

Determination of Total Phenolic, Flavonoid Content and Antioxidant Activity of Campolay (*Pouteria campechiana* (Kunth) Baehni) Extract

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Abstract—Objectives: The purpose of this research was to determine the antioxidant activity, total phenolic and total flavonoid content of ethanol 70% extract fruit peel, pulp, seeds and leaves *Pouteria campechiana* (Kunth) Baehni. **Method:** extraction was performed using maceration by 70% ethanol. The extract was evaporated using a rotary evaporator. Antioxidant activity was tested using DPPH assay and determined the inhibitory concentration 50 (IC₅₀) DPPH scavenging activity. **Determination of total phenolic and flavonoid content** were performed by UV-visible spectrophotometry. **Results and Discussion:** Ethanol 70% extract of leaves was given the highest of total flavonoid content (0.781 gQE/100g extracts) and phenolic content (40.00 g GAE / 100 g extract). Extracts of fruit peels, pulp, seeds and leaves of *Pouteria campechiana* (Kunth) Baehni can be classified as very strong antioxidants because they have IC₅₀ DPPH < 50 µg/ml. The lowest IC₅₀ DPPH scavenging activity was given by ethanol 70% extract of seed *Pouteria campechiana* (Kunth) Baehni (0,5 µg / mL).

Keywords: motor ability, long jump, adolescent

I. INTRODUCTION

Antioxidants are compounds that are important for health. The antioxidant compounds can be found in various medicinal plants. The presence of natural antioxidant compounds obtained from the medicinal plant will neutralize free radicals in the body [1]. Free radicals in the body can be damaged cells and tissues. Therefore, it is very important to look for medicinal plants that have potential as a source of natural antioxidants.

Genus of *Pouteria* is commonly found in tropical countries. Some species of *Pouteria* are reported to have anti-inflammatory activity, treat skin damage, analgesic activity and anti-ulcer [2]. One of species of the genus *pouteria* which grows in Indonesia is campolay (*Pouteria campechiana*).

Fruit and leaves of *Pouteria campechiana* contain phenolic and flavonoid compounds [3]. Phenolic and flavonoids can be affected by antioxidant activity [4]. Water extracts of fruit flash, fruit peel, and seeds *Pouteria campechiana* have antioxidant activity [5]. Besides that, chloroform extract and ethanol 70% extract of fruit flash and fruit peel have antioxidant activity [5]. The existence of this information, *Pouteria campechiana* is one of the main medicinal plants as a potential source of natural antioxidants. The different parts of a plant can produce a different biological activity. Comparison of IC₅₀ values of DPPH scavenging by ethanol 70% extract of fruit peel, fruit pulp, seed and leaf from maceration result has not been reported.

In the research carried out, we determine antioxidant activity which would be expressed with IC₅₀ value and total phenolic and

flavonoid content of ethanol 70% extract fruit peel, fruit pulp, seed, and leaf *Pouteria campechiana* that grew in West Java, Indonesia.

II. MATERIAL AND METHOD

A. Material

The raw material used fruit peel, fruit pulp, seeds and leave of *Pouteria campechiana* were collected from a conservation park in Subang, West Java, Indonesia. *Pouteria campechiana* was determined at Herbarium Bandungense, Faculty of Biology, Padjadajaran University. The chemical used DPPH as free radicals, methanol Pro analysis, quercetin as standard flavonoid compound, gallic acid as a standard phenolic compound, and ascorbic acid as antioxidant standards. Data Analysis

B. Procedure

Preparation samples and extraction

Fruit peel, fruit pulp, seeds and leaves of *Pouteria campechiana* were cleaned and dried using an oven at 60°C. 50 g dried peel, pulp, seeds, and leaves of *Pouteria campechiana* then extracted using a macerator with 70% ethanol solvent. Liquid extract was evaporated using a rotary evaporator. So, by obtained four extracts, namely peel extract (P_A), pulp extract (P_B), seeds extract (P_C), and leave extract (P_D).

Phytochemical screening

Phytochemical screening is carried out for P_A, P_B, P_C, and P_D using FeCl₃ reagent for phenolic compound, amyl alcohol for flavonoid compound, gelatin for tannins, dragendorff and chloroform reagents for alkaloid, KOH 5% for quinones, Lieberman-Bouchard for triterpenoid and steroids, sulfate vanillin 10% for diterpenes and sesquiterpenes [6].

