TOTAL CAROTENOID CONTENT AND ANTIOXIDANT ACTIVITY OF SAWO WALANDA (Pouteria campechiana Kunth. Baehni) EXTRACT FROM VARIOUS SOLVENTS

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TOTAL CAROTENOID CONTENT AND ANTIOXIDANT ACTIVITY OF SAWO WALANDA (*Pouteria campechiana* Kunth. Baehni) EXTRACT FROM VARIOUS SOLVENTS

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ABSTRACT

Pouteria campechiana, also known as sawo walanda in Indonesia, is known for its high abundance of carotenoids, which are recognized for their antioxidant properties. The extraction of these constituents involves a crucial step of selecting an appropriate solvent, as indicated by several studies. Therefore, this study aims to assess the antioxidant activity as well as determine the total carotenoid content of P. campechiana fruit and leaf extract in various solvents. The study methodology involved extraction using the maceration method with n-hexane and ethyl acetate as solvents. Antioxidant activity and determination of total carotenoid were carried out using UV-Vissible spectrophotometry. DPPH was used as a free radical, while β-carotene served as the standard for total carotenoid content. The results showed that n-hexane fruit (NF) extract had the highest total carotenoid concentration (70.028 gBEQ/100 g extract), while n-hexane leaf (NL) extract had the lowest (39.540 gBEQ/100 g extract). Ethyl acetate leaf (EL) extract exhibited better antioxidant activity with IC_{50} of 3.094 µg/mL±0.82 compared to NL (9.270 µg/mL±1.201), ethyl acetate fruit (EF) (31.516 µg/mL±1.786), and NF (45.382 µg/mL±2.31) extracts. Based on the statistical analysis results, the coefficient correlation of total carotenoid content with IC_{50} DPPH was $r = 0.855$, $p<0.01$. This indicated that an increase in total carotenoid content did not always lead to a proportional increment in the inhibition of DPPH. These findings revealed that n-hexane solvent was more suitable for extracting carotenoids from the fruit part, while ethyl acetate was more appropriate for the leaf. Furthermore, the fruit and leaf extract of P. campechiana had potential antioxidant activity as natural ingredients in the food and pharmaceutical industries.

Keywords: *Sawo walanda, Pouteria campechiana, carotenoid, antioxidant activity*

INTRODUCTION

The family Sapotaceae, which is distributed worldwide, includes the genus Pouteria. Pouteria species have a rich history in traditional medicine, being used for the treatment of various ailments such as back pain, ulcers, skin eruptions, and inflammation.[1] Among the medicinal plants within the Sapotaceae family, *Pouteria campechiana,* commonly referred to as sawo walanda in Indonesia, has been reported to hold significant importance.

According to a previous study, the fruit of *P. campechiana* (Kunth. Baehni) is a significant source of carotenoids[2] and has gained recognition for its anti-inflammatory, antioxidant, and hepatoprotective properties.[3,4] Furthermore, extracts obtained from its leaf and fruit have been found to contain phenolics, flavonoids, and terpenoids, exhibiting significant antioxidant activity.[5]

Medicinal plants serve as a valuable source of raw materials containing phenolics, flavonoids, polyphenols, and terpenoids. These compounds have been reported to be responsible for the various bioactivities exhibited by plants. The specific phytochemical component produced depends on the extraction technique and the solvent employed. Carotenoids are compounds belonging to a group of secondary metabolites known as terpenoids. Some of the common

solvents used for the extraction of carotenoids include acetone, chloroform, hexane, methanol, methylene chloride, ethyl acetate, and diethyl ether.[5] The choice of solvent is largely dependent on the type and concentrations of carotenoids being extracted. Therefore, the selection of the appropriate solvent is very important. Therefore, this study aims to assess the antioxidant activity as well as determine the total carotenoid content of P. campechiana fruit and leaf extract in various solvents. The correlation of total carotenoid content with antioxidant activity was analyzed using Pearson's method.

MATERIAL AND METHODS

Material

The materials used in this study included DPPH (2,2-diphenyl-1 pichydrazyl) (Sigma-Aldrich), ascorbic acid (Sigma-Aldrich), beta carotene (β-carotene) (Sigma-aldrich), Sawo walanda (*Pouteria campechiana* Kunth. Baehni), methanol, ethyl acetate, n-hexane, and other analytical grade reagents, which were obtained from Merck.

