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Research Article

Total Polyphenols, Total Flavonoids, Antioxidant Activity and Inhibition of Tyrosinase Enzymes from Extract and Fraction of *Passiflora ligularis* Juss

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ABSTRACT

Background: Sweet granadilla (*Passiflora ligularis* Juss) grows in the cool highlands of Indonesia, one of which is the province of West Sumatera. Sweet granadilla has potent antioxidant activity and can inhibit the tyrosinase enzyme. **Objective:** This study was performed to determine content of total polyphenols, total flavonoids, antioxidant activity, tyrosinase inhibition in different part of *P. ligularis* extract and fraction. **Materials and Methods:** Leaves, stems, peels and seeds *P. ligularis* were separately extracted by the ultrasound-assisted extraction (UAE) method using 70% ethanol. Then, the ethanol extract was fractionated using n-hexane, ethyl acetate and distilled water. The ethanol extract and active fraction were determining antioxidant activity using FRAP and DPPH method, inhibition of tyrosinase enzyme, total polyphenol and total flavonoid content. This study was equipped with analysis of light microscopy, SEM microscopy and LC-MS. **Results:** The highest total polyphenol content was found in the seed extract 176.22 ± 1.51 mg GAE/g extract and total flavonoid content was found in leaves extract 5.77 ± 0.48 mg QE/g extract. The highest antioxidant activity by FRAP method was found in seeds extract 80.79 ± 1.29 g Fe_2SO_4 equivalent/100 g extract and DPPH method was found in stem extract with IC_{50} value 9.00 ± 0.09. The highest percentage of tyrosinase inhibition (1 mg/ml) was found in seed extract 52.4 ± 2,55 %. In fraction of seed extract show that ethyl acetate fraction most active than others. **Conclusion:** These results indicate that ethyl acetate fraction of seed *P. ligularis* has potent antioxidants and good inhibition of the tyrosinase enzyme.

Key words: Antioxidant, *Passiflora ligularis* Juss, Tyrosinase, Ultrasound-assisted extraction (UAE), Sweet granadilla.

INTRODUCTION

More than 500 species of the genus Passiflora, growing as lianas or vines climbing vines, or as arboreous or shrub species.[1] Latin America has the highest number of these species, Colombia, Brazil, Ecuador and Peru are the countries with the highest species diversity.[2] Passiflora plant parts have shown high antioxidant capacity. In general, the antioxidant capacity of passion fruit plant parts aligns with their content of phenolic compounds. Polyphenols act against the side effect caused by ultraviolet (UV) rays can act as radical scavengers in biological systems, and have the potential for photo-protection of human skin. The antioxidant capacity and presence of phenolic compounds, the sustainable benefits are very relevant and can be exploited when used in cosmetic products for skin protection and anti-aging.[3-5]

In Indonesia, passion fruit (Passiflora spp.) Indonesia is a horticultural plant cultivated from the family Passifloraceae.[6] Indonesia has three types of passion fruit, namely purple fruit (*Passiflora edulis f. edulis* Sims), yellow passion fruit (*Passiflora edulis Sims f. flavicarpa* Deg) and sweet granadilla (*Passiflora ligularis* Juss).[7] Sweet granadilla grows in the cool highlands. Its distribution is in Indonesia, New Guinea, Jamaica, Sri Lanka, India, and several other tropical areas in the world.[8] Indonesia, especially West Sumatera, has sweet granadilla (*P. ligularis*) with superior varieties of super solinda and gumanti.[9]

Non-conventional extraction methods such as Ultrasonic Assisted Extraction (UAE) stand out as a sustainable alternative that requires less solvent and energy. In addition, it is easy to use, safe, economical and reproducible.[10] Sepúlveda *et al* (2020) have extracted leaves from *P. ligularis* using the UAE extraction method.[11]

Research about *P. ligularis* is still limited. Other researchers have carried out several studies on the leaves, peels, and seeds of *P. ligularis* separately from different countries.[11,12] Therefore, this study was conducted to compare different *P. ligularis* plant parts related to their potential as antioxidants and tyrosinase inhibitors using the UAE extraction method.

MATERIALS AND METHODS

Plant material

The plant materials used were leaves, stems, peels and seeds of sweet granadilla (*Passiflora ligularis* Juss) obtained from Batu Dalam village, Danau Kembar sub-district, Solok district, West Sumatera province and determined at Herbarium Bogoriensis, LIPI, Indonesia.

Chemical

Chemicals used include the enzyme tyrosinase (Sigma Aldrich), gallic acid, quercetin, ascorbic acid, kojic acid, 3,4-Dihydroxy-L-phenylalanine (L-DOPA) (Sigma Aldrich, United States of

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America), HCl, distilled water (Brataco Chemika, Indonesia), DMSO (Merck, Germany), 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (TCI, Japan), aqua pro injection, methanol p.a, 70% ethanol, ethanol P (95% ethanol), folin-ciocalteu (Merck, Germany), potassium dihydrogen phosphate (Merck, Germany), sodium hydroxide (Merck, Germany), aluminum chloride (Merck, Germany), sodium acetate (Merck, Germany), 2,4,6-Tris(2-Pyridyl)-S-Triazine (TPTZ) (Sigma Aldrich, United States), iron (III) chloride hexahydrate (Merck, Germany), iron (II) sulfate heptahydrate (Merck, Germany).

Micromorphology

Microscopical features of leaves/stems/peels/seeds powder were observed under a light microscope (euromex holland) at the Pharmacognosy and Phytochemical Laboratory, Faculty of Pharmacy UI and Field-Emission Scanning Electron Microscopes (SEM) at CMPFA, Faculty of Engineering UI.