Determination of Total Phenolic Content

Determination of total phenol content using the Pourmurad method [7] with the addition of folin ciocalteu reagents. Gallic acid is used as a phenolic standard. Measurement of standard absorbance and extract using UV-Vis spectrophotometry at a wavelength of 765 nm. The total phenol content of the extract was obtained using a linear regression equation from a standard curve with a value of R² = not less than 0.99. Total phenol content was expressed as equivalent gallic acid per 100 grams of extract (g GAE / 100 g extract).

Determination of Total Flavonoid Content

Determination of total flavonoid content using the Chang method [8] with the addition of AlCl₃ reagents. Quercetin used as a quercetin standard. Quercetin made into a series of concentrations in methanol P.a. Measurement of absorbance of standard solutions and extracts using UV-Vis spectrophotometry at a wavelength of 415 nm. Total flavonoid content of extract calculated using a linear regression equation from a standard curve with a value of R² = not less than

0.99. Total flavonoid content was expressed as equivalent quercetin per 100 grams of extract (gQE / 100 g extract).

Antioxidant activity

Determined of antioxidant activity used the modified Blois method [9]. The solution of 50 ppm DPPH in methanol was used as a control solution and measured an absorbance (A_0). Determine of antioxidant activity of each sample made to a series concentration of the solution in methanol Pro Analysys, then a 50 ppm DPPH solution was mixed with each concentration series of PA, PB, PC, and PD with a volume ratio (1.5 mL: 15 mL).

III. RESULTS

Phytochemical screening results of each extract showed extracts of fruit peel, fruit pulp, seeds and leaves of *Pouteria campechiana* have phenol and flavonoid compound.

The total phenolic content of PA, PB, PC, and PD could be seen in Figure 2. The linear regression equation of the gallic acid standard curve (Figure 1.) using the calculated total phenol content of PA, PB, PC, and PD. PD was the highest total phenol content and PB was the smallest.

Total flavonoid content in PA, PB, PC, and PD can be seen in Figure 4. The linear regression equation of the standard quercetin curve (Figure 3.) used to calculate the total flavonoid content. The highest total flavonoid content is in PD while the smallest is in PA.

Antioxidant activity

IC_{50} values of fruit peel extract, fruit pulp, seeds and leaves of *P. camechiana* can be seen in Figure 5. Seed extract (PC) has the smallest IC_{50} value when compared to PA, PB, and PD. This means that 70% ethanol seed extract of *P.campechiana* has the highest antioxidant activity.

