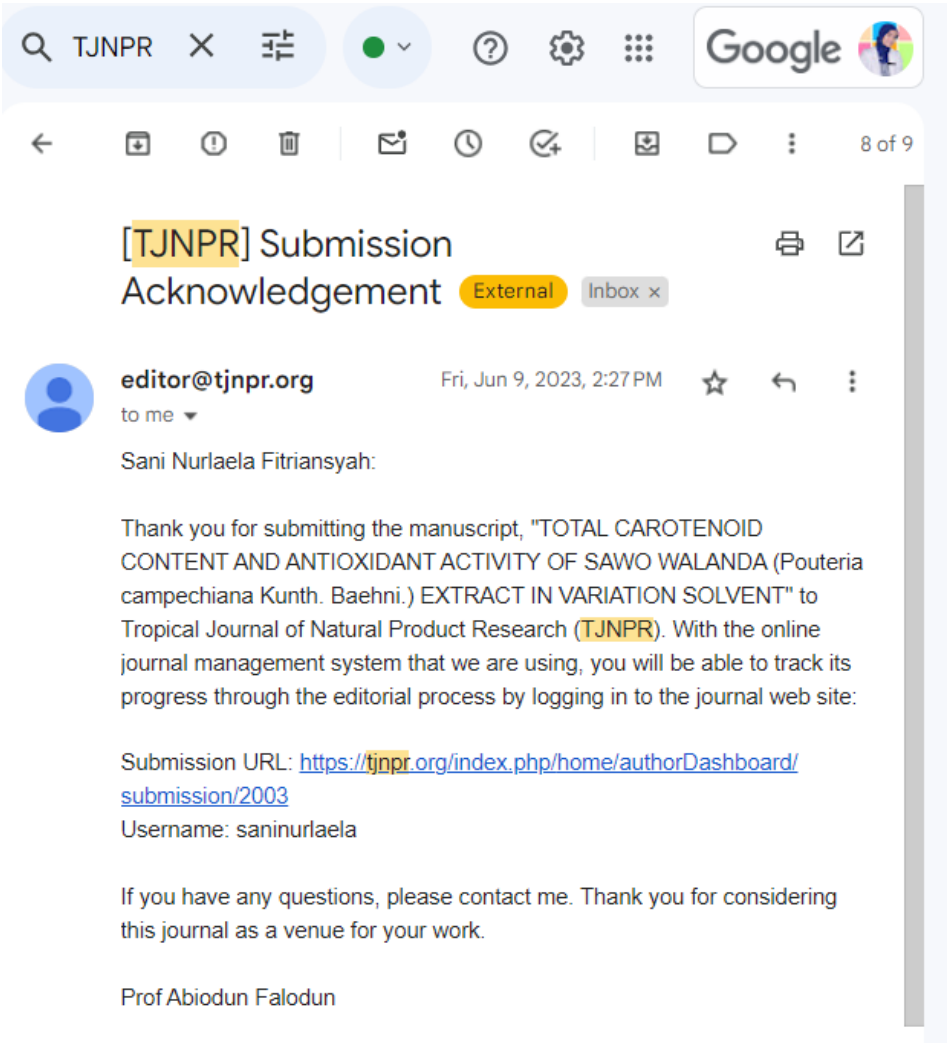
BUKTI KORESPONDENSI JURNAL INTERNASIONAL

Tropical Journal of Natural Product Research

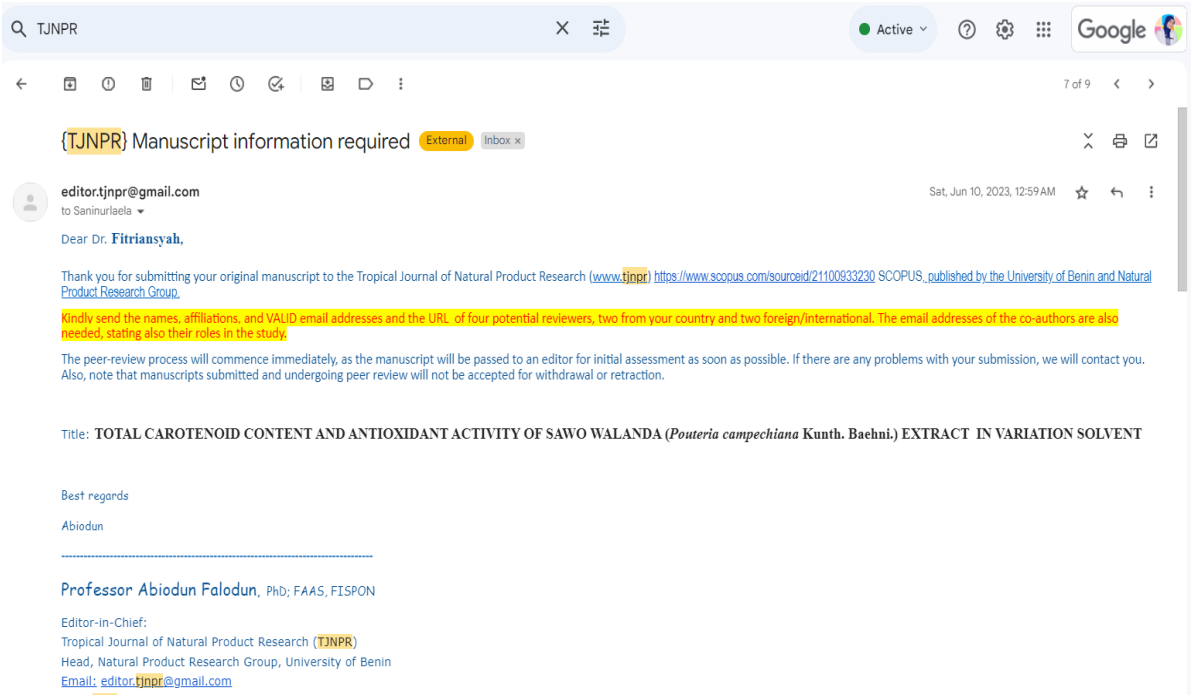
Total Carotenoid Content and Antioxidant Activity of Sawo Walanda (Pouteria campechiana Kunth. Baehni) Extract from Various Solvent