LC-MS test

LC-MS test of seed ethyl acetate fraction was analyzed in Characterization Laboratories Serpong, National Research and Innovation Institute through E- Layanan Sains, Badan Riset dan Inovasi Nasional.

Extraction

Leaves/stems/peels/seeds extract of *P. ligularis* was obtained by extraction using the Ultrasound-Assisted Extraction (UAE) method with an ultrasonic bath and 70 % ethanol as a solvent. 10 g dried powder of leaves/stems/peels/seeds of *P. ligularis* was put into a 100 ml Erlenmeyer container, then filled with 70% ethanol [powder (g): solvent ratio (ml) = 1:10] with a frequency of 40 kHz, temperature ± 40°C for 30 minutes.[13] The leaf/stem/peel/seed extract was filtered to take the supernatant, then evaporated using a rotary vacuum evaporator and a water bath until a thick extract was obtained.

Determination of total polyphenols

The procedure for determining total flavonoids is listed in the Indonesian Herbal Pharmacopoeia[13] with concentration adjustment. Weigh carefully ± 100 mg of extract, put into a vial, add 25 mL of methanol p.a, stir for 30 minutes with sonication. Filter into a 25 mL volumetric flask, add methanol p.a through a filter to volume. Then, pipette 1 ml of the test solution and each of the gallic acid reference solution series in a vial, add 5 mL of aqueous Folin-Ciocalteu LP (7.5% in water) and wait for 8 minutes, add 4 mL of 1% NaOH, incubate for ± 1 hour protected from light at room temperature. The absorption of each solution was measured at the maximum absorption wavelength of gallic acid at 737 nm using T80+ UV-Vis spectrophotometry. Perform blank measurements with the same procedure without adding the test solution. Make a calibration curve and calculate the concentration of the test solution. The process was carried out in triplicates.

Determination of total flavonoids

The procedure for determining total flavonoids is listed in the Indonesian Herbal Pharmacopoeia[13] with concentration adjustment. Accurately weigh ± 100 mg of extract, put it in a vial. Then, add 25 mL of ethanol P, stir for 30 minutes with sonication. Filter into a 25 mL volumetric flask, add ethanol P through a filter to volume. Separately pipette 1 mL of the test solution and each series of quercetin comparison solutions into a vial. Add to each 3 mL of ethanol P, 0.1 mL of 10% aluminum chloride, 0.2 mL of 1 M sodium acetate and 5.6 mL of double-distilled water. Shake it and wait for ± 30 minutes at room temperature. The absorption was read at the maximum wavelength of quercetin at 437 nm using T80+ UV-Vis spectrophotometry. The blank was read in the same procedure without adding aluminum chloride. Make a calibration curve and calculate the concentration of the test solution. The process was carried out in triplicates.

Fractionation

Fractionation (liquid-liquid partition) extract of the active part of sweet granadilla was carried out using a separating funnel. The solvents used were n-hexane (nonpolar), ethyl acetate (semipolar) and distilled water (polar). 850 mL of warm distilled water was added to 85 grams of ethanol extract of sweet granadilla. Then 850 mL of n-hexane was added to partition; The mixture was shaken three times for 5 minutes each time in a separating funnel, and allowed to stand for 1-hour until two layers were formed [distilled water (bottom), n-hexane (top)]. The two layers are separated. This process is repeated three times until no more compounds can be separated. The combined aqueous layer was partitioned further, as before, using ethyl acetate. The remaining fraction of the n-hexane, ethyl acetate and distilled water partition were obtained by evaporation in a water bath.

Antioxidant activity

FRAP method

Testing of antioxidant activity with the FRAP method follows the procedure that has been done with slight modifications.[14,15]

A total of 10 mg of extract from each plant part was weighed and added methanol up to 10 ml. Then, 5 mg of fraction from seed was weighed and added methanol up to 10 mL for distilled water fraction add with 50 µL DMSO p.a. Sonication was carried out for ± 5 minutes to help dissolve the extract. Then the dilution was carried out and the extract concentration series was made. Ascorbic acid was used as a positive control.

The FRAP I reagent solution for the calibration curve was prepared by mixing acetate buffer, TPTZ and distilled water (10:1:1). Meanwhile, the FRAP II reagent solution for the extract or positive control was prepared by mixing a solution of acetate buffer, TPTZ, and iron(III) chloride hexahydrate (10:1:1).

To make a calibration curve, 150 µL of FRAP I reagent solution was added to 50 µL of standard solution or distilled water (blank I). As for testing samples or positive controls, 150 L of FRAP II reagent solution was added to 50 µL of extract or ascorbic acid solution. As a blank sample or positive control (blank II), the mixture of 50 µL of methanol and 150 µL of FRAP II reagent solution was used. The mixture was shaken in a shaker on a microplate reader for 10 seconds, then incubated at 37°C for 8 minutes in the dark. Absorption measurements were carried out at a wavelength of 590 nm and shake for 30 seconds using an Elisa microplate reader (Biotek 800 TS Absorbance Reader). The test was carried out in triples.