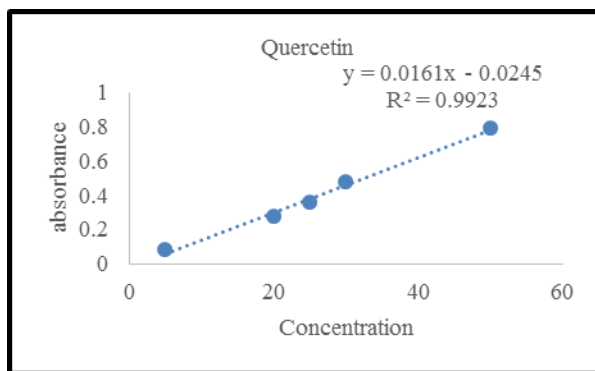


Figure 3. Standard Curve of Quercetin

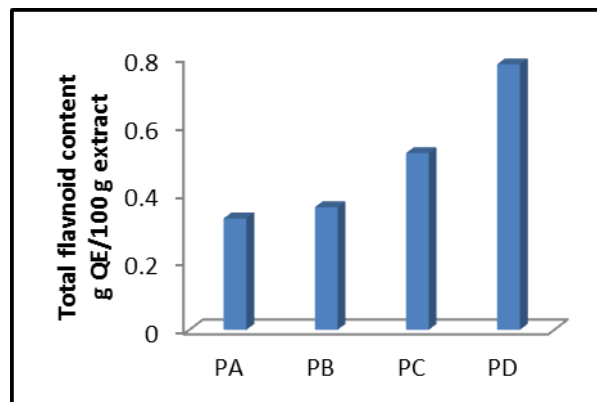


Figure 4. Total flavonoid content

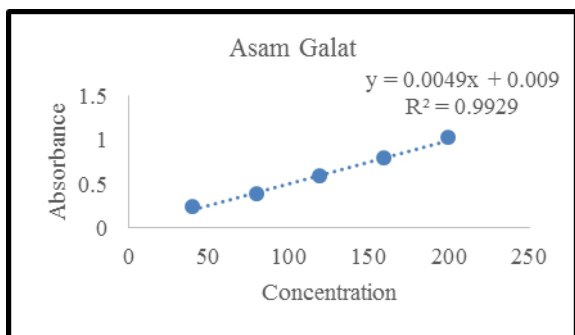


Fig. 1 Standard curve of gallic acid

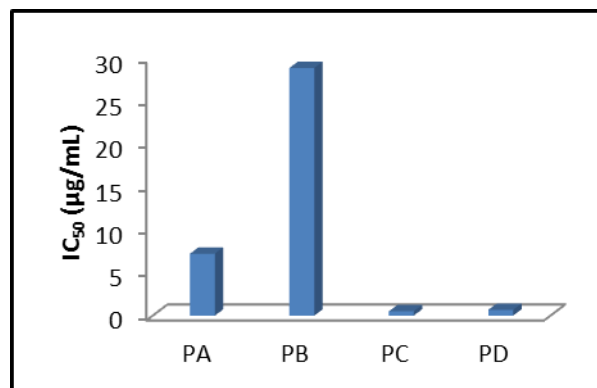


Figure 5. IC_{50} scavenging activity of DPPH

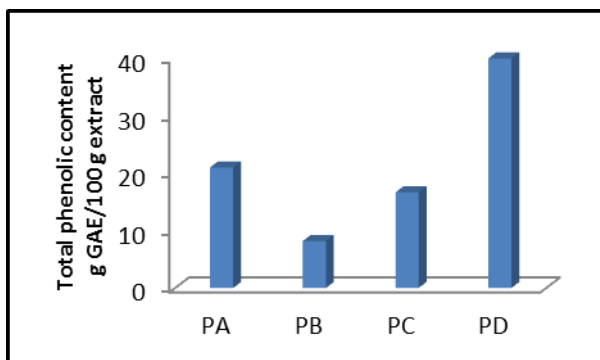


Figure 2. Total phenolic content

IV. DISCUSSION

Based on phytochemical screening, extracts of fruit peel, fruit pulp, seeds and leaves of *Pouteria campechiana* have phenol and flavonoid compounds. This information is preliminary information that extracts of fruit peel, fruit pulp, seeds and leaves of *P.campechiana* have antioxidant activity. Qualitatively, there was no difference in chemical compound content between the extracts of fruit peels, fruit pulp, seeds and leaves of *Pouteria campechiana*.

PD was the highest total phenol content and PB was the smallest. So far, it has not been seen the types of phenol compounds contained in each extract of PA, PB, PC, and PD. Phenolic compounds are the largest compounds in plants which can be caused some biological activity [10]. The biological activity depends on the type of phenolic

compound. Phenolic compounds a major role in antioxidant activity [10].

However, the results of the study, the total phenol content in PA, PB, PC, and PD extracts did not contribute significantly to antioxidant activity. This is shown, PD has a greater total phenol content compared to PC, and is directly proportional to IC₅₀ value of PD greater when compared to IC₅₀ value in seeds extract. This shows that the antioxidant activity of PD is smaller than PC, even though PD has a greater total phenol content. This can be caused by differences in the types of phenolic compounds in each extract PA, PB, PC, and PD. Where this type of phenol compound will contribute to the strength of antioxidant activity.

The highest total flavonoid content is in PD while the smallest is in PA. Antioxidant activity in PA, PB, PC, and PD is not only caused by the contribution of flavonoid compounds. This can be seen in the total flavonoid content directly proportional to the IC₅₀ value of DPPH scavenging by PA, PB, PC, and PD.

Although PD was the highest total flavonoid content, it does not have the smallest IC₅₀ value. Flavonoid compounds was a role in the strength of antioxidant activity. OH group with ortho position on C-3 'and C-4' can be affected the higher antioxidant activity [11] when compared to OH groups on C-3 atom and the double bond between C-2 and C-3 [12; 10].

