J. Pijar MIPA, Vol. 17 No.5, September 2022: 696-700 ISSN 1907-1744 (Cetak)
DOI: 10.29303/jpm.v17i5.3463 ISSN 2460-1500 (Online)

DETERMINATION OF FLAVONOID CONTENT OF ETHANOL AND ETHYL ACETATE EXTRACT FROM PURPLE PASSION FRUIT PEEL

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Received: April 7, 2022. Accepted: May 20, 2022. Published: September 30, 2022

Abstract: Purple passion fruit is a fruit that is widely cultivated in Indonesia for syrup and juice making. Purple passion fruit has a high nutritional value, such as antioxidants. Passion fruit contains vitamin A and C, β-carotene, flavonoid, and fiber components. However, passion fruit peel is a waste that can not be appropriately facilitated, so it becomes a waste that can pollute the environment. In addition, the passion fruit peel contains flavonoids that can be used as antidiabetic drugs. This study aimed to determine the difference in levels of flavonoid in the peel extract of purple passion fruit by using ethanol and ethyl acetate solvents. The test of total flavonoid using the $AlCl_3$ method and the absorbance was measured using a UV-Vis spectrophotometer. The results showed that the total flavonoid levels of passion fruit peel extract with ethanol and ethyl acetate solvents of 15.01 mg QE/g samples and 1.27 mg QE/g extract. The highest total flavonoid contents were obtained in ethanol extract of 15.01 mg QE/g extract.

Keywords: *Purple Passion Fruit Extract, Total Flavonoids, UV-Vis Spectrophotometer*

INTRODUCTION

Passion fruit is a plant that lives long and is included in vines. Passion fruit plants have slightly woody, posting stems and many overlapping branches. In young plants, the branches are green and, after old, become brownish-green. The shape of the leaves of this plant is ting, jagged, green, and shiny, with a stalk length of 2-3 centimeters, leaf length of 9-12 centimeters, and width of 7-9 centimeters [1]. Purple passion fruit is a fruit that is widely cultivated in Indonesia for syrup and juice making. Purple passion fruit has a high nutritional value, such as antioxidants. Passion fruit contains vitamin A and C, β-carotene, fiber, and flavonoid [2].

Passion fruit peel is a waste that can not be appropriately facilitated, so it becomes a waste that can pollute the environment. In North Sumatra, a factory produces drinks from passion fruit. In a day, the factory can produce 10-15 tons of waste in seeds and fruit peels, as much as 2-3 tons per day [3]. The waste needs to be used properly because the passion fruit peel contains highly active substances, including alkaloids, pectin, polysaccharides, and flavonoids [4].

Flavonoids are the most abundant phenolic compounds in all plants [5]. All plant parts contain flavonoids, including fruits, peels, roots, and stems. All plants that contain flavonoids can be used as antiviral, antibacterial, anti-inflammatory, anticancer, and antiallergic [6]. On the other hand, flavonoid compounds also have potential as antioxidants. The antioxidants can prevent free radicals that cause damage to the system of body's immune and the oxidation of lipids and proteins [7].

Before testing flavonoids, a sample of passion fruit peel must be extracted first. In this study, researchers used a maceration extraction method. Extraction is done to get extracts that contain highly active substances. The extraction effectiveness depends on the solvent, solvent sample, and extraction time [8]. The effectiveness of extraction of a compound by a solvent depends on the solvent solubility, like dissolve principle. *A* compound will be dissolved in a solvent with the same properties [9]. Researchers need to determine the highest flavonoid levels on the peel of passion fruit by using ethanol and ethyl acetate solvents based on the description. Flavonoid tests using the UV-Vis Spectrophotometry method.

RESEARCH METHODS

The tools used in this research are a rotary evaporator vacuum, UV-Vis spectrophotometer, and glassware. The ingredients used in this study are purple passion fruit peel, ethanol, methanol, acetyl acetate, quercetin solution, $AlCl_3$ 10%, sodium acetate 1 M, and aquadest.