Sample Preparation

P. campechiana fruit and leaf were freshly collected from Bandung, West Java, Indonesia, in January 2019. The sample was identified in the Herbarium Jatinangor, the Plant Taxonomy Laboratorium, Biology Department, UNPAD, with reference number 123/HB/01/2020. Subsequently, the fruit and leaf were sorted, washed, dried at 40°C – 45°C, and ground into powder.

Extraction

A total of 300 grams of the powdered fruit and leaf was extracted using maceration. Each sample was extracted using n-hexane and ethyl acetate solvent, and the procedure was repeated in triplicate. Furthermore, each extract was concentrated using a rotary evaporator at 50°C to produce n-hexane fruit (NF), ethyl acetate fruit (EF), n-hexane leaf (NL), and ethyl acetate leaf (EL) extracts.

Phytochemical Analysis

To assess the presence of secondary metabolites in *P. campechiana* fruit and leaf extract, phytochemical screening was carried out to determine the presence of alkaloids, flavonoids, tannins, polyphenols, monoterpenes/sesquiterpenes, triterpenoids/steroid, quinones, and saponins.[6]

Antioxidant Activity

Antioxidant activity test was carried out using the DPPH method proposed by Fidrianny, (2018).[7] The standard for contrasting antioxidant chemicals was ascorbic acid, and the concentration of DPPH used was 50 µg/mL, which served as the control. The percentage of free radical inhibition was measured by mixing the DPPH solution with the sample in a (1:1) ratio. After incubating the sample for 30 min, the absorbance was measured at 517 nm, and this procedure was repeated in triplicate for each sample.

$$\%\,Inhibition = \frac{(Blank\ abs - Sample\ abs)}{Blank\ abs}\ x\ 100\%$$

Determination of IC_{50} Extract to DPPH

A calibration curve connecting the various sample concentrations to the percentage of sample inhibition against DPPH was used to calculate the IC_{50} value against DPPH. The IC_{50} was then determined using the calibration linear regression value, with the x-value being used for calculations.

Determination of Antioxidant Activity Index

The antioxidant group of a sample was identified using the antioxidant activity index (AAI).[8] (Scherer), which was calculated using the formula below:

$$AAI = \frac{Final\ concentration\ of\ DPPH}{Final\ concentration\ \mathrm{IC50}}$$

Determination of Total Carotenoid Content

Thaipong[9] and Fidrianny[10] proposed a measuring method to assess the overall carotenoid content. The extracts were diluted in n-hexane, and their absorbance was measured at a wavelength of 470 nm. Furthermore, the analysis was carried out in triplicate for each extract. To establish a standard curve, beta carotene was employed as a standard at concentrations ranging from 10 to 40 µg/mL. The total carotenoid content was determined using the linear regression equation of the calibration curve, and the results were expressed as beta carotene equivalent of 100 g extract (g BE/100 g extract).

Data Analysis

Analyses of each sample were carried out in triplicates, where the averages and standard deviations of at least three separate experiments were used to calculate all the results. Subsequently, Pearson's method was used to determine a correlation between the total carotenoid concentration and antioxidant activity.[10]

RESULT AND DISCUSSION

Extraction

Extraction was carried out using maceration with different polarity solvents. The amount of metabolite content of each *P. campechiana* extract is presented in Table 1. The results showed that ethyl acetate solvent could extract a higher amount of metabolites from *P. campechiana* fruit and leaf. Furthermore, primary and secondary metabolites could be among the extracted compounds.

Phytochemical Analysis

Table 2 shows the results of the phytochemical screening on the crude and each extract. Phenolic compounds, flavonoids, and saponin were absent in NF and NL, while Monoterpene/sesquiterpene was not found in EF and EL. Phenol and saponins were semipolar to polar metabolites, but monoterpenes/sesquiterpenes tended to be nonpolar to semipolar. Several studies showed that *P. campechiana* was be rich in phenolic compounds, flavonoids, and terpenoids. The leaf contained high levels of flavonoids stilbenoids and tannins[11], while the fruit was rich in terpenoids, including carotenoid[12] and phenolic acid[13]. According to Sangeetha[14], flavonoids and saponins were also absent in the fruit extract. Acetone extract leaf *P. campechiana* contained alkaloids, flavonoids, saponins, steroids, tannins, and terpenoids[14].

Carotenoid Total Content in Extract *P. campechiana*

Total carotenoid content was calculated based on the standard curve beta carotene with linear regression y=0.0352x – 0.103, R^2 = 0.992 (Fig. 1). In measuring the total carotenoid content, beta-carotene was frequently employed as a standard. The highest content of (70,028 ± 2,341) g BE/100 g extract was found in NF, while the smallest was exhibited by NL at (39,40 ± 1,76) g BE/100 g extract, as shown in Fig. 2. The intensity of the carotenoids was attributed to their conjugated double bonds, which led to a vibrant yellow-orange shade.[15] NF and EF gave stronger orange-yellow color compared to NL and EL.

