JURNAL ILMIAH MEDICAMENTO

Vol.12 No.1 (March 2026)
page 29-37
https://doi.org/10.36733/medicamento.v12i1.12213
e-ISSN: 2356-4814

Original Article

Chemical and DPPH Free Radical Scavenging Stability of Avocado Seed Acetone Extract Against pH Changes

I Gede Made Suradnyana[1*], Debby Juliadi [1], Ni Nyoman Yudianti Mendra[2], Ni Made Dharma Shantini Suena [1]

[1]Department of Pharmaceutic, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Jln. Kamboja, No. 11 A, Denpasar, 80223, Indonesia
[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Jln. Kamboja, No. 11 A, Denpasar, 80223, Indonesia

Submitted : 15-07-2025
Revised : 10-02-2026
Accepted : 04-03-2026

Keywords: avocado seed extract, chemical stability, DPPH free radical scavenging activity, total phenolic content

Correspondence:
I Gede Made Suradnyana
gedemadesuradnyana@unmas.ac.id

Abstract

Background: Avocado seeds contain phenol, flavonoid and anthocyanidin compounds that have antioxidant activity. The stability of phenolic compounds depends on the pH of the solution. A decrease in total phenolic levels causes a decrease in antioxidant activity. The chemical stability and DPPH free radical scavenging activity of avocado seed extract against pH are not yet known.
Objective: This study aimed to determine the effect of pH on the chemical stability and DPPH free radical scavenging activity of avocado seed extract.
Methods: Chemical stability was observed from changes in total phenolic levels and stability of DPPH free radical scavenging activity was observed from the difference in the percentage of DPPH reduction of the extract solution in phosphate-citrate buffer pH 3.24, 5.52 and 7.44 before and after being heated at 60°C for 2 hours.
Results: The average decrease in total phenolic levels of the extract in buffer pH 3.24, 5.52 and 7.44 before and after being heated was 5.76%, 3.89% and 22.08%, respectively. The decrease in DPPH free radical scavenging activity of the extract in buffer pH 3.24, 5.52 and 7.44 before and after heating was 24.78%, 1.96% and 56.09% respectively. There is a significant difference in the total phenolic content and DPPH free radical scavenging activity of the acetone extract solution in phosphate–citrate buffer at pH 3.24 and 7.44 before and after heating, but there is no significant difference at pH 5.52.
Conclusion: Therefore, in the development of formulations containing acetone extract of avocado seeds, buffering at a pH close to 5.22 is required.

How to Cite: (*citation style* AMA 11th Ed.)
Suradnyana IGM, Suena NMDS, Juliadi D, Mendra NNY. Chemical and DPPH Free Radical Scavenging Stability of Avocado Seed Acetone Extract Against pH Changes. *J. Ilm. Medicam.*, 2026;12(1): 29-37. https://doi.org/10.36733/medicamento.v12i1.12213

INTRODUCTION

Avocado is a dicotyledonous plant belonging to the Lauraceae family, originating from south-central Mexico. Avocado (*Persea americana*) is widely cultivated in Indonesia due to its high economic value. The fruit is rich in nutrients such as folic acid, unsaturated fats, and potassium, and it contains essential secondary metabolites that can help prevent cancer. The processing and consumption of avocados leave behind solid waste, accounting for approximately 21–30% of the fruit's total weight.[1]

One of the byproducts of avocado processing waste is the avocado seed. Avocado seeds contain secondary metabolites, including phenolic compounds, tannins, flavonoids, and alkaloids.[2,3] The phenolic compounds identified in avocado seeds include catechin, caffeic acid, chlorogenic acid, (epi)catechin, ferulic acid, kaempferol, kaempferide, procyanidins, rutin, trans-5-O-caffeoyl-D-quinic acid, and vanillic acid.[4] The flavonoid compounds identified include apigenin, kaempferol, catechin, quercetin, phloridzin, cinchonain, quercetin glucuronide, procyanidin dimer A, procyanidin dimer B, and procyanidin trimer A.[5] The phenolic, flavonoid, and anthocyanidin compounds present in avocado seeds exhibit antioxidant activity, where higher levels of these compounds correspond to greater antioxidant activity.[6]