Sample Preparation

The purple passion fruit peel was weighed. Next, the sample was washed with clean water and cut into small pieces. After that, the sample was dried by wind. The dried fruit peel was mashed with a blender and sold until a powder size of 60 mesh was obtained.

Extraction

Sixty grams of the powder was soaked using 120 mL ethanol solvent at room temperature for twenty-four hours. Then, it was filtered until it separated between filtrate and dreg. Next, Fitrat is

put in a container, and the dregs are re-macerated using ethanol as much as 120 mL. The maceration process is carried out for three days. Filtrate has been collected. After that, filtrate was concentrated using a rotary evaporator at 60 ^{0}C until concentrated extract was obtained [10]. The same treatment is also applied to maceration with ethyl acetate solvents. The yield calculation uses the formula:

$$\% \text{ Yield} = \frac{\text{Extract weight (g)}}{\text{Powder weight(g)}} \text{ x } 100\%$$

Determination of Total Flavonoid Levels

25 mg standard quercetin was weighed and dissolved with 25 mL ethanol. Then, the standard quercetin solution was made in the concentration of 10 – 50 mg/L. Then, it was added with 1 mL of each concentration and 0.1 mL of aluminium chloride 10%, 0.1 mL of CH_3COONa 1 M, and 2.8 mL of aquadest. The incubating process of each solution was carried out for 22 minutes. The absorbance was read at UV-Vis spectrophotometry with a wavelength of 444 nm [10].

25 mg of ethanol and ethyl acetate extract was weighed. Each extract was dissolved with 25 mL of ethanol and ethyl acetate to obtain a concentration of 1000 mg/L [10]. Then, each 2.5 mL test solution of ethanol extract and ethyl acetate concentrated 1000 mg/L was pipetted and added with 0.1 mL of aluminium chloride 10%, 0.1 mL of CH_3COONa 1 M, and 2.8 mL of aquadest. Then, the solution was incubated for 22 minutes. Finally, the absorbance was read at wavelength of 444 nm using UV-Vis spectrophotometry [10].

RESULTS AND DISCUSSIONS

Passion Fruit Peel Extraction

Extraction in this research used the maceration using ethanol and ethyl acetate solvents. Using two solvents with different polarities determines the yield and obtains the active compound based on polarity. The passion fruit peel powder was weighed and soaked with solvent until the powder was ideally submerged. Once the solvent was ideally submerged, it was stored in a dark room and was not exposed to sunlight for 24 hours. After being silenced for 24 hours, the dregs and filtration were obtained. The dreg obtained was re-macerated again using the same solvent. Maceration was done four times to attract the active compound as a whole to obtain more extracts [11-12]. The yield of passion fruit peel extract is shown in Table 1. The percentage of ethyl acetate extract yielded from the purple passion fruit peel is greater than that of ethanol extract from purple passion fruit peel. More active compounds from the purple passion fruit peel were extracted into semi-polar solvents such as ethyl acetate.

Table 1. The yield of extract of purple passion fruit peel

Extracts types	Colour	Extract weight (g)	Sample Weight (g)	Yield (%)
Ethanol	Dark brown green	0.818	60	1.36
Ethyl Acetate	Brownish-yellow	1.596	100	1.59

Determination of Total Flavonoid Levels

First, absorbance measurements were taken in a standard solution used to compare the measurement of total flavonoid compounds in the sample. Quercetin was chosen as the standard solution because it includes flavonoid compounds that effectively counteract free radicals. Quercetin is equivalent to mg QE/g of dry matter and is used as a standard for flavonoids [13] [14]. The manufacture of standard curves aims to know the relationship between the concentration and its absorbance value so that the concentration of the sample is known. An R-value close to one indicates a good correlation between quercetin levels and absorbance. The correlation coefficient (r) indicated a linear relationship between the two variables. This study obtained an r-value of 0.9981, which can be interpreted as linear or good because the result is close to 1. Based on Figure 1, the linear regression equation y = 0.0182x - 0.0072 can be used to calculate the total flavonoid level value in the test sample.