1. Submit ke Journal Tropical Journal of Natural Product Research (9 Juni 2023)
2. Permintaan melengkapi data dari Editor TJNPR (10 Juni 2023)
3. Revisi pertama, Accepted dengan revisi sedang (moderate revision) (17 Juni 2023)
4. Editorial and Reviewer Comment (5 Juli 2023)
5. Submit revisi pertama (9 Juli 2023)
6. Proofread dokumen diterima (15 Juli 2023)
7. Submit revisi kedua dan artikel diterima : minor revisi (17 Juli 2023)
8. Check galley proof (25 Juli 2023)
9. Article published online (1 Agustus 2023)

1. Submit ke Journal Tropical Journal of Natural Product Research (9 Juni 2023)



2. Permintaan melengkapi data dari Editor TJNPR (10 Juni 2023)



TJNPR

Active

Google

7 of 9

{TJNPR} Manuscript information required External Inbox ×

editor.tjnpr@gmail.com
to Saninurlaela

Sat, Jun 10, 2023, 12:59 AM

Dear Dr. **Fitriansyah,**

Thank you for submitting your original manuscript to the Tropical Journal of Natural Product Research (www.tjnpr) https://www.scopus.com/sourceid/21100933230 SCOPUS, published by the University of Benin and Natural Product Research Group.

Kindly send the names, affiliations, and VALID email addresses and the URL of four potential reviewers, two from your country and two foreign/international. The email addresses of the co-authors are also needed, stating also their roles in the study.

The peer-review process will commence immediately, as the manuscript will be passed to an editor for initial assessment as soon as possible. If there are any problems with your submission, we will contact you. Also, note that manuscripts submitted and undergoing peer review will not be accepted for withdrawal or retraction.

Title: **TOTAL CAROTENOID CONTENT AND ANTIOXIDANT ACTIVITY OF SAWO WALANDA (*Pouteria campechiana* Kunth. Baehni.) EXTRACT IN VARIATION SOLVENT**

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email: editor.tjnpr@gmail.com

sani nurlaela fitriansya <saninurlaela@stfi.ac.id>
to editor.tjnpr

Sun, Jun 11, 2023, 4:15 PM

Dear Editor,
Here I attach the reviewer's recommendations:
1. Dr. Diki Prayugo, with email : dikiprayugo@stfi.ac.id
2. Siti uswatun hasanah, with email : sitiuswatunhasanah@stfi.ac.id
3. Saida hanoun, with email : saida.hanoun@univ-khenchela.dz
4. Trang naguyen, with email : nguyenhadieutrang@iuh.edu.vn

Thank You,

•••

--
Best regard,
Sani Nurlaela Fitriansyah, M.Si., Apt
Sekolah Tinggi Farmasi Indonesia
Bandung, West Java

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>
to me

Mon, Jun 12, 2023, 2:36 AM

Thank you for the information.

Best regards

Abiodun

3. Revisi pertama, Accepted dengan revisi sedang (moderate revision) (17 Juni 2023)

TJNPR

Active

Google

6 of 9

1 of 25,962 (TJNPR) Editor Decision External Inbox ×

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>
to sani

Sat, Jun 17, 2023, 9:52 PM

Dear Dr. Fitriansyah,

The manuscript submitted to the Tropical Journal of Natural Product Research www.tjnpr.org
https://www.scopus.com/sourceid/21100933230 has been carefully reviewed by competent experts.

I am pleased to inform you that the manuscript has been accepted for publication in Tropical Journal of Natural Product Research.

Find attached the details of the decision.

Please send your response urgently to the Editor-in-Chief, to enable us to process your manuscript for the next issue Vol 7 issue 6, 2023.
Kindly acknowledge the receipt of the mail.

Title: TOTAL CAROTENOID CONTENT AND ANTIOXIDANT ACTIVITY OF SAWO WALANDA (*Pouteria campechiana* Kunth. Baehni.) EXTRACT IN VARIATION SOLVENT

Authors: Sani Nurlaela Fitriansyah*, Tiara Yollanda, Hesti Riasari

TJNPR Editorial Decision: accepts with moderate revisions

Thank you very much for choosing to publish with Tropical Journal of Natural Product Research.

TJNPR is now Q3

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email: editor.tjnpr@gmail.com
www.tjnpr.org SCOPUS, SCImago SJR Q4 0.13
https://www.scopus.com/sources.uri

Professor of Pharmaceutical Chemistry, FAAS
Fellow, Fulbright (USA)
Deputy Vice-Chancellor (Academic) 2014-2016
Faculty of Pharmacy
University of Benin
Phone: +234-807-318-4488;
email: faloabi@uniben.edu; abiodun.falodun@fulbrightmail.org
Google Scholar Citations
SCOPUS https://www.scopus.com/authid/detail.uri?

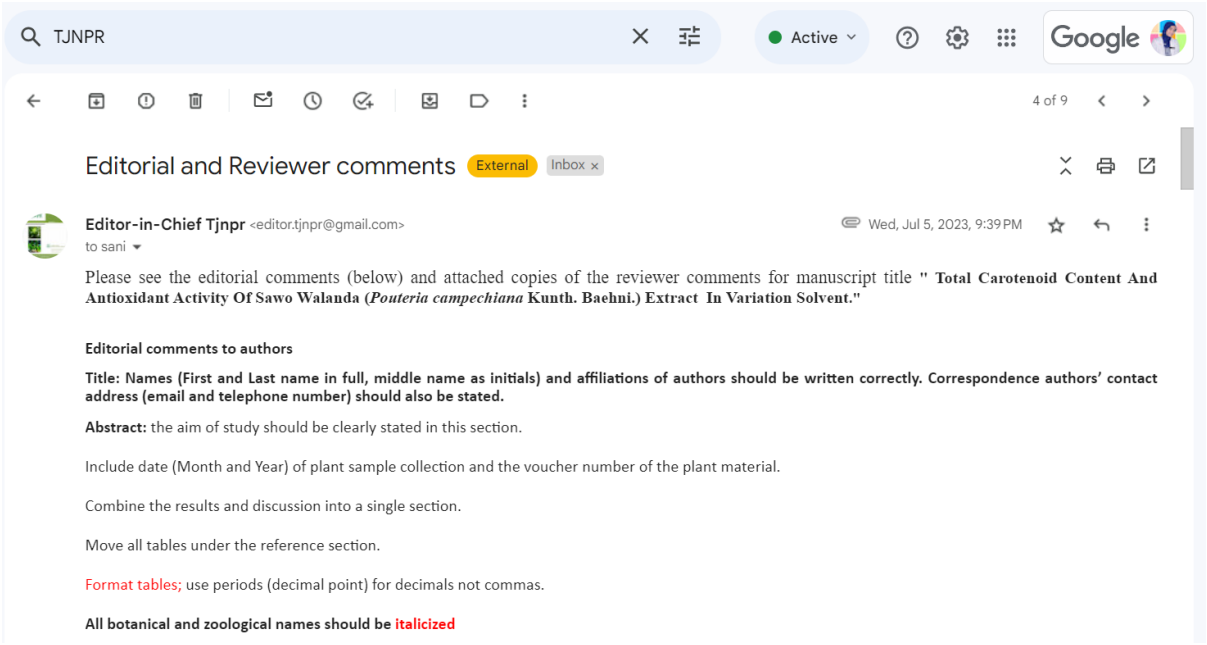
https://orcid.org/0000-0003-2929-3305authorId=12794326500#top

University of Benin TJNPR SCOPUS Q4
www.uniben.edu www.tjnpr.org

One attachment • Scanned by Gmail

Provisional accep...

