was conducted to compare the phytochemical compound and antioxidant activity of *Passiflora foetida* leaves and fruits extracts.

MATERIALS AND METHODS

Plant material

Leaves and fruits of *Passiflora foetida* were collected from the Mangrove forest region, Wonorejo, Surabaya. Leaves and fruits of *Passiflora foetida* used for this study has a different classification for *Passiflora foetida* leaves were green color, has a length of±9 cm and width±10 cm, intact, and not perforated while the classification for *Passiflora foetida* fruits was green color, has a diameter of±1.5 cm, intact, and flat skin surface. The plant was authenticated in the Herbarium of Biology and Food Industry Microbiology Laboratory at the Department of Food Technology, Agricultural Technology Faculty, the Widya Mandala Catholic University of Surabaya with voucher specimen no FTP-UKWMS-0002 for future reference.

Chemical reagent

Aquabidest, aquadest, sodium hydroxide, chloroform, ammonia, sulfuric acid, mercury chloride, potassium iodide, iodine, methanol, ethanol, ether, acetic acid, magnesium powder, hydrochloric acid, n-amyl alcohol, ferric chloride, copper (II) sulfate, potassium sodium tartrate, gallic acid, Folin Ciocalteu, sodium carbonate, (+)-catechins, sodium nitrite, aluminum chloride, DPPH, sodium phosphate monobasic, sodium phosphate dibasic, potassium ferricyanide, chloroacetic acid.

Passiflora foetida leaves extraction

Passiflora foetida leaves that have been collected were dried at ambient temperature, ground and sieved with 28 mesh size. The dried flour of *Passiflora foetida* leaves was measured moisture content. For the extraction process, 2 g of *Passiflora foetida* leaves dried flour was packed in a tea bag and extracted with 100 ml of hot water (95 °C). Parameters were analyzed, including phytochemical content, total phenol, total flavonoid, DPPH free radical scavenging activity, and ferric reducing power.

Passiflora foetida fruits extraction

Passiflora foetida fruits that have been collected were weighted 250 g and crushed with the addition of aqua dest (fruit: aquadest= 1:3). The mixture of crushed fruit was macerated with a magnetic stirrer at ambient temperature for 3 h. The mixture was filtered and the filtrate was dried with a freeze dryer for±72 h. Dried powder of

Passiflora foetida fruits extracts weighed 1 g and dissolved in 50 ml water. Parameters were analyzed, including phytochemical content, total phenol, total flavonoid, ferric reducing power, and DPPH free radical scavenging activity.

Moisture content

Moisture content of *Passiflora foetida* dried leaves flour and fruit are determined with the thermogravimetric method [4]. One gram of samples is measured with the oven at 105 °C. The difference weight between before and after heating was the moisture content of the sample.

Yield analysis

Yield analysis of *Passiflora foetida* fruits was measured with the comparison between the weight of dried fruit extract and initial fresh fruit weight (% w/w dry base). The yield of *Passiflora foetida* fruits was used to determine the concentration of the antioxidant compound in fresh fruits.

Phytochemical identification

Phytochemical identification was done to determine phytochemical content in samples such as alkaloid, flavonoid, phenolic, sterol, triterpenoid, saponin, tannin, and cardiac glycoside in *Passiflora foetida* leaves and fruits extracts [5].

Total phenol analysis

Total phenol analysis was determined by spectrometry method [6]. 100 µl sample was added with 1 ml Folin Ciocalteu 10% and 2 ml Sodium Carbonate 7.5%. The mixture was added with water in a 10 ml volumetric flask and shook. The solution was incubated at ambient temperature for 30 min and the absorbance of the sample was measured at λ 760 nm. The total phenolic content of the sample was stated by gallic acid equivalence (GAE)/g sample dry base.

Total flavonoid analysis

Total flavonoid analysis was determined by the $AlCl_3$ colorimetry method [7]. 200 µl sample was added with 0.3 ml $NaNO_2$ 5% (b/v), 0.3 ml $AlCl_3$ 10% (b/v), and 2 ml NaOH 1 M in 10 ml volumetric flask. The mixture shook and diluted with water until volume 10 ml. The absorbance of the sample was measured at λ 510 nm. Total flavonoid content of the sample was stated by catechin equivalence (CE)/g sample dry base