Carotenoids referred to a class of compounds with both polar and nonpolar characteristics. The hydrocarbon carotenoids, namely beta carotene and lycopene were examples of the nonpolar category. Meanwhile, polar carotenoids, such as lutein, canthaxanthin, astaxanthin, and fucoxanthin were oxygenated derivatives of hydrocarbon carotenoids, as shown in Fig. 3.[16] Carotenoids were typically extracted using organic solvents, such acetone, chloroform, hexane, methanol, and diethyl ether. The sample matrix and its components, moisture content, the functional group (polarity), and the chain length of the existing compounds were significant factors during the extraction.[16] The most frequent methods used for extracting both polar and nonpolar carotenoids simultaneously included acetone, ethanol, ethyl acetate, and n-hexane,[17] with hexane and acetone being the common solvents.[17]

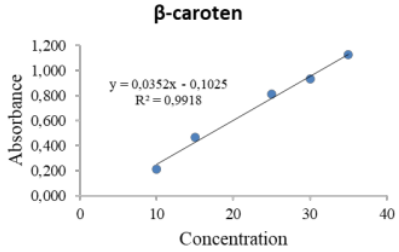

Fig. 1 Standard Curve β-carotene

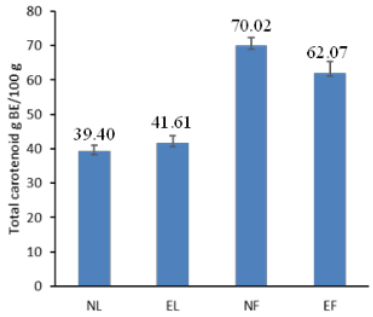

Fig. 2 Total carotenoid content *P. campechiana* extract

The results showed that *P. campechiana* n-hexane and ethyl acetate fruit and leaf extracts contained terpenoids based on the phytochemical analysis. Carotenoids were a category of terpenoids found in the samples. A previous study showed that *P. campechiana* contained beta-carotene, beta-cryptoxanthin, and violaxanthin (Fig. 3)[18]. Furthermore, the total carotenoid content in petroleum ether fruit *P. campechiana* was 278 µg/g extract.[19]

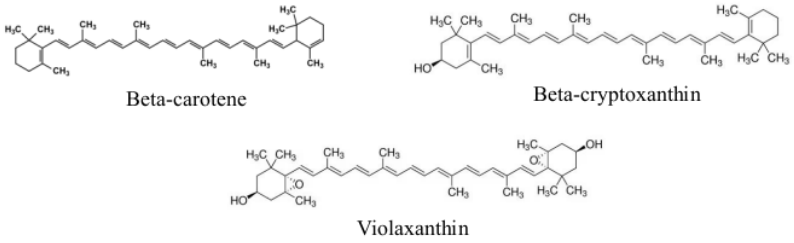

Fig. 3 The carotenoid in *P. campechiana*[18]

Antioxidant Activity and Correlation With Total Carotenoid Content

The antioxidant activity was expressed in terms of IC_{50} and Antioxidant Activity Index (AAI), as shown in Table 3. The IC_{50} was the number of samples required to neutralize 50% of the free radical DPPH, and the lowest value indicated the strongest antioxidant activity. The IC_{50} of DPPH scavenging capacities of NF, EF, NL, and EL were lower compared to DPPH of ascorbic acid standard. Furthermore, LE had better antioxidant activity compared to NE, NF, and EF. The IC_{50} DPPH of ascorbic acid was 1,98 µg/ml, NL and EL ranged from 3.094 to 9.27 µg/mL, while NF and EF ranged from 31,516 to 45,382 µg/mL.