FRAP value (µmol/g sample) = (C x V x Fp) / m

Where C is the concentration of standard solution $Fe_2SO_4.7H_2O$ which is equivalent to the sample (µmol/mL), is obtained by entering the net absorption value of the sample into the calibration curve equation. V is the sample volume (mL), Fp is the dilution factor, and m is the weight of the sample (g). The antioxidant activity of the sample is expressed as grams of Fe_2SO_4 equivalent per 100 grams of extract, which is calculated by the following equation:

Antioxidant activity = FRAP value x 10-6xMr $Fe_2SO_4.7H_2O$ x 100

DPPH method

The antioxidant activity test using the DPPH method followed the procedure done with slight adjustments.[16] 5 mg extract was dissolved in a 10 ml volumetric flask with methanol p.a to obtain of 500 µg/ml and then sonicated until all the extract dissolved. Then a series of solutions of different concentrations were made for each extract of the plant parts. The extract concentration series solution was pipetted as

much as 8 ml, then put into a dark-colored vial and added 2 ml of 0.3 mM DPPH solution. Then, it was vortexed for 20 secs and incubated at room temperature for 30 min in a dark room. Ascorbic acid was used as a positive control and then measured at a maximum wavelength of 516 nm DPPH using T80+ UV-Vis spectrophotometry. Each procedure was repeated three times.

$$\%\ Inhibition = \frac{(\text{Control absorbance} - \text{Sample absorbance})}{\text{Control absorbance}} \text{ x } 100\ \%$$

After obtaining the percentage of inhibition from each concentration, then the equation y = a + bx is determined by calculating a linear regression curve where x is the concentration (µg/ml) and y is the percentage of inhibition (%).

$$IC50 = \frac{(50 - a)}{b}$$

Tyrosinase inhibitory effect

The testing procedure was carried out based on previous research with slight modifications.[16] Each of 80 µL phosphate buffer 50 mM with pH 6.5, 40 µL of extract solution and fraction 200-1000 µg/ml (40-200 µg/ml in well), 40 µL of L-DOPA solution (4 mM), and 40 µL of tyrosinase enzyme solution (75 U/mL) were pipetted into a 96-well microplate. The solution mixture was incubated for 30 minutes at 25˚C. The absorbance was read using a microplate reader at a wavelength of 490 nm and shake for 60 seconds in Glomax microplate reader. Control samples were made without the addition of the tyrosinase enzyme.

The extract and fraction solution were prepared by dissolving with phosphate buffer for the leaves, stems, peels, n-hexane fraction and distilled water fraction. The exception for seed extract and fraction ethyl acetate were dissolved by adding 0.5 ml of DMSO 5 % and then made up to 5 ml in the vial.

Blanks were prepared by pipetting 120 µL of 50 mM phosphate buffer pH 6.5, 40 µL (4 mM) of L-DOPA solution, and 40 µL (75 U/mL) of tyrosinase enzyme solution into a 96-well microplate. The control blank was made without the addition of the mushroom tyrosinase. The test was carried out in triples.

The 5 % DMSO made with 0.5 ml of DMSO p.a and 4.5 ml of phosphate buffer 50 mM pH 6.5 was added to the vial. Blank was prepared by pipetting 80 µL of 50 mM phosphate buffer pH 6.5, 40 µL of 5 % DMSO, 40 µL of L-DOPA solution (4 mM), and 40 µL of tyrosinase enzyme solution (75 U/ml) into a 96-well microplate (GloMax). The blank control was made without the addition of the mushroom tyrosinase. The test was carried out in triples.

$$\%\ Tyrosinase\ Inhibition = \frac{(A-B)-(C-D)}{(A-B)} \text{ x } 100\ \%$$

Description: A = absorbance of blank solution with enzyme (blank), B = absorbance of blank solution without enzyme (blank control), C =