4. Editorial and Reviewer Comment (5 Juli 2023)



TJNPR

Active

Google

4 of 9

Editorial and Reviewer comments External Inbox ×

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> Wed, Jul 5, 2023, 9:39 PM

to sani

Please see the editorial comments (below) and attached copies of the reviewer comments for manuscript title " **Total Carotenoid Content And Antioxidant Activity Of Sawo Walanda (*Pouteria campechiana* Kunth. Baehni.) Extract In Variation Solvent."**

Editorial comments to authors

Title: Names (First and Last name in full, middle name as initials) and affiliations of authors should be written correctly. Correspondence authors' contact address (email and telephone number) should also be stated.

Abstract: the aim of study should be clearly stated in this section.

Include date (Month and Year) of plant sample collection and the voucher number of the plant material.

Combine the results and discussion into a single section.

Move all tables under the reference section.

Format tables; use periods (decimal point) for decimals not commas.

All botanical and zoological names should be italicized

All comments/corrections made by reviewers should be completely addressed, point by point, and make appropriate changes in the manuscript, or provide a suitable rebuttal to any specific request for change that has not been made.

All corrections/changes made in the manuscript should be **highlighted in yellow** when submitting the manuscript in the revised form on or before 9th July 2023

The authors should carefully revise and correct the manuscript taking into consideration the comments of all the reviewers.

50% of the references cited should be between 2016-2020. The revised and corrected manuscript should be subjected to plagiarism checker (17% allowed in TJNPR) and English language editing. Evidence of the checks should be attached when submitting the revised/corrected manuscript.

During submission of the revised manuscript include another file labelled "**Responses to reviewers' comments**" (a matrix) clearly showing your responses to each of the issues raised by the reviewers; mention the section, page and paragraph/lines where and how the changes/corrections have been made.

Strictly adhere to the author guidelines. Make sure that all the facts and information provided in the manuscript are correct. Check grammar, spelling, spacing, other information and facts including scientific names, formulae, symbols, equations, etc.

Ensure that all the references are correctly cited in the text and list. Verify all the references from their original sources. Confirm correctness of the citation info such as authors' names (surnames, initials, spelling, arrangements, etc), year, title, journal, volume, pages, punctuation, etc. The numbers and units must be presented according to the journal style. Use clearly distinguishable patterns for the illustrations/figures (e.g., graphs and charts) such that they should be legible even for black and white printing or when reduced in size.

Proofread the whole document after effecting all the corrections. The revised version should be approved by all the co-authors before submitting it.

A manuscript not complying with these and other instructions will not be processed and may be rejected.

Please find the attached review comments for your revisions.

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email: editor.tjnpr@gmail.com
www.tjnpr.org SCOPUS, SCImago SJR Q4 0.13
https://www.scopus.com/sources.uri

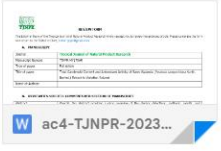
Professor of Pharmaceutical Chemistry, FAAS
Fellow, Fulbright (USA)
Deputy Vice-Chancellor (Academic) 2014-2016
Faculty of Pharmacy
University of Benin
Phone: +234-807-318-4488;
email: faloabi@uniben.edu; abiodun.falodun@fulbrightmail.org
Google Scholar Citations
SCOPUS https://www.scopus.com/authid/detail.uri?

https://orcid.org/0000-0003-2929-3305authorId=12794326500#top

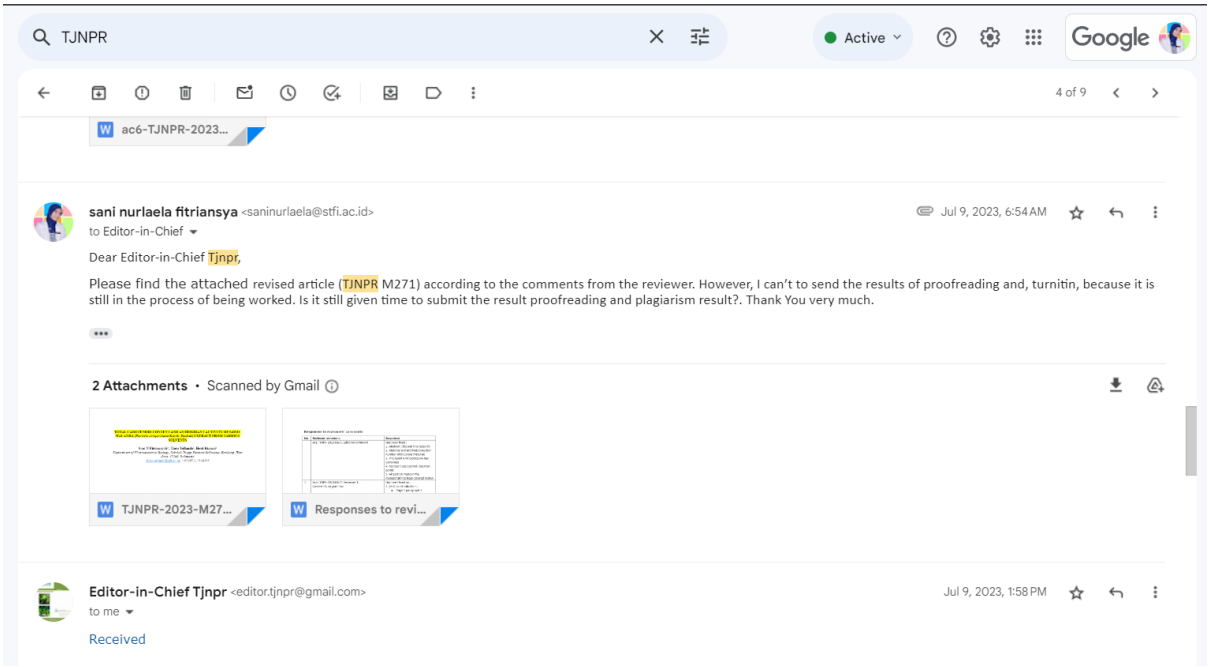
https://orcid.org/0000-0003-2929-3305authorId=12794326500#top

