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Antioxidant Activity of *Passiflora edulis* (Passion fruit) Seed Extracts Obtained from Maceration and Ultrasonic Assisted Extraction Method

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ABSTRACT

Passion (*Passiflora edulis*) fruit belongs to the Passifloraceae family and has high-value economics. The extraction efficiency is affected by the phytochemical's properties of plant, the solvent and extraction method used to extract the desired compounds. The seeds of passion fruit exhibit an oil content, the valuable bioactive compound as antioxidant activity. The passion fruit seed were the waste obtained from the passion fruit syrup company in Makassar City. This study aims to determine the effect of different extraction methods on antioxidant activity of passion fruit seed extracts. The seed was macerated with methanol at room temperature for 5 days, and the ultrasonic-assisted extraction (UAE) process was performed for 30 minutes. The antioxidant capacity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The results showed that the percentage yield of extract from UAE was 6.45% and the extract yield from maceration method was 5.458%. The result of antioxidant activity (IC₅₀ value) revealed that the methanol extract obtained from UAE and maceration were 71.672 ug/mL and 144.901 ug/mL, respectively. These results indicate that the UAE method is a suitable extraction method to produce strong antioxidant activity from *Passiflora* seed extract.

Keywords: passion fruit; ultrasonic assisted extraction method; antioxidant activity

INTRODUCTION

Passion (*Passiflora edulis*) fruit is one of the important commodities in South Sulawesi, especially Makassar because one of the souvenir icon in Makassar is passion fruit syrup. The syrup process produces waste in the form of skins and seeds. Passion fruit contains as much as 13.6% seeds, so the more syrup produced; the more seed waste will be produced. The passion fruit syrup industry produces about 40% of seed waste and 100% of this waste isn't used. Meanwhile, the passion fruit seeds oil contain high fatty acid, tocopherol, flavonoid, vitamin C, and others nutrients as well as plant-based vegetables (Porras et al., 2012). However, passion fruit waste is only disposed of and not used, so this is an opportunity to be managed and developed into an economically valuable

product. Passion fruit seeds oil (PFSO) contains flavonoids and piceatannol which can inhibit the tyrosinase enzyme and melanin biosynthesis (Charissa et al., 2017). The tyrosinase enzyme converts tyrosine into 3,4-dihydroxyphenylalanine (DOPA) and into dopaquinone which is further synthesized into melanin pigment characterized by black/brown spots on the skin (Porras et al, 2022). In addition, PFSO contains 87.59% unsaturated fatty acids, and other compounds, like tocopherol (499.3 mg/kg), phenolic (314.13 mg GAE/kg), and vitamin C. Phenolic compounds in PFSO are capable of depigmentation of the skin by directly inhibiting tyrosinase activity in the melanogenesis process. The binding of flavonoids to copper and their antioxidant effects are reported to play a role in inhibiting the action

of the tyrosinase enzyme (Porrás et al., 2017). The combination of tocopherol and phenolic compounds in passion fruit oil will be very effective in overcoming skin damage. The passion seed has antioxidant activity with a radical scavenging mechanism (EC_{50}) 108 ± 1.58 (Malacrida & Jorge, 2012; Pereira et al., 2019; Silva et al., 2015). Therefore, Passion oil is very potential in preventing free radicals that trigger premature skin aging. Environmental influences such as ultraviolet light, cigarette smoke, pollutants, temperature, nutrition, and an unhealthy lifestyle can contribute to the formation of free radicals and Reactive Oxygen Species (ROS). This stimulates skin inflammation which triggers a series of biochemical reactions in the skin and causes damage to the dermis collagen network resulting in premature aging (photoaging/ premature skin aging) (Fonseca et al., 2022). In this section, it is necessary to explain the description of the specific related to the scheme which is strong and able to reduce skin damage due to UV-B, besides that it can also inhibit photo carcinogenesis by preventing the formation of cyclopyrimidine dimmers in the epidermal P53 gene and inhibiting the process of melanogenesis (Nautiyal & Wairkar, 2021). Furthermore, Fonseca et al also reported that passion fruit (*Passiflora edulis*) seed extract prevented an increase in the amount of skin melanin equivalent to 4% hydroquinone in guinea pigs (*Cavia porcellus*) exposed to UVB light.