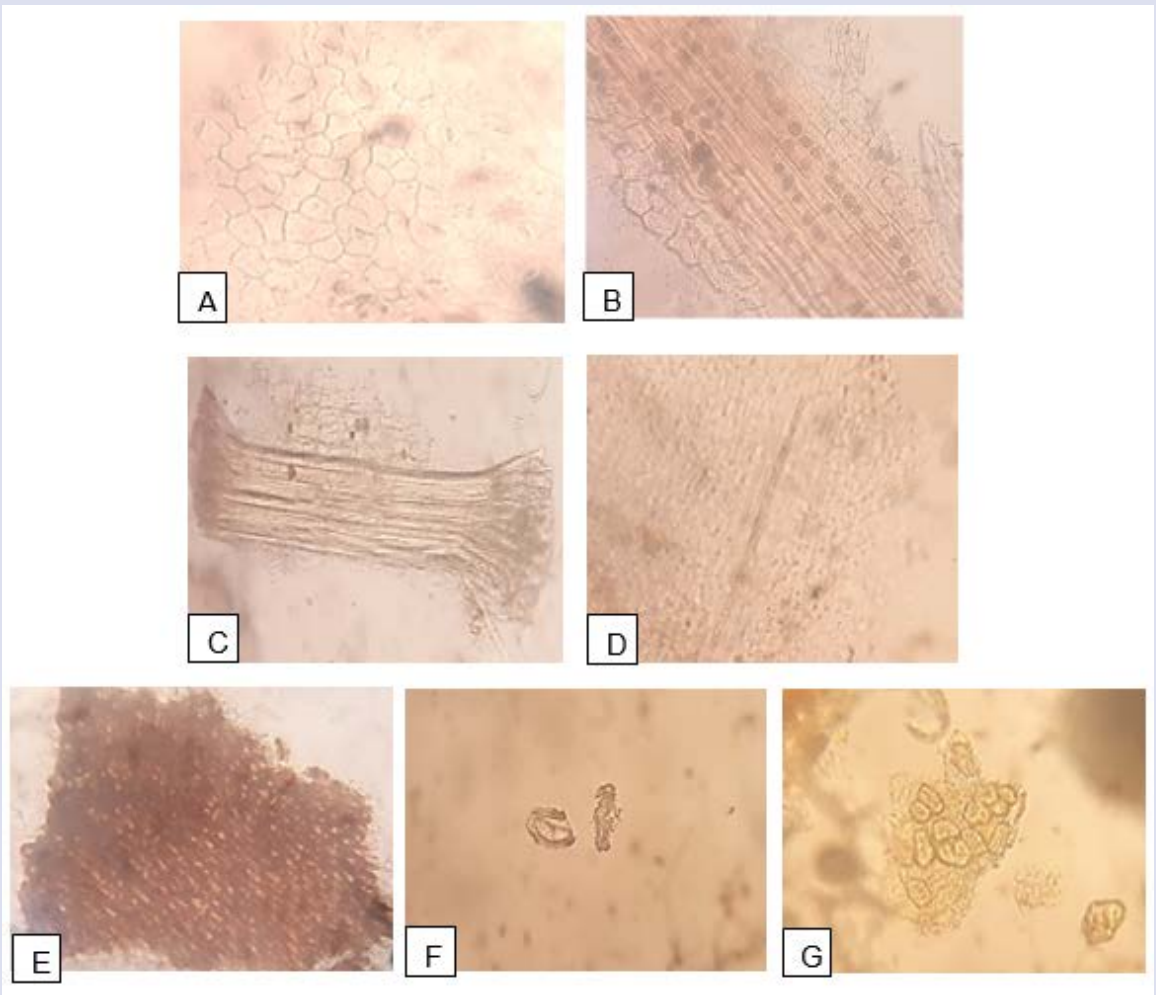

Figure 1: Morphology (A) Anisocytic stomata on leaves (B) Parenchyma with druse calcium oxalate crystals on leaves (C) Wood-fiber and calcium oxalate crystals on stems (D) Thickening of secondary walls on stems (E) Mesocarp on peels (F) Stone cells on peels and (G) Stone cells on seeds.

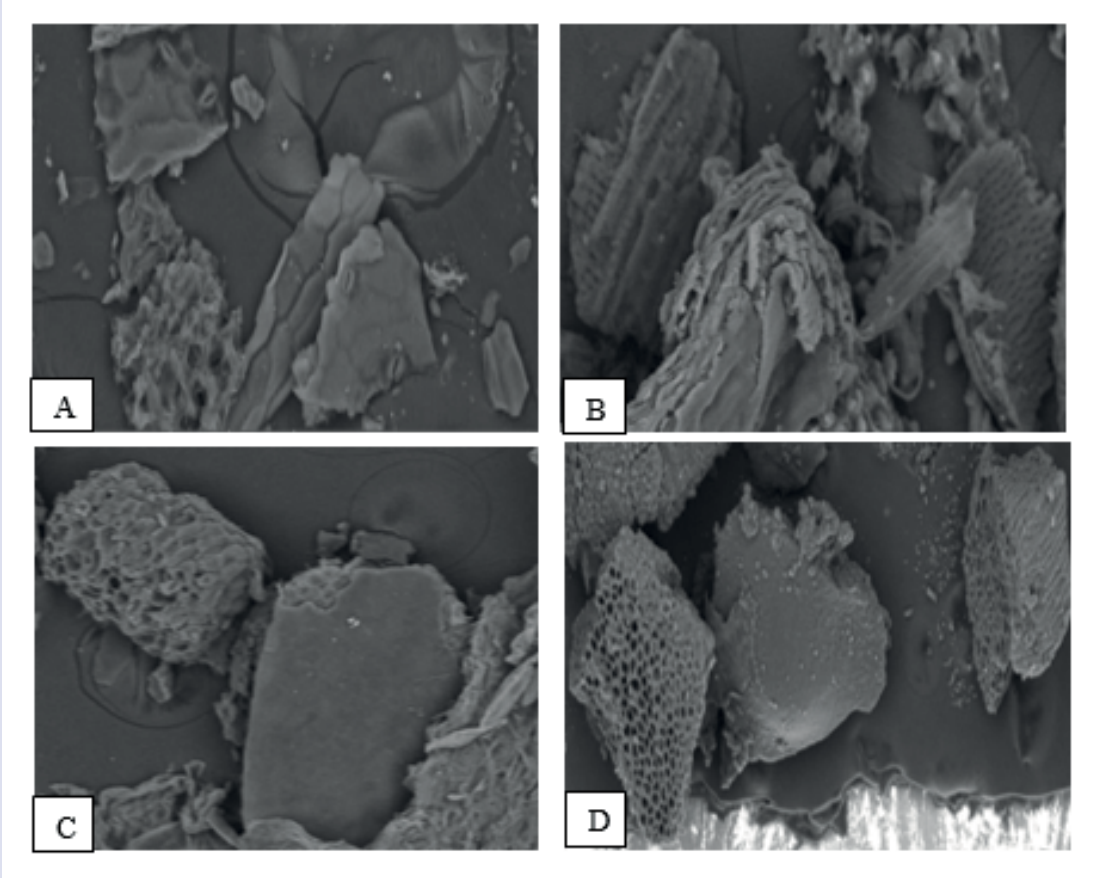

Figure 2: SEM microscopy (A) Anisocytic stomata in leaves (B) Trachea vessel in stems (C) Parenchyma in peels (D) Sclerenchyma tissue in seeds.