University of Benin TJNPR SCOPUS Q4
www.uniben.edu www.tjnpr.org

6 Attachments • Scanned by Gmail



5. Submit revisi pertama (9 Juli 2023)



6. Proofread dokumen diterima (15 Juli 2023)

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

Sani N Fitriansyah[1*], Tiara Yollanda[1], Hesti Riasari[1]
Department of Pharmaceutical Biology, Sekolah Tinggi Farmasi Indonesia
Bandung, West Java, 40266, Indonesia
Saninurlaela@stfi.ac.id, +62 (022) 7514355

Style Definition: Hyperlink: Font color: Custom Color(RGB(5;99;193))
Style Definition: Comment Reference
Style Definition: Comment Text: Font: (Default) Calibri
Style Definition: Comment Subject: Font: (Default) Calibri
Style Definition: Balloon Text: Font:
Style Definition: HTML Preformatted
Style Definition: Header: Font: (Default) Calibri
Style Definition: Footer: Font: (Default) Calibri
Formatted: Centered

ABSTRACT

~~Sawo~~Pouteria campechiana, also known as sawo walanda ~~is rich~~ in ~~carotenoid. According to reports, carotenoid can act as antioxidants. Solvent selection is important in extracting the carotenoid.~~Indonesia, is known for its high abundance of carotenoids, which are recognized for their antioxidant properties. The ~~objective of~~extraction of these constituents involves a crucial step of selecting an appropriate solvent, as indicated by several studies. Therefore, this study ~~was~~aims to ~~evaluate~~assess the antioxidant activity ~~and determined~~ as well as determine the total carotenoid content of P. campechiana fruit and ~~leaves~~leaf extract in various ~~solvent~~solvents. The ~~research methods included the~~study methodology involved extraction ~~used~~using the maceration method with n-hexane and ethyl acetate ~~solvent~~as solvents. Antioxidant activity and determination of total carotenoid were carried out using UV-Vissible spectrophotometry. DPPH ~~is~~was used as a free radical ~~and~~, while β-carotene ~~is used~~served as the standard for total carotenoid content. The ~~findings revealed~~results showed that ~~the~~ n-hexane fruit (NF) extract had the highest total carotenoid concentration (70.028 gBEQ/~~100g~~100 g extract~~) and the~~), while n-hexane leaf (NL) extract had the lowest (39.540 gBEQ/~~100g~~100 g extract). ~~Antioxidant activity was expressed with IC~~$_{50}$ ~~(µg/ml). The result of antioxidant activity showed that the ethyl~~Ethyl acetate leaf (EL) extract ~~had~~exhibited better antioxidant activity with IC$_{50}$ ~~(~~of 3.~~094µg~~094 µg/mL±0.82~~) than the n-hexane leaves~~ compared to NL (9.~~270µg~~270 µg/mL±1.201), ~~the~~ ethyl acetate fruit ~~extract~~(EF) (31.~~516µg~~516 µg/mL±1.786), and ~~the n-hexane fruit extract~~NF (45.~~382µg~~382 µg/mL±2.31~~).~~) extracts. Based on the statistical analysis results, the coefficient correlation of total carotenoid content with IC$_{50}$ DPPH ~~is~~was r = 0.855, p<0.01. This ~~means~~indicated that an increase in total carotenoid content ~~is~~did not always ~~followed by an increase~~lead to a proportional increment in the inhibition of DPPH. ~~In our work, it can be concluded~~These findings revealed that n-hexane solvent ~~is~~was more suitable for extracting carotenoids from the fruit part, while ethyl acetate ~~is~~was more ~~suitable~~appropriate for the ~~leaves. Fruit~~leaf. Furthermore, the fruit and leaf extract of P. campechiana ~~have~~had potential antioxidant activity as natural ingredients in the food and pharmaceutical industries.

~~Key word~~ ***Keywords:*** *Sawo walanda, Pouteria campechiana, carotenoid, antioxidant activity*

INTRODUCTION

The family Sapotaceae, which is ~~extensively~~ distributed ~~globally~~worldwide, includes the genus Pouteria. ~~In traditional medicine,~~ Pouteria species have ~~been~~ a rich history in traditional medicine, being used ~~to treat~~for the treatment of various ailments such as back pain, ulcers, skin eruptions, and inflammation.[1] ~~One of~~Among the ~~Sapotaceae family's~~ medicinal plants ~~is~~within the Sapotaceae family, *Pouteria campechiana* ~~which was known~~, 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 ~~is well known~~has gained recognition for its anti-inflammatory, antioxidant, and hepatoprotective properties.[3,4] ~~*Pouteria campechiana*~~ Furthermore, extracts obtained from its leaf and ~~fruits extract contains phenolic group, flavonoid, and terpenoid group and~~ fruit have been found to contain phenolics, flavonoids, and terpenoids, exhibiting significant antioxidant activity.[5]

~~Sources~~Medicinal plants serve as a valuable source of raw materials ~~with phenolic~~containing phenolics, flavonoids,~~ other~~ polyphenols, and terpenoids~~ include medicinal plants. A phytochemical molecule may~~. These compounds have been reported to be ~~the reason for a plant's biological action.~~responsible for the various bioactivities exhibited by plants. The specific phytochemical component produced ~~will depend~~depends on the extraction technique and the solvent employed. Carotenoids ~~belong~~are compounds belonging to ~~the~~a group of secondary metabolites ~~called~~known as terpenoids. ~~The following~~Some of the common solvents

~~are employed to extract~~used for the extraction of carotenoids~~:~~ include acetone, chloroform, hexane, methanol, methylene chloride, ethyl acetate, and diethyl ether.[5] The ~~appropriateness of the chosen~~choice of solvent ~~will depend~~is largely dependent on the ~~kinds~~type and concentrations of carotenoids ~~that are intended for extraction. So choosing the right~~being extracted. Therefore, the selection of the appropriate solvent ~~for the extraction process becomes~~ is very important ~~in carotenoid extraction.~~

~~In~~, Therefore, this study~~, the~~ aims to assess the antioxidant activity as well as determine the total carotenoid content of P. campechiana fruit and leaf extract in ~~n-hexane and ethyl acetate extract fruit and leaves *P.campechiana* was calculated, antioxidant activity were tested and the~~ various solvents. The correlation of total carotenoid content with antioxidant activity ~~were~~was analyzed ~~use Pearson'~~using Pearson's method.