DPPH radical scavenging activity

Passiflora foetida leaves and fruits extract antioxidant activity was measured by spectrophotometer [8]. 3 ml of DPPH solution (4 mg/100 ml methanol) was added to 100 µl sample in a test tube and diluted until 5 ml. The mixture was incubated in ambient temperature for 30 min. The absorbance of the sample was measured at λ 517 nm. DPPH radical scavenging activity was stated as % inhibition using the equation:

$$\% \text{ inhibition: } \frac{Abs_{t=0} - Abs_{t=30}}{Abs_{t=0}} x\ 100\%$$

$Abs_{t=0}$= Control absorbance

$Abs_{t=30}$= Sample absorbance

Ferric reducing power

Ferric reducing power of *Passiflora foetida* leaves and fruits extracts were measured by spectrophotometer [9]. 200 µl sample mixed 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide 1% and incubated at 50 °C for 20 min. 2.5 ml chlorogenic acid 10% was added to the solution and the mixture centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was added with 2.5 ml aquabidest and 0.5 ml ferric chloride 0.1% and incubated for 10 min. The absorbance of the sample was measured at λ 700 nm. High absorbance indicates an increased ferric reducing power. The ferric reducing power of the sample was stated as a gallic acid equivalence (GAE)/g sample dry base.

RESULTS AND DISCUSSION

Passiflora foetida dried leaves flour and fruits had moisture around 13.51±0.34% and 85.36±0.36%, respectively. The yield obtained from fruits extraction with aqua dest was 8.81±1.36%. *Passiflora foetida* leaves and fruits extracts contained phytochemical compounds that were shown in table 1. Data-informed that both leaves and fruits extract contained alkaloid, flavonoid, phenolic compound and cardiac glycoside. The difference was saponin content in leaves extract that wasn't detected in fruit extract.

Tannin, terpenoid, and sterol weren't detected in both extracts. Terpenoid and sterol was nonpolar compound [10] and the solvent used to extract was aqua dest which is a polar solvent. Tannin is a water-soluble active compound that can be found in the plant. In this study, tannin wasn't detected in both extracts. A different result was obtained from [11] who found tannin in both extract and [12] found tannin in *Passiflora* leaves. The difference result was caused by a different place of plant growth that can influence the nutritional value and phytochemical content of plants [13].

Total phenol, Flavonoid, DPPH scavenging activity, and ferric reducing power shown in fig. 1, 2, 3 and 4, respectively. Total phenol and flavonoid of Passiflora leaves extract (22.92±0.18 mg GAE/g Sample dry base and 7.01±0.10 mg CE/g Sample dry base respectively) was higher compared with *Passiflora* fruits extract (6.53±1.02 mg GAE/g Sample dry base and 1.56±0.27 mg CE/g Sample dry base respectively). Consequently leaves extract of *Passiflora* leaves extract scavenging activity and reducing power was higher (2.76±0.01 and 3.20±0.04 mg GAE/g Sample dry base respectively) than *Passiflora* fruits extract (1.00±0.15 and 1.12±0.17 mg GAE/g Sample dry base respectively).

Table 1: Phytochemical compound in passiflora foetida leaves and fruits extracts

Sample	Alkaloid	Flavonoid	Phenolic	Saponin	Tannin	Cardiac glycoside	Terpenoid	Sterol
Leaf extract	+	+	+	+	-	+	-	-
Fruit extract	+	+	+	-	-	+	-	-

Note: +detected based on color change,-not detected based on color change

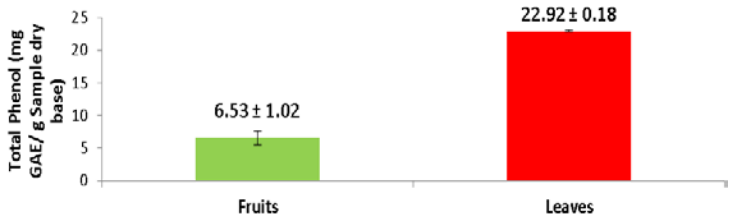

Fig. 1: Total phenol in passiflora leaves and fruits extracts, note: each sample was replicated 5 times with results for fruits and leaves respectively was 6.53±1.02 and 22.92±0.18

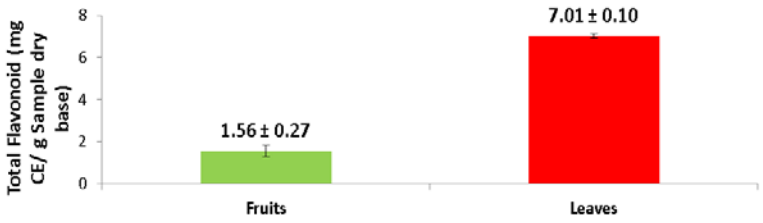

Fig. 2: Total flavonoid in passiflora leaves and fruits extract, *note: each sample was replicated 5 times with results for fruits and leaves respectively was 1.56±0.27 and 7.01±0.10*