The determination of flavonoid levels in this study used a method of colorimetry with the reagent $AlCl_3$. The aluminium chloride forms a stable acidic complex with the C4 keto group and C3 or C5 hydroxyl groups of flavones and flavonols. The formation of a complex that occurs so that the shift of the wavelength towards the solution marked by the visible (visible) produces a more yellow color. [15].

In addition, $AlCl_3$ also forms a labile acid complex with orthodihydroxyl groups in rings A or B of flavonoids to have maximum absorption at a wavelength of 444 nm [10]. It then measured its uptake using a maximum wavelength ultraviolet-visible (UV-Vis) spectrophotometer. The addition of sodium acetate to flavonoid tests aims to maintain wavelengths in the visible area. The test solution was incubated for 22 minutes before the

measurement to make the reaction run perfectly so that the intensity of the resulting color was maximal. The results of this study obtained the total flavonoid levels of ethanol extract and ethyl acetate as follows table 2.

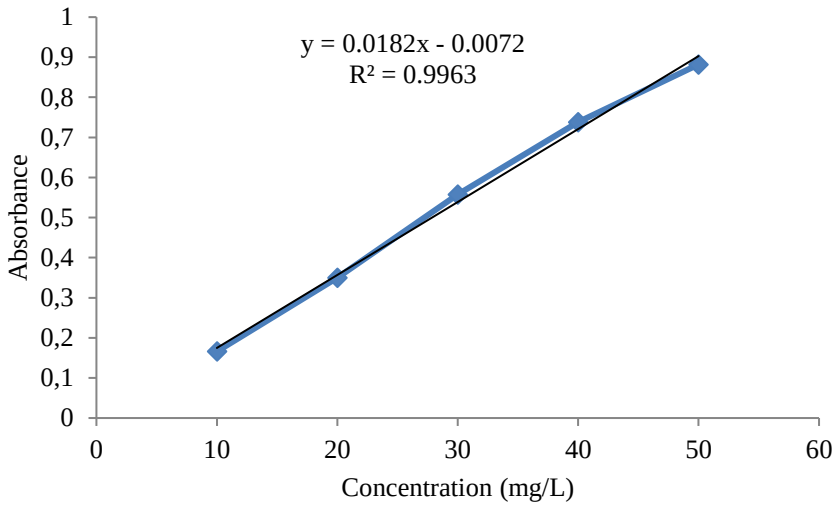

Figure 1. Quercetin standard curve

Table 2. Total flavonoid levels of purple passion fruit peel extract

Solvent	Sample Weight (g)	Sample volume (L)	Dilution factor	Absorbance	Concentration (mg/L)	Total Flavonoids (mg QE/g extract)
Ethanol	0.025	0.0025	10	0.266	15.01	15.01
Ethyl Acetate	0.025	0.0025	10	0.016	1.27	1.27

Based on Table 2, the total flavonoid levels of each test extract had different results. The total flavonoid levels of the ethanol extract was greater (15.01 mg QE/g extract) than the ethyl acetate extract (1.27 mg QE/g extract). The highest total levels of flavonoids are found in ethanol extracts because flavonoid compounds found in the passion fruit peel have better levels of polarity and solubility in ethyl acetate solvents. In addition, ethanol also has a polarity close to the polarity of phenols, so it is used as a solvent at extraction. In principle, polar compounds can dissolve in the same compounds, namely polar. Therefore, the polar ethanol solvents attract better flavonoid compounds that are polar. However, flavonoid compounds are divided into several groups with different polarity levels. This is due to the amount and position of each flavonoid group's hydroxyl group (-OH), thus affecting the solubility of flavonoids in the solvents [16]. The greater total flavonoid levels of ethanol extract than ethyl acetate extract agreed with previous studies [17]. In addition, it has also been reported that *Limnophila aromatica* ethanol extract has the largest total flavonoid levels compared to water extracts, methanol, and acetone [18]. The phenolic and flavonoid content of the ethanol extract of Tagetes erecta L. was the highest followed by the ethyl acetate and n-hexane extracts [19-21]. In another study, it was also reported that the ethanolic extract of P. emblica leaves had the highest total flavonoid content (3.594 g QE/100 g) followed by ethyl acetate and n-hexane extract [22-23]. Some of these reports suggest that ethanol solvent is suited to extract total flavonoids from plants.