Based on the description, Passion oil has potential in the pharmaceutical field for the development of pharmaceutical preparations, especially herbal cosmetics. But the selection of extraction methods is important to extracts the desired active compounds effectively. Furthermore, the type and amount of solvent used can also affect the number of active compounds that can be drawn, where compounds with polar properties will dissolve in polar solvents and non-polar compounds will dissolve in non-polar solvents (Ahmad et al., 2022). Therefore, this study aims to determine the effect of different extraction methods on antioxidant activity of passion fruit seed extracts.

METHODS

The passion fruit seed samples were obtained from the passion fruit syrup factory waste in the city of Makassar. The specimen of herbarium has identified and deposited at determination unit No. 0371 laboratory of pharmacognosy and phytochemistry, Faculty of Pharmacy, Universitas Muslim Indonesia. Passion fruit seeds are obtained, dried and powdered, and ready for extraction. The solvent was methanol, hexane, ethyl acetate from Brataco, and DPPH (Sigma Aldrich).

The Extraction Method

The extraction process of the passion seeds was performed using maceration and ultrasonic-assisted extraction methods. As much as 500 g of powdered passion seeds were dissolved with methanol then stored at room temperature for 5 days. The UAE method for 30 minutes.

Qualitative Phytochemical Analysis

Procedures to identify the constituents, Alkaloids, Flavonoids, Tannin, and Phenol (Sigma). Solvent phase n-hexane: Ethyl Acetate (1:3), sprayed with $AlCl_3$, UV light 254 nm and 366 nm.

Thin Layer Chromatography (TLC) Assay

The plant extracts were prepared and dissolved in methanol to be spot on pre coated TLC by using capillary tubes. The line was drawn on the TLC plate surface as a place to spot each extract. The TLC plate then place on the chamber containing mobile phase solvents, n-hexane : Ethyl Acetate (1:3), until the mobile solvent reaches upper line. TLC plate were air dried and observed under ultra violet light. They were later sprayed with iodine vapors for the development of the separate bands the movement. Analyte detection after drying the plate and exposed with iodine vapor all plates were visualized with the help of UV and all different spots that were observed was calculated.

Antioxidant Assay

A fresh solution of DPPH was prepared by dissolving 10 mg of DPPH in 100 mL of methanol. Each extract (2.5 mL) at different concentrations and the DPPH solution (2.5 mL) were mixed in a test tube. The test tube was incubated in the dark for 30 minutes at room temperature. Absorbance was recorded at 517 nm using a UV-VIS spectrophotometer. The percentage inhibition of radicals was obtained using the following formula.

$$\text{Inhibition activity} = \frac{\text{Absorbance control}}{\text{Absorbance (sampel-control)}} \times 100\%$$

RESULTS AND DISCUSSION

The sample has been determined in Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia with document number 0039/C/UD-FF/UMI/V/2023. There are many steps to obtain phytochemicals from plants such as milling, grinding, homogenization, and extraction. Among these steps, extraction is the main step for recovering and isolating phytochemicals from plant materials. The extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances (Ahmad et al, 2023). The yield of extraction depends on the solvent, pH, temperature, and extraction time of the method. The same solvent and composition of the sample are known as the most important parameters. In this work, passion fruit seed extracts were obtained by using different

extraction methods of methanol. Extraction yields ranged from 5.458% for methanol extract (maceration) to 6.45% for UAE extract (Table 1). This shows that the increase of extract yield related to the extraction method used. These results indicate that the extract yield correlates to the extraction method used. This may be attributable to the higher solubility of moderate polarity in methanol extract UAE.

Table 1. Passion Seed Extracts Yield from Maceration and Ultrasonic Assisted Extraction Methods

Extraction Method	Extract (g)	Yield extract (%)
Maceration	27.290	27.290
UAE	12.89	6.45

The extract was separated and observed with TLC. The results of the observation of flavonoid compounds by using TLC (Figure 1) showed a few spots (Figure 1a), observation under UV light 254 nm (Figure 1b) and the spot colour becomes dark. After spraying with AlCl₃ reagent, the appearance of the spots was clarified with UV light 366 nm with the results of the chromatogram profile shown in Figure 1c. The fluorescence yellowish green or green at UV light 366 nm may be auron compounds that do not contain free 4'-OH and flavanones without free 5-OH or flavonols that contain free 3-OH and accompanied or without free 5-OH. Light blue, fluorescent spots are likely to be flavone and flavanone compounds that do not contain 5-OH.

