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PHYTOCHEMICAL IDENTIFICATION AND ANTIOXIDANT ACTIVITY OF JUWET FRUIT (*Syzygium cumini* L. Skeels) ETHANOLIC EXTRACT

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ABSTRACT

Juwet (*Syzygium cumini* L. Skeels) is one of Indonesia's local fruits which its existence is hard to find now. All parts of this plant can be used for many treatments, one of which is as an antioxidant. The purpose of this study was to determine the compounds contained in the fruit and to determine the antioxidant activity as well. Phytochemical analysis was performed using the gas chromatography while antioxidant activity tests were carried out quantitatively by DPPH method. The results showed that the juwet fruit was contained phenol 284.47 mg/100 g GAE, flavonoids 379.07 mg/100g, tannins 3888.67 mg/100 g TAE and anthocyanins 40.28 mg/100 g which consisted of 66 types of chemical compounds. The results of the antioxidant analysis showed that the ethanolic extract of juwet fruit could inhibit DPPH radicals with IC_{50} of 72,96 µg/ml.

Keywords: Juwet fruit, antioxidant, phytochemical, DPPH.

INTRODUCTION

Indonesia is one of the countries that has the highest diversity of medicinal types in the world. This is due to the Indonesian territory is crossed by the equator and has a relatively stable climate which directly affects tropical forests biodiversity. Indonesia's tropical forest areas has the second-highest biodiversity in the world after Brazil's. Experts estimate that no less than 25,000 types of flora grow and spread throughout Indonesia (Primarck *et al*., 1998), one of which is juwet (java plum) fruit.

Juwet fruit is tasted sour and has oval shape. There are several color types of mature juwet fruit, including black, red, and white. In general, Bali's juwet fruit is consumed freshly as salad. According to Swami *et al*. (2012) juwet fruit can be used as traditional medicine for diabetes mellitus, anti-inflammation, and antidiarrhea.

Secondary metabolites commonly present in all types of plants are phytochemicals that play a role in body defence against biotic and abiotic disorders is also found in juwet fruit. For human needs, such metabolites are being sought and developed for a variety of benefits, therefore it is important to know the content of secondary metabolites of the juwet fruit. The method used to determine the metabolite content of plants is phytochemical analysis.

Isolation and identification of phytochemical compounds of juwet fruit can be carried out through extraction methods, phytochemical screening, analysis using gas chromatography (GC), and testing of antioxidant activity using DPPH. Extraction is the process of separating a substance based on the differences of its solubility in two different insoluble liquids.

A maceration system as cold method is usually used to extract the metabolites. Maceration is an extracting process conducted in room temperature and consist of maintaining contact between the plant powder and the solvent for a period of time. Solvent used in this study was 96% ethanol, not only because it is a volatile polar compound which suitable to use as an extracting solvent, however the ethanol is easy to be obtained and used.

MATERIALS AND METHODS

Place and Time of the Research

This research was conducted from March 2020 to June 2020. Juwet fruit was collected from Dauh Peken Village-Tabanan which its temperature was 28°C, on elevation of 143 meters above sea level, and its coordinate is (-8.52637; 115.1204183). The research sample analysis was conducted in the Laboratory of Genetic Resources and Molecular Biology of Udayana University and Analytical Laboratory of Udayana University.

Materials and Tools

The materials used in this study was black mature of juwet mesocarp (the fleshy middle layer of the fruit pericarp). The tools used were gas chromatography (GC), spectrophotometry, beaker glass, blender, cork borer, erlenmeyer, measuring cup, inoculation needle, camera, label paper, autoclave, filter paper, laminar airflow cabinet, oven, petri dish, micropipettes, test tubes, electric scales, vacuum rotary evaporators, and other equipment used in laboratories.

Preparation of test materials

Fresh juwet fruit was washed under running water, then separate the seeds to take the mesocarp, and cut into medium size using a knife. Then, dried the sample in the oven for 3x24 hours with the temperature of 50^{o}C. Furthermore, the dried juwet fruit was oven-dried at 40°C to obtain the constant weight (Tapotubun, 2018). Finally, the dried juwet was grinded into powdered using a cutting machine.