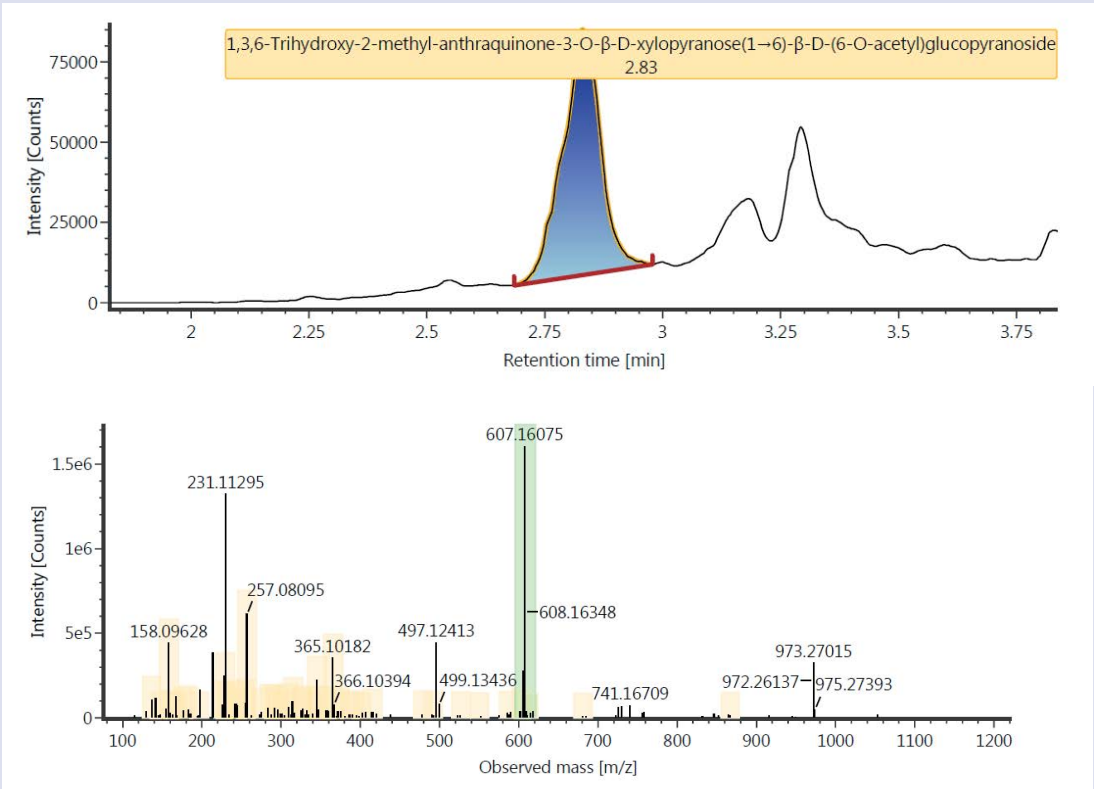

Figure 3: LC-MS of 1,3,6-Trihydroxy-2-methyl-anthraquinone-3-O-β-D-xylopyranose(1 6)-β-D-(6-O-acetyl) glucopyranoside.

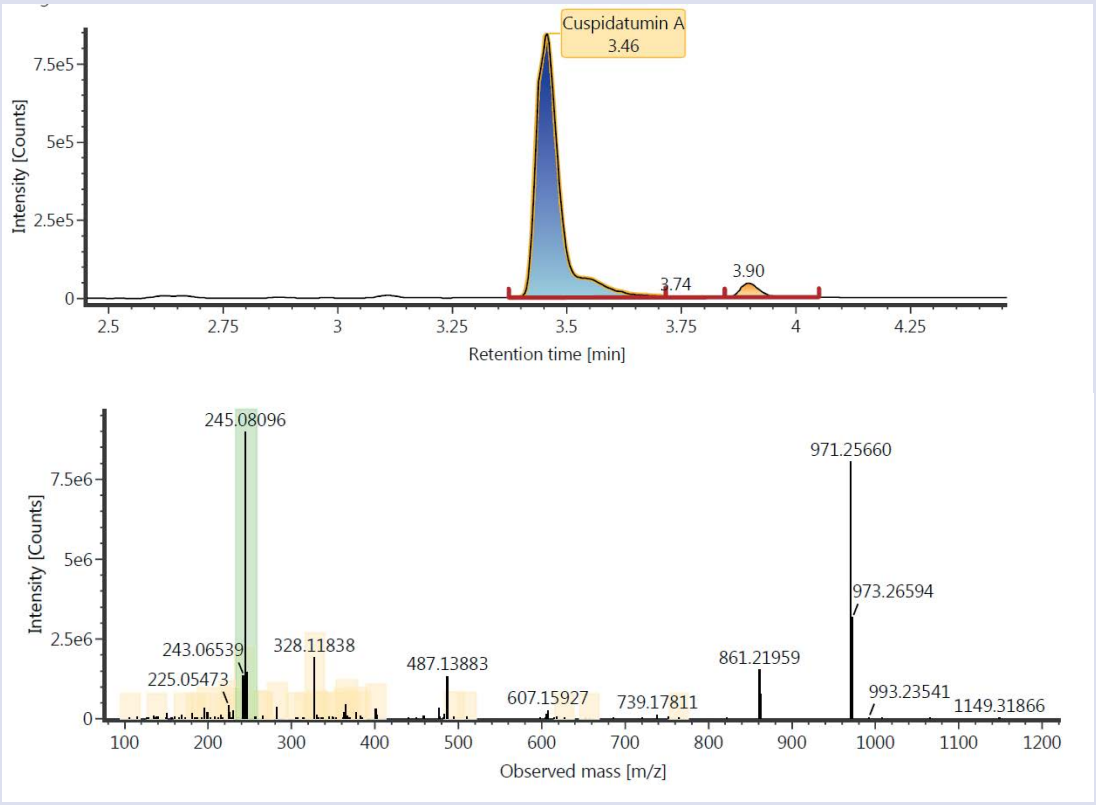

Figure 4: LC-MS of Cuspidatumin A.