Formatted: Font: 11 pt, Font color: Black

Formatted: Font: 11 pt, Font color: Black

Formatted: Font: 11 pt, Font color: Black

MATERIAL AND METHODS

Material

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

Sample ~~preparation~~Preparation

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

Extraction

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

Phytochemical ~~analysis~~Analysis

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

Antioxidant ~~activity~~Activity

Antioxidant activity test was carried out using the DPPH ~~adopted from~~method proposed by Fidrianny, (2018~~.~~).[7] The standard for contrasting antioxidant chemicals was ascorbic acid~~. The~~, and the concentration of DPPH used was 50 µg/mL, which ~~serves~~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 ~~and after incubation during~~. After incubating the sample for 30 min ~~subsequently measuring~~, the absorbance was measured at 517 nm~~. This~~, and this procedure was repeated in triplicate for each sample.

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

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

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. ~~It~~The IC_{50} was then determined using the calibration linear regression value~~. The IC50 value was calculated using~~, with the x-value ~~from the linear regression~~being used for calculations.

Determination of Antioxidant Activity Index

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

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

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

Determination of ~~total carotenoid content~~Total Carotenoid Content

Thaipong[9] and Fidrianny[10] ~~decided to measure~~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. ~~The~~Furthermore, the analysis was ~~conducted~~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~~The total carotenoid content was determined ~~utilizing~~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 ~~three copies. The~~triplicates, where the averages and standard deviations of at least three separate experiments ~~are~~were used to calculate all ~~of~~ the results. ~~The~~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 ~~by~~using maceration with different polarity solvents. The amount of metabolite content of each *P. campechiana* extract ~~can be seen~~is presented in Table 1. The ~~findings show~~results showed that ethyl acetate solvent ~~may~~could extract a higher amount of metabolites from *P. campechiana* fruit and ~~leaves. Both~~leaf. Furthermore, primary and secondary metabolites ~~may~~could be among the extracted compounds.

Phytochemical ~~analysis~~Analysis

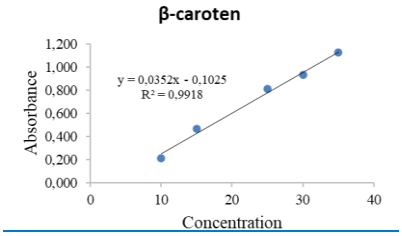
Table 2 ~~lists~~shows the results of the phytochemical screening on the crude and each extract. Phenolic compounds, flavonoids, and saponin were absent in NF and NL~~.~~, while Monoterpene/sesquiterpene was ~~absent~~not found in EF and EL. Phenol and saponins ~~are~~were semipolar to polar metabolites, ~~while~~but monoterpenes/sesquiterpenes ~~tend~~tended to be

nonpolar to semipolar. Several studies showed that *P. campechiana* ~~is known~~was be rich in phenolic compounds, flavonoids, and terpenoids. ~~*P. campechiana*~~ The leaf ~~is rich in flavonoid, stilbenoid,~~contained high levels of flavonoids stilbenoids and ~~tannin[11], whereas in~~tannins[11], while the fruit was rich in terpenoids, including carotenoid[12] and phenolic acid[13]. According to Sangeetha[14] ~~related to our work, which flavonoid~~, flavonoids and ~~saponin~~saponins were also absent in the fruit extract. Acetone extract leaf *P. campechiana* ~~presents alkaloid, flavonoid, saponin, steroid, tannin~~contained alkaloids, flavonoids, saponins, steroids, tannins, and ~~terpenoid[14]~~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 ~~is~~was frequently employed as a standard. The highest ~~total carotenoid~~ content ~~was found in NF of~~ (70,028 ± 2,341) g BE/100 g extract ~~and~~was found in NF, while the smallest was ~~indicated~~exhibited by NL at (39,40 ± 1,76) g BE/100 g extract, as shown in Fig. 2. The intensity of the carotenoids ~~is~~was attributed to their conjugated double bonds, which ~~result in~~led to a vibrant yellow-orange shade.[15] NF and EF gave stronger orange-yellow color ~~than~~compared to NL and EL.

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



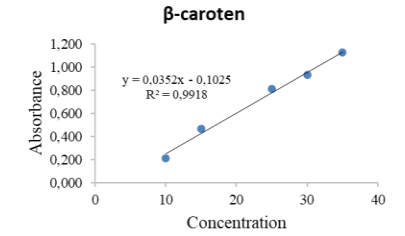


Fig. 1 Standard Curve β-carotene

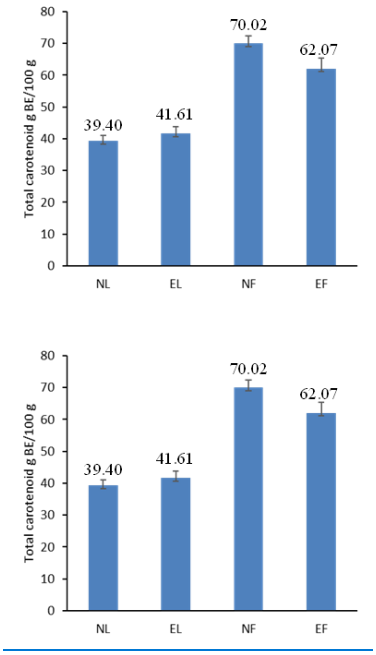


Fig. 2 Total carotenoid content ~~*Pouteria*~~*P. campechiana* extract

~~*P.*~~ The results showed that *P. campechiana* ~~conducted terpenoid in~~ n-hexane and ethyl acetate fruit and leaf extracts contained terpenoids based on the phytochemical analysis. ~~Category~~Carotenoids were a category of ~~terpenoid~~terpenoids found in the samples. A previous study showed that ~~contains carotenoids.~~ *P. campechiana* ~~exhibited~~contained beta-carotene, beta -cryptoxanthin, and violaxanthin~~, according to a prior study~~ (Fig.4 3)[18]~~, and~~. Furthermore, the total carotenoid content in petroleum ether fruit *P. campechiana* ~~showed~~was 278 µg/g extract.[19]