CONCLUSION

The results showed total flavonoid levels of passion fruit peel extract with ethanol and ethyl acetate solvents of 15.01 and 1.27 mg QE/g extract, respectively. Based on the research results from both samples, the ethanol extract has higher total flavonoid contents.

REFERENCES

[1] Septiani, N. (2018). Uji Aktivitas Antibakteri Ekstrak Etanol dan Ekstrak Etanol dan Fraksi-Fraksi Kulit Buah Markisa Ungu (*Passiflora edulis* Sims) terhadap Bakteri *Staphylococcus epidermidis* di Laboratorium Fitokimia dan Mikrobiologi Farmasi USU. Universitas Sumatera Utara.

[2] Kusumastuty, I. (2014). Sari Buah Markisa Ungu Mencegah Peningkatan MDA Serum Tikus dengan Diet Aterogenik. *Indonesian Journal of Human Nutrition*, *1*(1), 50–56.

[3] Rozali. (2017). Studi Pemanfaatan Tepung Kulit Buah Markisa (*Passifloraedulis Sims*) sebagai Bahan Pengawet Minyak Goreng Curah. Univ. Muhammadiyah Sumatera Utara.

[4] Widodo, T. B. N. (2021). Aktivitas Kombinasi Ekstrak Etanol Kulit Markisa (*Passiflora edulis* Sims) dan Kulit Alpukat (*Persea americana* Mill) terhadap Kelarutan Kalsium Oksalat. *Jurnal Kimia (Journal Of Chemistry)*, *15*(2): 121–130.

[5] Azizah, D. N., Kumolowati, E. & Faramayuda, F. (2014). Penetapan Kadar Flavonoid Metode $AlCl_3$ pada Ekstrak Metanol Kulit Buah Kakao (*Theobroma cacao* L.). *Kartika Jurnal Ilmiah Farmasi*, 2(2):45–49.

[6] Sapitri, R. (2020). Analisis Kadar Senyawa Flavonoid Total dan Tanin Total Ekstrak Etanol Kulit Buah Jeruk Manis (*Citrus aurantium* L.) Menggunakan Metode Spektrofotometri UV-VIS. Universitas Al-Ghifari

[7] Aminah, A., Tomayahu, N. & Abidin, Z. (2017). Penetapan Kadar Flavonoid Total Ekstrak Etanol Kulit Buah Alpukat (*Persea americana* Mill.) dengan Metode Spektrofotometri UV-Vis. *Jurnal Fitofarmaka Indonesia*, *4*(2): 226–230.

[8] Sa'adah, H., Nurhasnawati, H. & Permatasari, V. (2017). Pengaruh Metode Ekstraksi Terhadap Kadar Flavonoid Ekstrak Etanol Umbi Bawang Dayak (*Eleutherine palmifolia* (L.) Merr) dengan Metode Spektrofotometri. *Borneo Journal of Pharmascientech*, *1*(1): 1–9.

[9] Sulistyo, S. (2019). Pengaruh Jenis dan Konsentrasi Pelarut terhadap Randemen Ekstrak Flavonoid Daun Sawo Duren (*Crysophillum cainito* L.) dengan Metode Maserasi. Universitas Muhammadiyah Surakarta.

[10] Pujiastuti, E. & El'Zeba, D. (2021). Perbandingan Kadar Flavonoid Total Ekstrak Etanol 70 % dan 96 % Kulit Buah Naga Merah *Hylocereus polyrhizus*. *Cendekia Journal of Pharmacy*, *5*(1): 28–43.