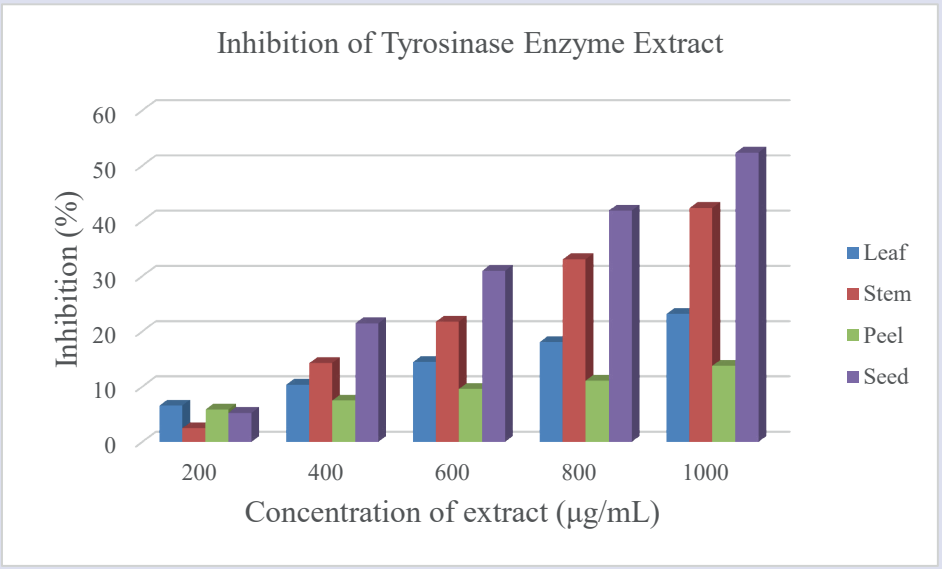

Figure 5: Inhibition of tyrosinase enzyme of *P.ligularis* extracts.

absorbance of sample solution with enzyme (sample), D = absorbance of sample solution without enzyme (sample control).

RESULTS AND DISCUSSION

LC-MS

Some of the compounds based on detector counts from the highest to lowest were Candidate Mass C28H22O8, Candidate Mass 971.2566, Candidate Mass C33H38O17, Cuspidatumin A, 1,3,6-Trihydroxy-2-methyl-anthraquinone-3-O-β-D-xylopyranose(1 6)-β-D-(6-O-acetyl) Glucopyranoside, Candidate Mass C99H11NO5, Candidate Mass C12H12N2O2, Sesamol and 5-Hydroxymethyl furoic acid.

Total polyphenol and flavonoid content

The study results showed that the highest levels of polyphenols were found in the seeds of *P. ligularis* compared to other plant parts with a value of 176.22 ± 1.51 mg GAE/g extract as shown in table 1 with concentration of sample 250-500 µg/ml. While the total flavonoid

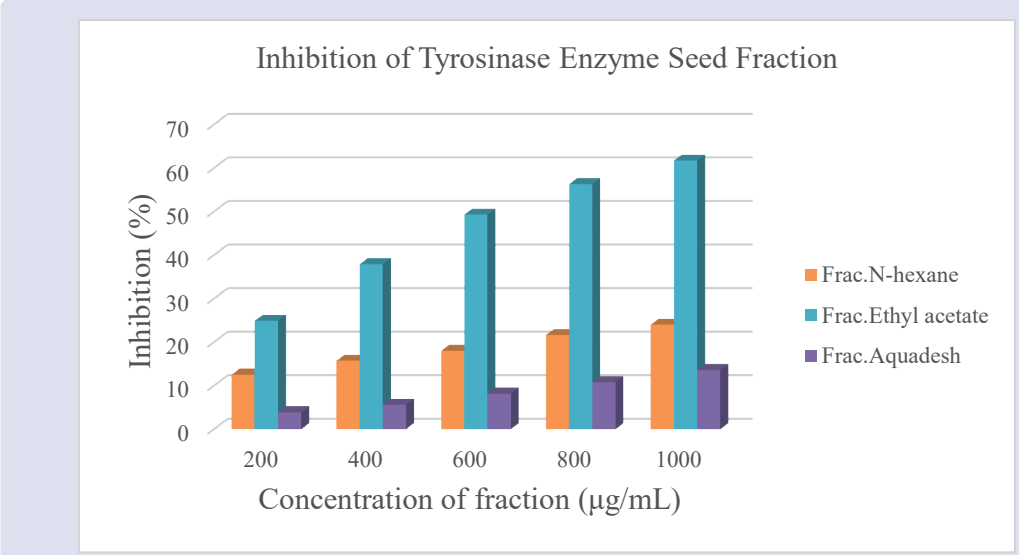

Figure 6: Inhibition of tyrosinase enzyme of *P.ligularis* seed fractions.

content based on the study results was primarily found in the leaf part of *P. ligularis* was compared to other parts with a value of 5.77 ± 0.48 mg QE/g extract as shown in table 2 with concentration of sample 1000-3000 µg/ml. This value was based on the maximum absorbance of the sample in the range of 0.2-0.8 with different concentrations.[13]

The antioxidant activity of phenolic compounds is related to their ability to scavenge free radicals, donate hydrogen atoms or electrons, or chelate metal cations.[8] Phenolic compounds are commonly found in every part of plants, and they have many biological effects.[17]

Fractionation

Based on the best results of the study of polyphenol levels, FRAP and tyrosinase enzyme inhibitory activity were found in the seeds of *P. ligularis* extract. the seed extract was fractionated using n-hexane (non polar), ethyl acetate (semi polar) and distilled water (polar).

Antioxidant activity

FRAP method

The study results of antioxidant activity testing using the FRAP method showed high antioxidant activity in the seeds extract of *P. ligularis* compared to other parts with antioxidant activity 80.79 ± 1.29 g Fe_2SO_4 equivalent/100 g extract as shown in table 3 with concentration of sample 50-200 µg/ml (12,5-50 µg/ml in well). In the seed fraction, the highest FRAP value was found in the ethyl acetate fraction with antioxidant activity 114.74 ± 7.33 g Fe_2SO_4 equivalent/100 g fraction as shown in table 4 with concentration of sample 25-100 µg/ml (6.25-25 µg/ml in well). These results are still lower than the positive control of ascorbic acid with antioxidant activity 404.71 ± 3.89 g Fe_2SO_4 equivalent/100 g extract with concentration 12 µg/ml (3 µg/ml in well).