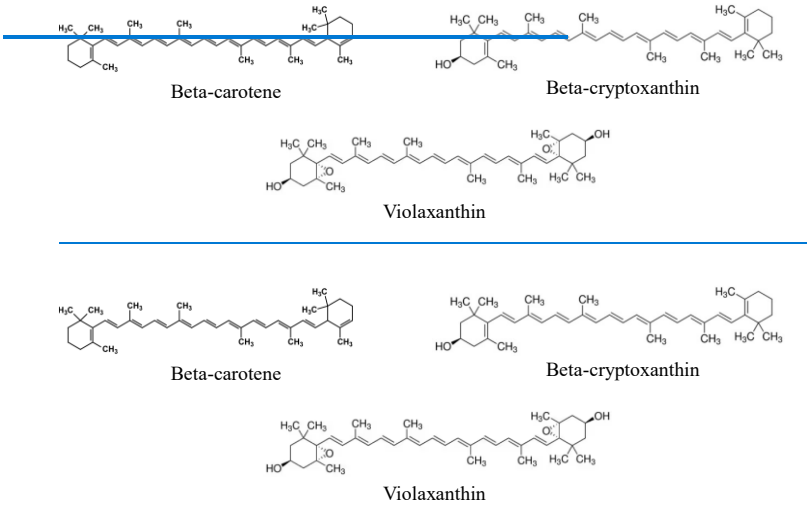


Fig. 3 The carotenoid in *P. campechiana*[18]

Antioxidant Activity and Correlation With Total Carotenoid Content

~~Antioxidant~~The antioxidant activity was expressed in terms of IC_{50} and ~~in~~ Antioxidant Activity Index (AAI), as shown in Table 3. -The IC_{50} ~~is~~was the number of samples required to neutralize 50% of the free radical DPPH~~. The IC_{50} value with~~ , and the lowest value ~~indicates~~indicated the strongest antioxidant activity. The IC_{50} of DPPH scavenging capacities of NF, EF, NL, and EL were lower ~~when~~ compared to ~~IC_{50} of~~ 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, ~~the IC_{50} DPPH values of NL,~~ 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 ~~some of the~~ previous studies, ~~the *Pouteria*~~*P. campechiana* ~~has~~had antioxidant properties. ~~In different extraction methods using Soxhlet with increasing polarity of the solvent, the~~The antioxidant activity of n-hexane, ethyl acetate, and ethanol leaf extract of ~~leaves of *P.campechiana*~~ the plant had been reported employing different extraction methods using Soxhlet with increasing polarity of the solvent.[20] The 70% ethanol ~~70%~~ extract of pulp, peel, and ~~leaves~~leaf of *P. campechiana* ~~showed antioxidant activity~~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 ~~can~~could also contribute to the antioxidant activity exhibited by the test plant.[22] Comparing the carotenoid and phenolic groups, the phenolic group ~~has~~had a higher potential for ~~antioxidant properties.~~this activity.[23] Beta carotene ~~has~~had antioxidant properties because it ~~contains~~contained conjugated double bonds. Through a chemical reaction between carotenoids and free radicals, the conjugated double-bond structure ~~is~~was directly damaged. ~~Carotenoids contain~~Furthermore, carotenoids contained long conjugated chains, ~~which makes them very reactive~~leading to the high reactivity of these compounds.[24] The phenolic group ~~can contribute~~also contributed to the antioxidant activity. Donating hydrogen atoms to DPPH,

phenolic groups, and flavonoids ~~may~~could reduce the damage caused by free radicals.[25] ~~Both cinnamic~~Cinnamic acid and benzoic acids significantly ~~increase~~increased the antioxidant activity. Compared to benzoic acid, cinnamic acid ~~may have~~had a greater impact on ~~antioxidant activity~~these properties.[26] The ~~existence of a biological activity approach to the content of secondary metabolites, can be one reason that can explain the~~ difference in IC_{50} values ~~in~~of NF, EF, NL, and EL was caused by the different biological activities of their secondary metabolites.

Fruit and ~~leaves~~leaf extract ~~*Pouteria*~~of *P. campechiana* ~~have the potential as~~had antioxidant activity ~~properties. The grouping antioxidant activity can~~potentials, which could be expressed with AAI value.[8] Based on the AAI value~~,~~ in Table 3, NF and EF ~~are~~were included in the moderate group ~~antioxidant activity~~, while NL and EL ~~are included~~were in the very strong ~~antioxidant activity~~group.

The percentage inhibition value of DPPH ~~is~~was inversely correlated with the IC_{50} value of DPPH. ~~The percentage inhibition value of DPPH increases with a decrease in the DPPH IC_{50} value.~~ Based on statistics, the Pearson's correlation coefficient between the total carotenoid total content of *P. campechiana* fruit and ~~leaves~~leaf extract with IC_{50} scavenging of DPPH gave a ~~value~~ positive and significant value (r = 0.855, p<0.01). ~~It means,~~This indicated that the total carotenoid content in the fruit and leaf extract ~~has not~~had no correlation with inhibition against DPPH potent.

Conclusion

~~The study result concluded~~In conclusion, the results showed that ~~the n-hexane~~NF extract ~~fruit~~of *Pouteria campechiana* ~~was a~~had higher carotenoid total content, followed by ~~ethyl acetate~~EF, EL, and NL extracts. Furthermore, EL extract ~~fruit, ethyl acetate leaves, and n-hexane extract leaves *Pouteria campechiana*. The ethyl acetate leaf extract has~~had better inhibition of DPPH. Based on statistics, total carotenoid content in the fruit and ~~leaves~~leaf extract of *Pouteria campechiana* ~~has not significantly contributed~~had no significant contribution to the inhibition of DPPH.

Conflict of Interest

The ~~Author~~author declares no conflict of interest.

~~Author's~~Authors' Declaration

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

Acknowledgments

The ~~author is~~authors are grateful to Sekolah Tinggi Farmasi Indonesia. This ~~work~~study was carried out during the academic year 2021/2022.

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.