Exraction

Extraction of the juwet fruit was carried out using maceration as follows: 150 g of finely juwet powder was macerated using two litres of ethanol 96%. The maceration process were carried out at room temperature for three days with regular shaking (Senja *et al*., 2014). After the filtration, the organic solvent was evaporated under vacuum

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evaporator with the temperature of 40°C to obtain crude extract (Putranti, 2013).

Gas chromatography analysis

The juwet fruit extract was analysed according to the GCMS procedure of the Forensic Laboratory Denpasar. The analysis was carried out using Agilent 7890B MSD 5977B, with the Wakosil ODS/5C18-200 silica column with a size of 4.6 x 200 mm using N_2 gas as a carrier. The injection temperature used was 290°C for 27 minutes with an injection rate of 1 µl/minute. Identification was done by comparing the retention time of each chromatograph peak with the database.

Phytochemical Screening

Quantitative test

Phytochemical screening was carried out by using the UV Spectrophotometry method to analyse the composition of a sample quantitatively based on the interaction between the material and light.

Determination of the Total Anthocyanin Contents

Anthocyanin content analysis was carried out using the pH differential method (Giusti *et al*., 2001). The first solution was dissolved in 0.025 M KCl buffer with the pH of 1.0 and the other solution was dissolved in 0.4 M sodium acetate buffer with the pH of 4.5. Prior of the test, scanning of sample also carried out by measuring sample in the wavelengths of 200-750 nm range of both buffers (KCl and sodium acetate) to determine the anthocyanin contents and the λvis-max of sample.

Determination of the Total Tannins Contents

Sample of 500 mg was placed into a flask, then add 50 ml of distilled water and stir with a mechanical shaker for 1 hour. After a filtration, placed the filtrate in a volumetric flask and adjusted by adding water into the volume of 50 ml. A volume of 5 ml of filtrate mixed by 0.8 ml of potassium hexacyanoferrate (III) 0.008 M in 0.1 N hydrochloric acid and 0.8 ml of ferric chloride of 0.1 M in 0.1 N hydrochloric acids. The absorption of the solution was measured using an ultraviolet-visible spectrophotometer at a wavelength of 420 nm.

Determination of the Total Phenolic Contents

Determination of phenolic content in the ethanol extract of juwet refers to the Chun *et al*. (2003) procedure. The sample of 10 mg was mixed with 10 ml ethanol, then 1 ml of the sample was mixed with 0.4 ml of Folin-Ciocalteau's reagent. Shake the solution and incubated for 4-8 minutes. The solution was added with 4.0 ml of Na_2CO_3 and shaked to homogenize the solution mix, then added with distilled water up to 10 ml. The solution incubated for 2 hours and the absorbance was measured at a wavelength of 750 nm which gave a blue complex. The repetition was done

three times to obtain the phenol content as mg gallic acid equivalent/g/extract.

Determination of the Total Flavonoids Contents

Put 10 g of the sample into an erlenmeyer and dissolved with 100 ml of 80% methanol-water at room temperature for 24 hours. Then the whole solution was filtered and transferred into a crucibles, and evaporated to dry on a water bath and then weighed.

Determination of Antioxidant Activity

The quantitative test was carried out using the DPPH method. Activity of the extract to free radical was evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical which provided a strong absorbent at 516 nm. Samples and standards dissolved in ethanol were added with DPPH solution with a volume ratio of 1:1 and incubated for 30 minutes at room temperature using a covered dark container lined with aluminium foil. The DPPH absorbent percentage was calculated using a formula: I (%) = (Ao-As)/Ao x 100%, which I = percentage of decrease of DPPH absorbent, Ao = absorbance of DPPH control solution, and As = absorbance of the sample solution after adding DPPH. Antioxidant activity was expressed by the IC_{50} value obtained from the linear regression constructed by data of 5 different extract concentrations. The IC_{50} value was determined as the concentration that cause inhibition of 50% DPPH (Ghasemi *et al*., 2009).

RESULTS AND DISCUSSION

Analysis of Juwet Fruit Extract

Gas chromatography (GC) analysis produced 66 types of compounds. Literature studies indicated that each compound has its use and function. The compounds were distinguished over its retention time. At the retention time of 2.486 was found d-glycero-d-glacto-heptose compound and its function is involved in the biosynthesis of lipopolysaccharide by G-negative bacteria (Mayer and Tanner, 2007). At the retention time of 3.176 found carbonodithioic acid and S-ethyl 0-(1-methyl ethyl) ester which acting as an antioxidant (Lauderback et al., 2009), while at the retention time of 3.640 was methyl compound such as gamma-octalactone (Z and E) which acts as an antineoplastic agent and antibacterial agent (PubCham, 2020).