The differences in the OH group substitution and the position of the double bonds in the flavonoid ring will distinguish the types of flavonoid compounds. Therefore, total flavonoid content in PA, PB, PC, and PD cannot be said to have a direct relationship with antioxidant activity.

Determination of antioxidant activity in research conducted using the Blois method [9] with DPPH. The antioxidant activity of the DPPH method basically measures the reduction value of DPPH by the antioxidant compounds in the extract. This value is expressed in percent inhibition. Antioxidant activity can be expressed by IC₅₀ value of DPPH scavenging activity by the extract. If IC₅₀ value is less than 50 µg / mL, then it belongs to a very strong antioxidant activity classified [9]. There is a correlation between IC₅₀ with antioxidant activity. The smaller IC₅₀ value of DPPH scavenging by extract, it shows the greater antioxidant activity of the extract. IC₅₀ values of fruit peel extract, fruit pulp, seeds and leaves of *P. campechiana* can be seen in Figure 1. Seed extract (PC) has the smallest IC₅₀ value when compared to PA, PB, and PD. This means that 70% ethanol seed extract of *P. campechiana* has the highest antioxidant activity. However, if seen IC₅₀ value of PA, PB, PC, and PD were below 50 µg / mL. This means that the antioxidant activity of PA, PB, PC and PD extracts is classified as very strong antioxidant activity. However, when compared to the IC₅₀ value of ascorbic acid, IC₅₀ value of PA, PB, PC, and PD have a greater than ascorbic acid.

Each part of a plant can be different types and concentrations of chemical compounds. This can be caused by different biological activities [13], including antioxidant activity. PA, PB, PC, and PD have the same secondary metabolite group when viewed qualitatively. But that does not mean PA, PB, PC, and PD have the same type and concentration of the compound. This statement is in line with the results of the study which showed differences in IC₅₀ values in PA, PB, PC, and PD. According to the previous study, IC₅₀ of *P. campechiana* fruit is 54 µg / mL in water extracts [14] and 0.88 mg / ml in methanol extracts [15]. In the study of Kong et al, 2013 [5] *P. campechiana* seed extract at 70% ethanol and 70% methanol had a greater DPPH inhibition percentage compared to fruit pulp extracts.

The DPPH method is very easy to use. The scavenging of free radicals by an extract is characterized by a change color in the DPPH solution [16] (no.23 in the correlation journal). The color change that

occurs in DPPH solution is the change in color from purple to a lighter color, for example yellow.

The fading color of DPPH solution, it can be the greater DPPH scavenging by extract. Chemical compounds in extracts that can be scave free radicals where capable donate hydrogen atoms to free radicals. Phenols, flavonoids are compounds that can donate hydrogen atoms to free radicals. [4].

Antioxidant compounds included in the phenolic group were acidic compounds, cinnamic acid, and benzoic acid. The three classes of compounds have different contributions to the antioxidant activity [17]. In flavonoids, the presence of OH groups and double bonds can increase antioxidant activity. The OH group on C-3 and the double bond between C-2 and C-3 on the flavonoid ring will increase antioxidant activity [18; 19]. The position of the OH group substitution and the double bond in the flavonoid ring can cause different types of flavonoid compounds. Therefore, although qualitatively the PA, PB, PC, and PD exhibit the same group of secondary metabolites, but that does not mean having the same strength of antioxidant activity.

V. CONCLUSION

Ethanol 70% extract of fruit peel, fruit pulp, seeds and leaves *Pouteria campechiana* has very strong antioxidant activity. The ethanol extract seeds had the highest antioxidant activity with IC₅₀ value of 0.5 µg / mL. Leaf extract has the highest of total phenol and flavonoid content when compared with fruit peel, fruit pulp and seeds extract. Antioxidant activity in the ethanol 70% extract fruit peel, fruit pulp, seeds and leaves of *Pouteria campechiana* is not only caused by the presence of flavonoid and phenol groups.

ACKNOWLEDGMENT

This article is a combination of a preliminary research result of S3 and research results of student creativity events. Thank you to the Directorate General and Learning of Kemenristek Dikti for the support of this research fund and thank you Sekolah Tinggi Farmasi Indonesia (*Indonesia School of Pharmacy*) for the facilities that have been provided to assist this research.

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