According to previous studies, *P. campechiana* had antioxidant properties. The antioxidant activity of n-hexane, ethyl acetate, and ethanol leaf extract of the plant had been reported employing different extraction methods using Soxhlet with increasing polarity of the solvent.[20] The 70% ethanol extract of pulp, peel, and leaf of *P. campechiana* also exhibited similar properties.[21] *P. campechiana* fruit extracts in methanol, acetone, ethanol, and water at various stages of ripeness (4, 8, 12, 16, and 24 weeks) were found to be effective antioxidants against DPPH.[15]

The carotenoid group could also contribute to the antioxidant activity exhibited by the test plant.[22] Comparing the carotenoid and phenolic groups, the phenolic group had a higher potential for this activity.[23] Beta carotene had antioxidant properties because it contained conjugated double bonds. Through a chemical reaction between carotenoids and free radicals, the conjugated double-bond structure was directly damaged. Furthermore, carotenoids contained long conjugated chains, leading to the high reactivity of these compounds.[24] The phenolic group also contributed to the antioxidant activity. Donating hydrogen atoms to DPPH, phenolic groups, and flavonoids could reduce the damage caused by free radicals.[25] Cinnamic acid and benzoic acids significantly increased the antioxidant activity. Compared to benzoic acid, cinnamic acid had a greater impact on these properties.[26] The difference in IC_{50} values of NF, EF, NL, and EL was caused by the different biological activities of their secondary metabolites.

Fruit and leaf extract of *P. campechiana* had antioxidant activity potentials, which could be expressed with AAI value.[8] Based on the AAI value in Table 3, NF and EF were included in the moderate group, while NL and EL were in the very strong group.

The percentage inhibition value of DPPH was inversely correlated with the IC_{50} value of DPPH. Based on statistics, the Pearson's correlation coefficient between the total carotenoid total content of *P. campechiana* fruit and leaf extract with IC_{50} scavenging of DPPH gave a

positive and significant value ($r = 0.855$, $p<0.01$). This indicated that the total carotenoid content in the fruit and leaf extract had no correlation with inhibition against DPPH potent.

Conclusion

In conclusion, the results showed that NF extract of *Pouteria campechiana* had higher carotenoid total content, followed by EF, EL, and NL extracts. Furthermore, EL extract had better inhibition of DPPH. Based on statistics, total carotenoid content in the fruit and leaf extract of *Pouteria campechiana* had no significant contribution to the inhibition of DPPH.

Conflict of Interest

The author declares no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for a claim relating to the content will be borne by them.

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References

1. Silva, CAM, Simeoni LA, Silveira D. Genus Pouteria: chemistry and biological activity. Rev. Bras. Farmacogn. 2009; 19(2A): 501–509.
2. Kanak-A F, Bakar MFA. Canistel-*Pouteria campechiana* (Kunth) Behni. Academic Press; 2018;107-111.
3. Kubola J, Siriamornpun S, Meeso N. Phytochemicals, vitamin C and sugar content of Thai wild fruits. Food Chem. 2011;126(3):972–981.
4. Aseervatham GSB, Sivasudha T, Sasikumar JM, Christabel HP, Jeya D, Ananth AD. Antioxidant and hepatoprotective potential of *Pouteria campechiana* on acetaminophen-induced hepatic toxicity in rats. J. Physiol. Biochem. 2014;(70):1–14.
5. Costa TS, Lopes R, Wondracek D, Vileria R. Carotenoids composition of canistel (*Pouteria campechiana* (Kunth.) Behni.). Revista Brasileria de Fruticultura. 2010;32(3):903-906.
6. Aulifa DL, Fitriansyah SN, Ardiansyah SA, Wibowo DP, Armiliani Y, and Christy DS. Phytochemical screening, antibacterial activity, and mode of action on *Morus nigra*. Pharmacogn J. 2018; 10(1): 167-171.
7. Fidrianny, I., Suhendy, H., Insanu, M. Correlation of phytochemical content with antioxidant potential of various sweet potato (Ipomea batatas) in West Java, Indonesia, Asian Pacific Journal of Tropical Biomedicine. 2018;8(1):25-30.
8. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2,2-diphenil-1-picrylhydrazyl method. Food Chem. 2009;112:654-658.
9. Thaipong K, Boonprakob U, Crosby K, Zevallos LC, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assay for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal. 2006;19:669- 675.
10. Hartati R, Nadifan HI, Fidrianny I. Crystal guava (Psidium guajava L. "Crystal"): evaluation in vitro antioxidant capacities and phytochemical content. Sci. World J. 2020; vol 2020:1-7.
11. Baky MH, Kamal AK, Elgindi MR, Haggag EG. A review on phenolic compounds from family sapotaceae. J Pharmacogn Phytochem. 2016;5(2):280-7.