Phytochemical Quantitative Analysis Screening results.

The phytochemical analysis found 4 phytochemical compounds contained in the ethanolic extract of juwet fruit flesh, namely phenolic compounds, flavonoids, tannins, and anthocyanin as shown in Table 1. The phenol content obtained was expressed as an equivalent to mg of gallic acid/100 mg of a fresh sample.

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Table 1. Phytochemical analysis of juwet fruit

No	Sample code	Total phenolic content (mg/100g GAE)	Flavonoid (mg/100g)	Tannin content (mg/100g TAE)	Anthocyanin content (mg/100g)
1	Extract of flesh fruit of juwet	284.47	379.07	3888.67	40.28

Table 2. Antioxidant activity of the juwet extract

Absorbance	Extract concentration (µg/ml)	Decreasing absorbance, I (%)	Regression equation (y = bx + a)	IC_{50} (µg/ml)
0.453	Blank	0	y = 40.839x + 47.02	72.96947
0.236	0.2	47.9029		
0.134	0.4	70.4194		
0.115	0.6	74.6137		
0.084	0.8	8.457		
0.076	1	8.223		

y=% inhibition, a=constant, b=gradient, x=DPPH (µg/ml), IC_{50}=effective concentration against 50% DPPH.

The results showed the total phenol ethanol extract of juwet fruit was 284.47 mg/100g/GAE. Phenol has a toxic and corrosive effect to human skin and at a certain concentrations may cause human health problems and death (Qadeer and Rehan, 1998).

Flavonoids has a conjugated aromatic system which showed a strong absorption band in the UV region. The flavonoid levels obtained was 379.07 mg/100 g. Flavonoids act as antioxidants by donating their hydrogen atoms or through their ability to chelate metals, in the form of glucoside (containing glucose side chains) or in the free form called aglycone (Cuppett *et al*., 1954).

Tannins consist of two types: condensed tannins and hydrolized tannins (Hovath, 1981). The results of this study showed that every 100 g of the extract contained 3041.60 mg of tannin/100 g of TAE.Anthocyanin is a water-soluble pigment that naturally accumulates in the epidermal cells of fruits, roots, and leaves. Anthocyanin can replace the use of synthetic carmoisine and amaranth dyes as red dyes in food products. The results of this study showed that every 100 g of extract containing anthocyanin of 40.28 mg/100 g. Anthocyanin acting as antioxidants in the body, makes them atherosclerosis fighters. Anthocyanin also protect the integrity of the endothelial cells

which lining the walls of blood vessels (Ginting and Sutomo, 2011).

Based on the data in Table 2, a regression equation was obtained as y = 40.839x + 47.02 and through this equation was obtained an IC_{50} value of 72.96 µg/ml. This means 72.96 µg/ml ethanolic extract of juwet fruit were able to act as antioxidants to inhibit DPPH (free radicals) by 50%. According to Phongpaichit *et al*. (2011) category, this IC_{50} value belongs to the moderate active category. Existing studies revealed that strawberries have an IC_{50} of 68.03 ppm or 68.03 µg/ml (Anggraini *et al*., 2017) which means the antioxidant of strawberry is almost the same as the juwet fruit. Based on the category, IC_{50} of >250 µg/ml is classified as not active antioxidant compound, IC_{50} of 100-250 µg/ml as weak, IC_{50} of 50-100 µg/ml as moderate, and IC_{50} of 10-50 µg/ml as active antioxidant compound.

CONCLUSIONS

The juwet fruit grown in Dauh Peken Village-Tabanan contained 284.7 mg/100g/GAE phenol, 379.07 mg/100 g flavonoids, 3888.67 mg/100 g/TAE tannins, and 40.28 mg/100 g anthocyanin which consisted of 66 types of chemical compounds. The fruit exhibit fairly antioxidant activity with IC_{50} of 72.96 µg/ml. The suggestion for further research is directed to determine the function of unknown compounds contained in juwet fruit.

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