Formatted: Indent: Left: 0 cm, Hanging: 0,75 cm, Numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0,63 cm + Indent at: 1,27 cm

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~~Extract (%)**
NF	2.82
EF	5.16
NL	2.4

EL	5.74

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

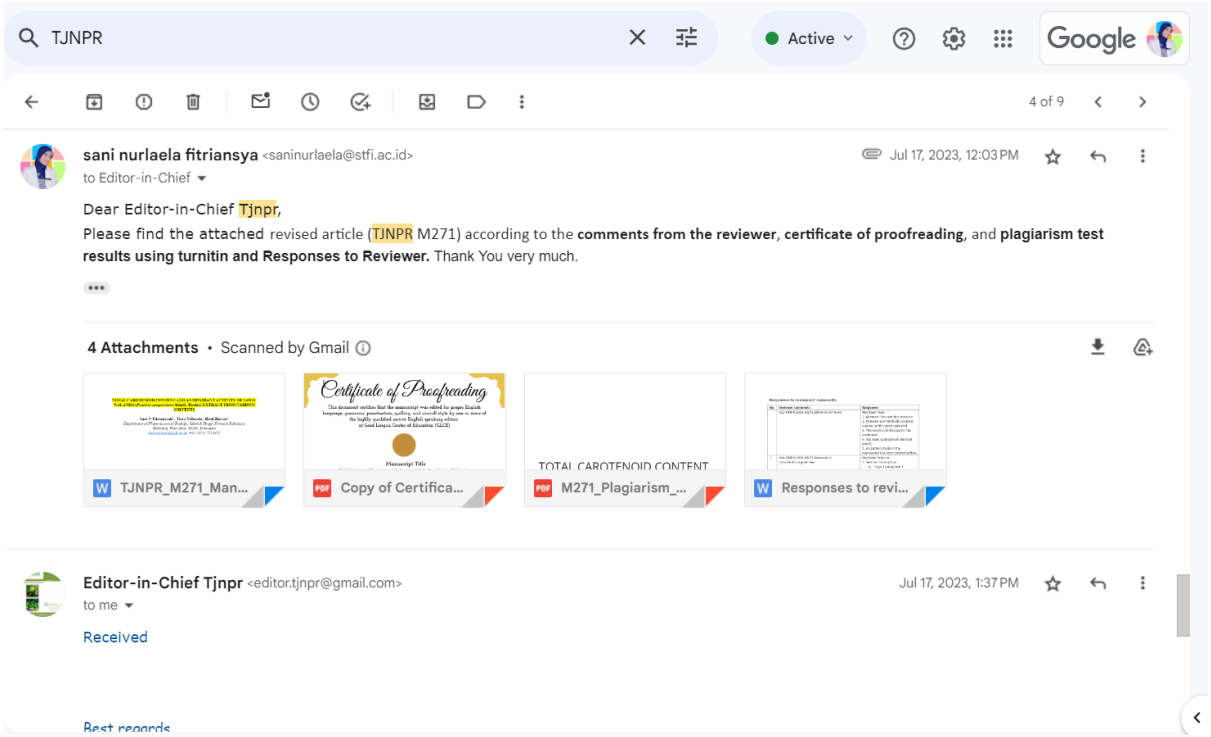
Metabolite	**Crude Drug**		**Extract**			
	Fruit	Leaf	NF	EF	NL	EL
Alkaloid	+	+	+	+	+	+
Phenol	+	+	-	+	-	+
Tanin	+	+	-	-	-	+
Flavonoid	+	+	-	+	-	+
Quinone	+	+	-	-	-	-
Saponin	-	-	-	-	-	-
Triterpenoid/steroid	+	+	+	+	+	+
Monoterpenoid/sesquiterpenoid	+	+	+	+	+	+

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

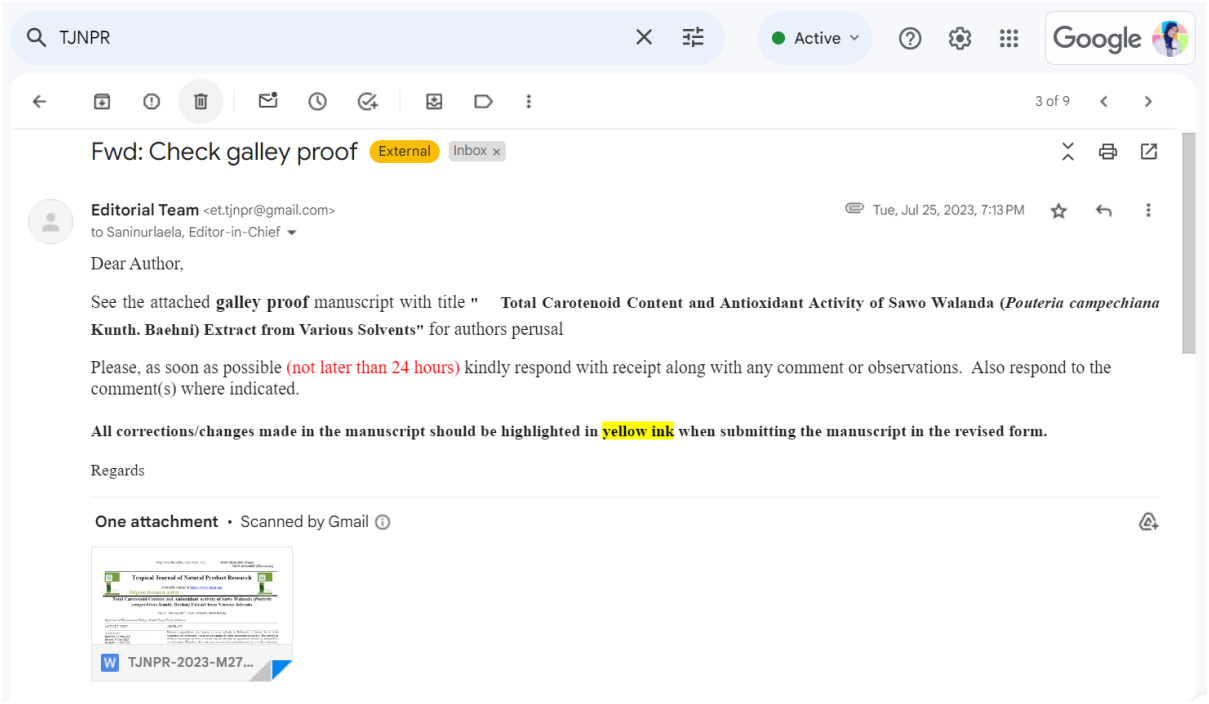
Sample	**IC_{50} µg/mL**	**Antioxidant Activity Index (AAI)**
NF	45.382 ± 2.31	0.551 ± 0.102
EF	31.516 ± 1.786	0.793 ± 0.098
NL	9.270 ± 1.201	2.697 ± 0.312
EL	3.094 ± 0.82	8.080 ± 0.221
As.acid	1.980 ± 0.230	12.626 ± 0.099