The results of previous research, the peel extract of *P. ligularis* has a higher FRAP value than the results of this study, which is 39.9 ± 4.3 µM Fe (II)/g.[18] But different in the seeds extract, this study has a higher FRAP value than other studies on pulp and seeds extract of *P. ligularis* with a FRAP value of 42.9 ± 3.8 µM Fe (II)/g.

The FRAP test measures the reduction of the TPTZ-Fe^{+3} complex (ferrous iron) to TPTZ-Fe^{+2} (ferrous iron) in the presence of antioxidants. Since the reduction of ferrous to ferrous iron occurs rapidly with all reducing agents with a half-reaction reduction potential above Fe^{+3}/Fe^{+2}, values in the FRAP test will reveal appropriate concentrations of electron-donating antioxidants.[19]

DPPH method

Based on the results of the study of antioxidant activity using the DPPH method, the antioxidant activity was very strong, successively at the stem extract with IC_{50} 9.00 ± 0.09 µg/mL; leaves extract 9.30 ± 0,04 µg/mL and seeds extract 13.83 ± 0.02 µg/mL. Meanwhile, the peel extract showed strong antioxidant activity with the value of IC_{50} 88.23 ± 0.83 µg/mL (table 5). This shows that the stems, leaves and seeds extract of *P. ligularis* have good potential as very strong antioxidants. In the seed fraction, the highest antioxidant activity was found in the ethyl acetate fraction with IC_{50} 6.62 ± 0.05 µg/mL as shown in table 6 with concentration of sample 2-100 µg/ml. This value is lower when compared to ascorbic acid as a positive control which has a very strong antioxidant activity IC_{50} of 3.23 ± 0.12 µg/mL.

Based on the results of previous studies, the IC_{50} of seeds extract has a better value when compared to studies that have been carried out[9] on *P. ligularis* seeds extract with an IC_{50} of 54.63 ± 0.14 µg/mL and other studies conducted[18] on the pulp and seeds extract of *P. ligularis* with IC_{50} of 73.9 ± 2.7 µg/mL. In addition, the peel extract from the results of this study has an IC_{50} value higher with lower activity antioxidant when compared to the research conducted[18] on the peel extract of *P. ligularis* with an IC_{50} of 61.3 ± 2.2 µg/mL. This difference can occur due to different areas of origin of the plant, so it can affect the content of the compounds.

Tyrosinase inhibitory effect

Based on the study results, the highest tyrosinase inhibition was found in the seeds extract of *P. ligularis* with an inhibition percentage of 52.4 ± 2.55 % with concentration of sample 200-1000 µg/ml (40-200 µg/ml in well). Meanwhile, other plant parts had a smaller percentage of inhibition, as shown in table 7. In the seed fraction, the highest tyrosinase inhibition was found in ethyl acetate fraction with an inhibition percentage of 61.7 ± 0.53 % as shown in table 8 with concentration of sample 200-1000 µg/ml (40-200 µg/ml in well). This value is still lower when compared to the positive control of kojic acid with an inhibition percentage of 72.9 ± 0.48 % with concentration 15-75 µg/ml (3-15 µg/ml in well).

The percentage of inhibition of *P. ligularis* seeds extract and fraction in this study were higher when compared to other passiflora species, such as research that has been done by Lourith *et al*[12] *P. edulis* seeds fraction

Table 1: Total polyphenol content of *P. ligularis* extract.

No.	Test Substance	Total Polyphenol Content (mg GAE/g Extract) ± SD*
1.	Leaf	168.29 ± 2.22
2.	Stem	68.78 ± 1.24
3.	Peel	55.15 ± 0.12
4.	Seed	176.22 ± 1.51

Note: * Data are expressed as mean values ± SD (n = 3).

Table 2: Total flavonoid content of *P. ligularis* extract.

No.	Test Substance	Total Flavonoid Level (mg QE/g Extract) ± SD*
1.	Leaf	5.77 ± 0.48
2.	stem	4.06 ± 0.14
3.	Peel	5.23 ± 0.13
4.	Seed	5.45 ± 0.14

Note: * Data are expressed as mean values ± SD (n = 3).

Table 3: Antioxidant activity by FRAP method of *P. ligularis* extract.

No.	Test Substance	FRAP (g Fe_2SO_4 equivalent/ 100 g extract) ± SD *
1.	Leaf	77.19 ± 4.74
2.	Stem	53.19 ± 0.72
3.	Peel	8.80 ± 0.25
4.	Seed	80.79 ± 1.29
5.	Ascorbic Acid	404.71 ± 3.89

Note: * Data were expressed as mean ± SD (n = 3).

Table 4: Antioxidant activity by FRAP method of *P. ligularis* seed fraction.

No.	Fraction	FRAP (g Fe_2SO_4 equivalent/ 100 g fraction) ± SD *
1.	N-hexane	30.23 ± 0.49
2.	Ethyl acetate	114.74 ± 7.33
3.	Aquadesh	10.84 ± 0.34

Note: * Data were expressed as mean ± SD (n = 3).

Table 5: Antioxidant Activity by DPPH method from *P. ligularis* extract.

No.	Test Substance	IC_{50} (µg/mL) ± SD*
1.	Leaf	9.30 ± 0.04
2.	Stem	9.00 ± 0.09
3.	Peel	88.23 ± 0.83
4.	Seed	13.83 ± 0.02
5.	Kojic Acid	3.23 ± 0.12

Note: * Data were expressed as mean ± SD (n = 3).