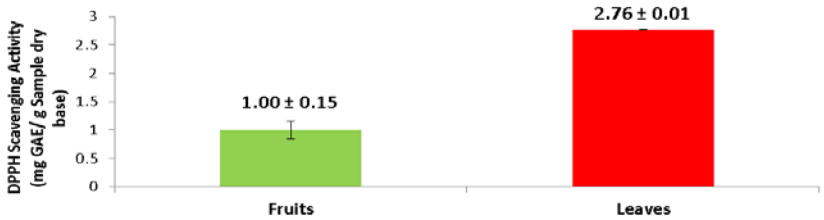

Fig. 3: DPPH scavenging activity in passiflora leaves and fruits extracts, Note: each sample was replicated 5 times with results for fruits and leaves respectively was 1.00±0.15 and 2.76±0.01

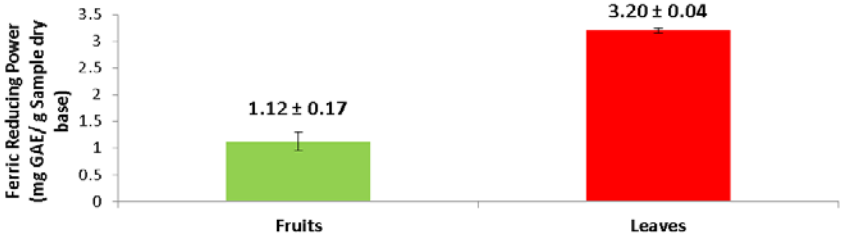

Fig. 4: Ferric reducing power in passiflora leaves and fruits extract, *Note: each sample was replicated 5 times with results for fruits and leaves respectively was 1.12±0.17 and 3.20±0.04*

Total phenolic content of determined by some factors like enzyme activity of Phenylalanine Ammonia-Lyase (PAL) and Chalcone Synthase (CHS) in plants [14] and the number of free hydroxyl group in the sample [15]. The formation of flavonoids is influenced by the action of CHS enzymes that form chalcone compounds which are subsequently isomerized by CHI enzyme (chalcone isomerase) into another flavonol compounds [16].

Total phenol and flavonoid from *Passiflora* leaves and fruits extracts were different because a different part of the plant has different functions and nutrition content. Leaves have a function for photosynthesis and place to storage nutrition [5] meanwhile fruit has the function to protect the seeds by surrounding it with flesh containing mineral, simple organic compound, and substrate and facilitate its dispersal [17].

Data in fig. 1 and 2 showed that flavonoid content in both extracts was too low compared to total phenolic content. 80% of the phenolic compound in the plant was flavonoid [3]. High results in total phenol assay correlated with the Folin-Ciocalteu method. Folin-Ciocalteu method wasn't a specific method to measure total phenol [18]. The presence of another reducing agent like aromatic amine, reducing sugar and ascorbic acid in a sample can increase the result of total phenol.

Antioxidant activity related to total phenol and flavonoid in *Passiflora* leaves and fruits extracts that can be measured as free radical scavenging activity and ferric reducing power. The measurement was based on the donation of a hydrogen atom or electron from the antioxidant compound to free radical. Total phenol and flavonoid were correlated with DPPH scavenging activity and ferric reducing power [3, 19, 20, 21]. DPPH assay was based on the decolorization of DPPH free radical from purple into yellowish color in the presence of antioxidant compounds [22, 23]. DPPH scavenging activity stated as inhibition rate that calculated from the difference of control and sample absorbance divided with control absorbance. Ferric reducing power was measured as a secondary antioxidant activity [24]. Ferric reducing power was measured based on the ability of an antioxidant compound to reduced ferric (III) iron ion to ferrous (II) iron ion that can be seen from the change of color from yellow to green-Prussian blue color [25]. Data showed that *Passiflora* leaves extract has higher DPPH free radical scavenging activity and ferric reducing power compared with fruit extract. This caused by a higher concentration of phenolic and flavonoid content in leaves extract. The higher concentration of the phenolic compound in the sample, the scavenging activity and ferric reducing power value will be increased [20].

CONCLUSION

The result obtained in this study showed that *Passiflora* leaves extract has higher total phenol, total flavonoid and antioxidant activity measured by DPPH scavenging activity and ferric reducing power value compared with *Passiflora* fruits extract.

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AUTHORS CONTRIBUTIONS

All authors have equal contributions.

CONFLICTS OF INTERESTS

Authors declare no conflicts of interest.

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