[11] Jovanović, A. A., Đorđević, V. B., Zdunić, G. M., Pljevljakušić, D. S., Šavikin, K. P., Gođevac, D. M., Bugarski, B. M. (2017). Optimization of the Extraction Process of Polyphenols from *Thymus serpyllum* L. Herb Using Maceration, Heat-and Ultrasound-Assisted Techniques. *Separation and Purification Technology*,179: 369–380.

[12] Chuo, S. C., Nasir, H. M., Mohd-Setapar, S. H., Mohamed, S. F. Ahmad, A., Wani, W. A. Muddassir, M. & Alarifi, A. (2020). Glimpse into the Extraction Methods of Active Compounds from Plants. *Critical Reviews in Analytical Chemistry*, 1–30,

[13] Ei, D. O. W. (2004). Antioxidant Activity of a Flavonoid-Rich Extract of *Hypericum perforatum* L. in Vitro. *Journal of Agricultural and Food Chemistry*, 52: 5032–5039.

[14] Sulaiman, M., Mohammed, S. & Manan, F. A. (2015). Analysis of Total Phenolics, Tannins and Flavonoids From *Moringa oleifera* Seed Extract. *Journal of Chemical and Pharmaceutical Research*, *7*(1) :132–135.

[15] Azizah, M. N. (2019). Penentuan Aktivitas Antioksidan dan Antidiabetes Ekstrak Daun Ndok-Ndokan (*Xanthophyllum Vitellinum*). Universitas Jember.

[16] Lestari, E. D. (2021). Penentuan Kadar Flavonoid Total Fraksi N-Heksan, Etil Asetat, dan Air Dari Ekstrak Etanol Bunga Cengkeh (*Syzygium aromaticum*) dengan Metode Spektrofotometri UV-VIS. Universitas Jenderal Achmad Yani Yogyakarta.

[17] Rebaya, A., Belghith, S. I., Baghdikian, B., Leddet, V. M., Mabrouki, F., Olivier, E., Cherif, J. K., Ayadi, M. T. (2015). Total Phenolic, Total Flavonoid, Tannin Content, and Antioxidant Capacity of *Halimium halimifolium* (Cistaceae). *Journal of Applied Pharmaceutical Science*, *5*(1): 052–057

[18] Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L. Huynh, L. H., Soetaredjo, F. E., Ismadji, S., Ju, Y. H. (2014). Effect of Extraction Solvent on Total Phenol Content, Total Flavonoid Content, and Antioxidant Activity of *Limnophila Aromatica*, *Journal of Food and Drug Analysis*, *22*(3): 296–302.

[19] Li, W., Gao, Y., Zhao, J. & Qi, W. (2007). Phenolic, Flavonoid, and Lutein Ester Content and Antioxidant Activity of 11 Cultivars of Chinese Marigold. *Journal of Agricultural and Food Chemistry*, *55*(21): 8478–8484.

[20] Candra, L. M. M., Andayani, Y., & Wirasisya, D. G. (2021). Pengaruh Metode Ekstraksi Terhadap Kandungan Fenolik Total dan Flavonoid Total Pada Ekstrak Etanol Buncis (Phaseolus vulgaris L.). *Jurnal Pijar Mipa*, *16*(3), 397-405.

[21] Calame, T. E. L., Kusmiyati, K., & Merta, I. W. (2021). The Effect of Young Papaya Seed Extract on The Motility and Abnormality of Male Mice Spermatozoes (Mus musculus). *Jurnal Pijar Mipa*, *16*(4), 555-561.

[22] Roanisca, O., Rani, R., & Mahardika, R. G. (2021). Phytochemical Screening and Antibacterial Potency of Jeruk Kunci Fruit Waste (Citrus x microcarpa Bunge) Extract Against Propionibacterium acnes. *Jurnal Pijar Mipa*, *16*(3), 387-392.

[23] Fitriansyah, S. N., Aulifa, D. L., Febriani, Y. & Sapitri, E. (2018). Correlation of Total Phenolic, Flavonoid and Carotenoid Content of *Phyllanthus emblica* Extract From Bandung With DPPH Scavenging Activities. *Pharmacognosy Journal*, *10*(3): 447–452.