Table 6: Antioxidant Activity by DPPH method from *P. ligularis* seed fraction.

No.	Fraction	IC_{50} (µg/mL) ± SD*
1.	N-hexane	16.07 ± 0.13
2.	Ethyl acetate	6.62 ± 0.05
3.	Aquadesh	186.23 ± 0.95

Note: * Data were expressed as mean ± SD (n = 3).

Table 7: Inhibition of tyrosinase enzyme from *P. ligularis* extract.

No.	Test Substance	Inhibition (%) ± SD*
1.	Leaf	23.2 ± 2.26
2.	Stem	42.4 ± 0.76
3.	Peel	13.8 ± 3.19
4.	Seed	52.4 ± 2.55
5.	Kojic Acid	72.9 ± 0.48

Note: * Data were expressed as mean ± SD (n = 3).

Table 8: Inhibition of tyrosinase enzyme from *P. ligularis* seed fraction.

No.	Fraction	Inhibition (%) ± SD*
1.	N-hexane	24.0 ± 1.05
2.	Ethyl acetate	61.7 ± 0.53
3.	Aquadesh	13.6 ± 0.42

Note: * Data were expressed as mean ± SD (n = 3).

with an inhibition percentage of fraction ethyl acetate 39.9 ± 0.0 % and fraction aqua 33.0 ± 0.5 % at a sample concentration of 1 mg/mL.

Tyrosinase is an enzyme that has an important role in melanin biosynthesis which can block melanin synthesis.[20] Therefore, the blocking of melanin synthesis through the inhibition of the tyrosinase enzyme is very important, and the seeds *P. ligularis* has good potential with a high inhibition value.

CONCLUSION

The UAE extraction method with 70% ethanol can extract various compounds from plant parts of *P. ligularis* from leaves, stems, peels and seeds. These four parts show different potencies. Seeds extract had the highest total polyphenol content with a value 176.22 ± 1.51 mg GAE/g extract, antioxidant activity with the highest FRAP was 80.79 ± 1.29 g Fe_2SO_4 equivalent/100 g extract and the highest potential for tyrosinase inhibition with the percentage reaching 52.4 ± 2.55. While the antioxidant activity test using the DPPH method was best found in stems with a value of IC_{50} 9,00 ± 0,09. In fraction of seed extract show that ethyl acetate fraction most active than others, with FRAP antioxidant activity 114.74 ± 7.33 g Fe_2SO_4 equivalent/100 g fraction, IC_{50} of DPPH 6.62 ± 0.05 µg/ml and tyrosinase inhibition 61.7 ± 0.53 %. These results indicate that *P. ligularis* seed has strong antioxidant potential and good inhibition of the tyrosinase enzyme. So that *P. ligularis* can be used in the pharmaceutical and cosmetic fields.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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ABBREVIATIONS

FRAP: Ferric Reducing Antioxidant Power

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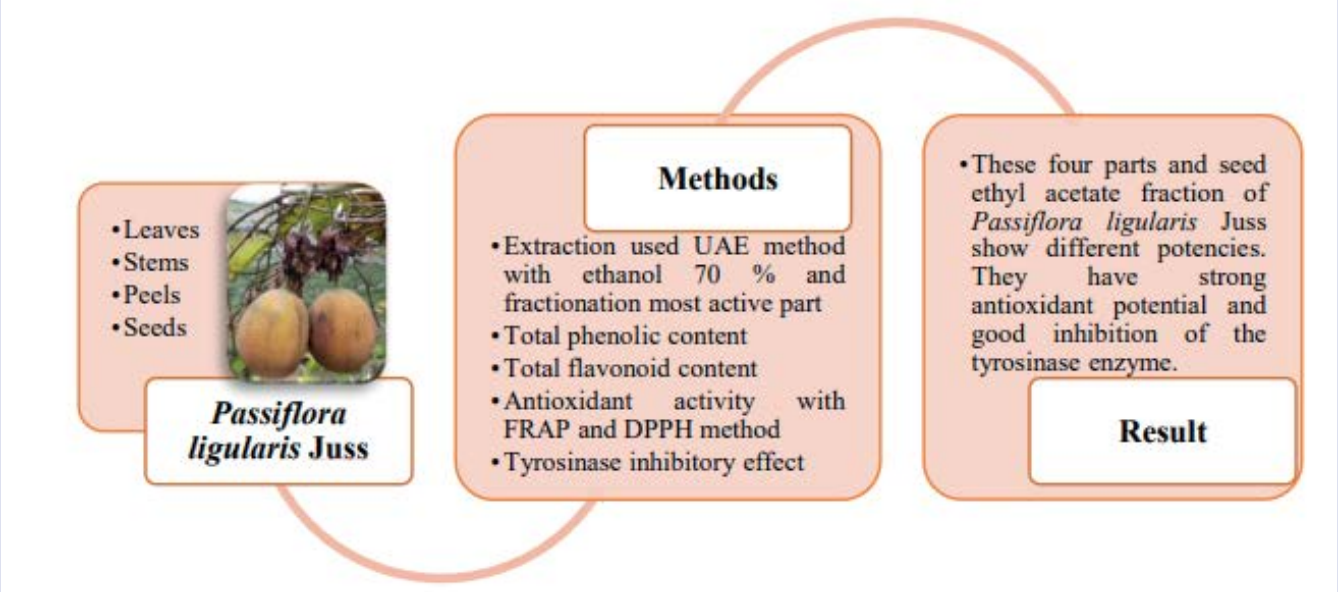
GRAPHICAL ABSTRACT



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