12. Lanerolle, S.de M, Priyadarshani, A.M.B, Sumithraarachchi, D.B, and Jansz, E.R. The carotenoids of Pouteria campechiana (Sinhala: ratalawulu). J.Natn.Sci. 2008;36(1):95-98.
13. Ma J, Yang H, Basile MJ, Kennelly EJ. Analysis of polyphenolic antioxidants from the fruit of three *Pouteria* species by selected ion monitoring liquid chromatography-mass spectrometry. J Agric Food Chem. 2004;52(19):873-878.
14. Sangeetha R, Pratheeba T, Ragavendran C, Natarajan D. *Pouteria campechiana* leaf extract and its bioactive compound myricitrin are mosquitocidal against Aedes aegypti and Culex quinquefasciatus. Asian Pac. J. Trop. Med. 2019;12(7):321–328.
15. Matsushita Y, Suzuki R, Nara E, Yokoyama A, Miyashita K. Antioxidant activity of polar carotenoids including astaxanthin-b-glucoside from marine bacterium on PC liposomes. Fisheries Sci. 2000;66:980-985.
16. Saini, R.K, Keum Y-S. Carotenoid extraction methods: a review of recent developments. Food Chemistry. 2017;(7):1-50.
17. Amorim-Carrilho, K. T., Cepeda, A., Fente, C., & Regal, P. Review of methods for analysis of carotenoids. TrAC Trends in Analytical Chemistry. 2014;56:49–73.
18. Ragasa CY, Labaclado LM, Rideout JA. Triterpenes and sterol from *Pouteria campechiana*. Philipp J. Sci. 2011;6(2):1–7.
19. Puspita D, Kurniawan YA, Aiboi Y. Kandungan KArotenoid Mentega dari Sawo Keju (*Pouteria campechiana*). Journal Food and Nutrition Science. 2019;3(1):1-9.
20. Fitriansyah SN, Hartati I, Fidrianny I. Effect of Different Solvent on Phytochemical Content, Tyrosinase Inhibition and Antioxidant Activities of Campolay (Pouteria campechaina Kunth. Baehni.). Open Acces Maced J Med Sci. 2022;10(A):158-163.
21. Hidayah N, Aulifa DL, Fitriansyah SN. Determination of Total Phenolic, Flavonoid Content and Antioxidant Activity of Campolay (*Pouteria campechiana*). Advances in Health Sciences Research. 2020;26:107-109.
22. Kalpana B, Vijay DW, Sanjay ST, Bhushan RS. Phytochemical, antimicrobial evaluation and determination of total phenolic and flavonoid contents of Sesbania grandiflora flower extract. Int J Pharm Pharm Sci. 2012;4(4):229-232
23. Fiedor J, Burda K. Potential role of carotenoids as antioxidant in human health and disease. Nutrients. 2014;6:466-488.
24. Sirvastava, R. Physicochemical, antioxidant properties of carotenoids and its optoelectronic and interaction studies with chlorophyll pigments. Scientific Reports. 2011; 11(18365):1-14.
25. Neeraj KS, Partap S, Priyanka, Keshari JK, Herman KS, Anil KS. Free radical scavenging activity of methanolic extract of Luffa cylindrica leaves. Int J Green Pharm. 2012;6(3): 231-236
26. Fidrianny I, Aristya T, Hartati R. Antioxidant capacities of various leaves extracts from three species of legumes and correlation with total flavonoid, phenolic, carotenoid content. Int J Pharmacogn Phytochem Res. 2015;7(3):628-634.

Table 1. Yield Extract *P. campechiana*

Extract	Yield of Extract (%)
NF	2.82
EF	5.16
NL	2.4
EL	5.74

Table 2. Phytochemical Screening of Crude Drug and Extract *P. campechiana*

Metabolite	Crude Drug		Extract			
	Fruit	Leaf	NF	EF	NL	EL
Alkaloid	+	+	+	+	+	+
Phenol	+	+	-	+	-	+
Tanin	+	+	-	-	-	+
Flavonoid	+	+	-	+	-	+
Quinone	+	+	-	-	-	-
Saponin	-	-	-	-	-	-
Triterpenoid/steroid	+	+	+	+	+	+
Monoterpenoid/sesquiterpenoid	+	+	+	+	+	+

Table 3. Antioxidant activity with IC_{50} extract *P. campechiana* to DPPH

Sample	IC_{50} µg/mL	Antioxidant Activity Index (AAI)
NF	45.382 ± 2.31	0.551 ± 0.102
EF	31.516 ± 1.786	0.793 ± 0.098

NL	9.270 ± 1.201	2.697 ± 0.312
EL	3.094 ± 0.82	8.080 ± 0.221
As.acid	1.980 ± 0.230	12.626 ± 0.099

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