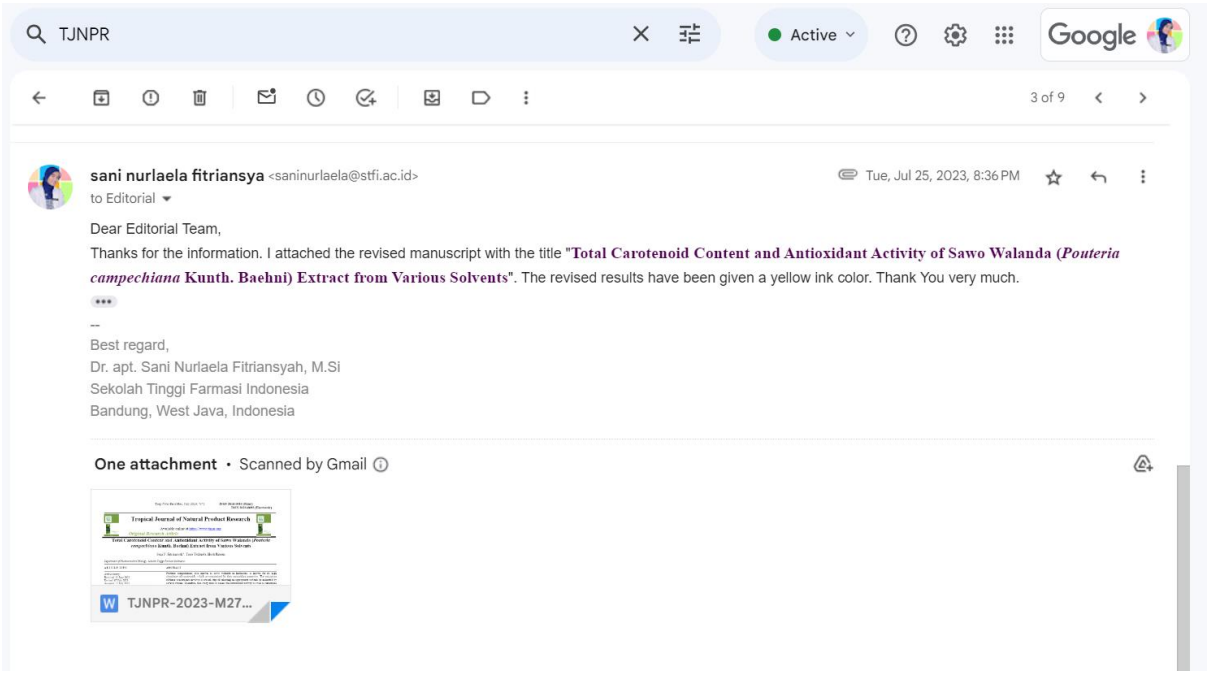
Formatted: Font: Bold

7. Submit revisi kedua dan artikel diterima : minor revisi (17 Juli 2023)



8. Check galley proof (25 Juli 2023)





9. Article published online (1 Agustus 2023)

Trop J Nat Prod Res, July 2023; 7(7):3478-3481 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Total Carotenoid Content and Antioxidant Activity of Sawo Walanda (*Pouteria campechiana* Kunth. Baehni) Extract from Various Solvents

Sani N. Fitriansyah*, Tiara Yollanda, Hesti Riasari

Department of Pharmaceutical Biology, Sekolah Tinggi Farmasi Indonesia.

ARTICLE INFO

Article history:
Received 11 June 2023
Revised 07 July 2023
Accepted 21 July 2023
Published online 01 August 2023

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.

*Corresponding author. E mail: Saninurlaela@stfi.ac.id
Tel: +62 (022) 7514355

Citation: Fitriansyah SN, Yollanda T, Riasari H. Total Carotenoid Content and Antioxidant Activity of Sawo Walanda (*Pouteria campechiana* Kunth. Baehni) Extract from Various Solvents. Trop J Nat Prod Res. 2023; 7(7): 3478-3481 http://www.doi.org/10.26538/tjnpr/v7i7.28

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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 (Figure 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 (Figure 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 (Figure 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]
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]

Table 1: Yield Extract *P. campechiana*

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

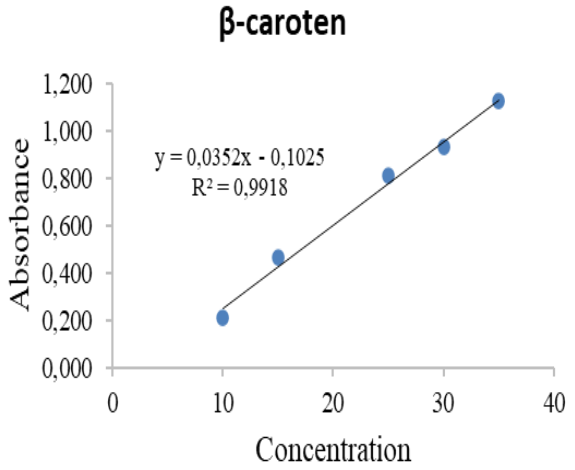


Figure 1: Standard Curve β-carotene

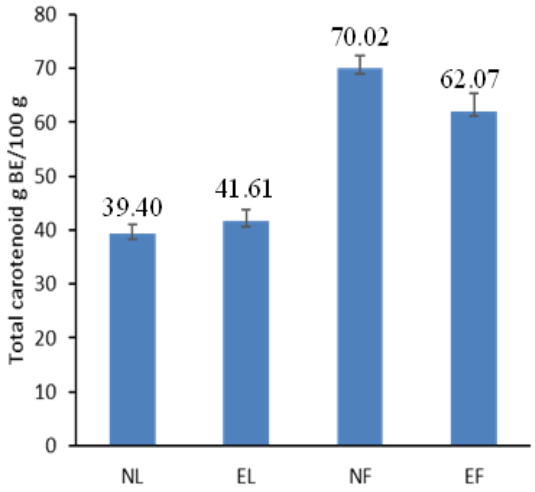


Figure 3: The carotenoid in *P. campechiana*[18]

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.

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

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

Acknowledgments

The authors are grateful to Sekolah Tinggi Farmasi Indonesia. This study was carried out during the academic year 2021/2022.

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, KT, Cepeda A, Fente C, Regal P. Review of methods for analysis of carotenoids. Trends Analyt Chem. 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*). J Food and Nutr. Sci. 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.