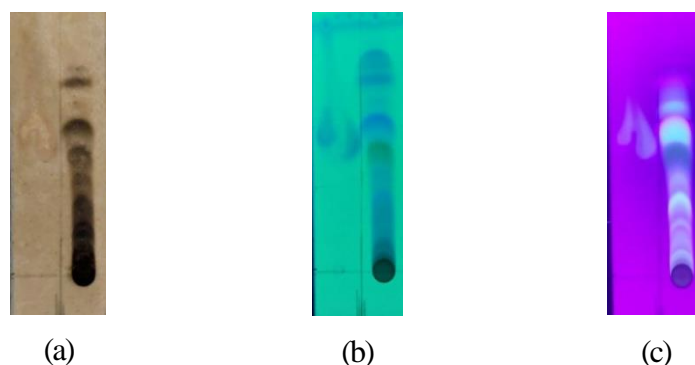


Figure 1. Thin Layer Chromatography with mobile phase n-hexane : Ethyl Acetate (1:3). (a) Sprayed AlCl₃, (b) UV light 254 nm, (c) UV light 366 nm.

The flavonoid content in the methanol extract has the ability of protecting cell structure, increasing vitamin C is effectiveness, being anti-inflammatory, preventing bone loss, and antibiotics. A number of medicinal plants containing flavonoids have been reported to have antioxidants, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer (Virsa et al., 2019). The antioxidant activity of flavonoids has been the focus of most recent studies. The ability of flavonoids to form complexes with metal ions such as iron may enhance their antioxidant effects under these conditions. Particular interest is the ability of flavonoids to inhibit macrophage-supported LDL oxidation, which promotes atherogenesis. The antioxidant properties of flavonoids may also support anti-inflammatory and antiplatelet effects and are attributed not only to their structural properties but also to their ability to interact with and penetrate the lipid layer of cell membranes. Flavonoids reduce nitric oxide radicals, superoxide anions and singlet oxygen. Like most other compound with antioxidant activity, flavonoids can also act as pro-oxidants under certain circumstances (Ahmad, et al., 2022). Tocopherol compounds in passion fruit are known as one antioxidant agent. An α -tocopherol is an exogenic antioxidant that can break the radical chain reaction to prevent ROS and oxidative stress. Supplementation of rats

under oxidative stress with α -tocopherol can considerably reduce malondialdehyde (MDA) levels, normalize seminiferous tubule epithelium and improve reproductive endocrine function (Lopez et al., 2023).

The effect of antioxidants on DPPH is thought to be due to their hydrogen-donating ability. Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer. DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, violet color DPPH solution is reduced to a yellow-colored product, diphenylpicryl hydrazine, by the addition of the extract in a concentration-dependent manner. Our results revealed that the methanolic extract had the highest scavenging activity when compared with other extracts (Table 2). This result showed the activity accordingly to the chemical constituents of the passion fruit extract. In addition, polyphenol contents and tocopherols scavenge the DPPH radicals by their hydrogen-donating ability. The results obtained in this study suggest that all the extracts showed radical scavenging activity by their electron transfer or hydrogen donating ability. Total polyphenols content and radical scavenging antioxidant activity are highly correlated (Malik et al, 2023).

Table 2. Antioxidant Activity of Passion Seed Extracts from Maceration and Ultrasonic Assisted Extraction Methods

Extraction method	Concentration (ppm)	Absorbance Blank	Absorbance Sample	% inhibition	IC ₅₀ (µg/mL)
Ultrasonic-Assisted Extraction	120	0.981	0.410	58.205	71.672
	140	0.981	0.373	61.977	
	160	0.981	0.340	65.341	
	180	0.981	0.308	68.603	
Maceration	120	0.981	0.506	48.420	144.901
	140	0.981	0.489	50.150	
	160	0.981	0.482	50.870	
	180	0.981	0.475	51.580	

CONCLUSION

This study reports the differences in yield extraction according to the extraction method. The data showed that UAE has the highest yield extract and has the highest antioxidant capacity.

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