In general, flavonoid compounds are unstable when exposed to heat.[7,8] A decrease in total flavonoid content positively correlates with a reduction in antioxidant activity.[9] Phenolic compounds such as catechin are highly unstable, whereas gallic acid and quercetin are relatively stable under heat exposure.[10] The total phenolic content, total flavonoid

content, and antioxidant activity of avocado leaf extract solutions at pH 4, 6, and 8 decrease after 15 minutes of storage at 70°C, 80°C, and 90°C, with the greatest reduction observed at 90°C.[11]

Currently, no studies have investigated the chemical stability and DPPH free radical scavenging activity of avocado seed extract. Since avocado seed extract has the potential for development into antioxidant-containing formulations, it is essential to first determine its stability under different pH conditions before formulation. Therefore, this study aims to examine the effect of pH on the chemical stability and DPPH free radical scavenging activity of acetone-extracted avocado seed extract.

METHODS

Tools and Materials

Tool. Oven (Memmert, Germany), blender, sieve No. 60, ultrasonic bath (Elma, Germany), Büchner funnel with vacuum filtration, rotary evaporator (Buchi, Switzerland), centrifuge (Ohaus, USA), pH meter (Ohaus, USA), water bath (Memmert, Germany), freezer (Biobase, China), magnetic stirrer (Thermo, USA), micropipette (Eppendorf, Germany), UV-Vis spectrophotometer (Shimadzu, Japan), a set of laboratory glassware, and 5 mL vials.

Materials. avocado seeds, acetone (Sekawan Bali Sejahtera, Indonesia), citric acid p.a. (Merck, Germany), disodium hydrogen phosphate p.a. (Merck, Germany), methanol p.a. (Merck, Germany), Folin-Ciocalteu reagent (7.5% solution in water), NaOH (Merck, Germany), ethanol p.a. (Merck, Germany), DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma, USA), gallic acid (Merck, Germany), and filter paper.

Research Procedure

Preparation of Avocado Seed Simplisia

Avocado seeds were separated from the seed coats, sliced into thin sections, and dried in a drying oven at 50°C until a constant weight was achieved. The dried seeds were then pulverized using a blender and sieved through a No. 60 mesh sieve to obtain a uniform particle size. The resulting powder (*simplisia*) was stored in sealed plastic bags at room temperature until further use.

Preparation of Acetone Extract from Avocado Seeds

A total of 370 g of avocado seed powder was placed into a maceration vessel, and 3,700 mL of 100% acetone was added. The mixture was intermittently stirred during the initial 6 hours of maceration and subsequently allowed to stand for an additional 18 hours at room temperature. The extract was then filtered using Whatman No. 4 filter paper, and the resulting filtrate was collected in a glass container protected from light with aluminum foil.

The residual plant material was subjected to remaceration twice, each time using 1,850 mL of 100% acetone for 24 hours, followed by filtration through Whatman No. 4 filter paper. The filtrates obtained from the second and third maceration steps were combined with the first filtrate. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C until a viscous extract was obtained. If necessary, further solvent removal was performed in an oven at 50°C.

This extraction procedure was repeated until a total of 740 g of avocado seed powder had been extracted. The mass of the concentrated extract was recorded, and the extraction yield was calculated.[12]

Preparation of Citrate-Phosphate Buffer Solutions at pH 3.24, 5.52, and 7.44

Citric acid monohydrate (5.2525 g) was accurately weighed and dissolved in CO_2-free distilled water in a 250 mL volumetric flask, then adjusted to volume to obtain a 0.1 M citric acid solution. Separately, disodium phosphate dihydrate (8.9025 g) was accurately weighed and dissolved in CO_2-free distilled water in a 250 mL volumetric flask and made up to volume to obtain a 0.2 M disodium phosphate solution.

Appropriate volumes of the 0.1 M citric acid solution and the 0.2 M disodium phosphate solution, as specified in **Table 1**, were mixed in a 250 mL beaker to prepare buffer solutions at pH 3.24, 5.52, and 7.44. The pH of each buffer solution was measured using a calibrated pH meter, and adjustments were made as necessary by the addition of small volumes of 0.1 M citric acid or 0.2 M disodium phosphate solution until the desired pH was achieved.

Table 1. Composition of Phosphate-Citrate Buffer Solution

pH Value	Volume of Citric Acid 0,1 M (ml)	Volume of Na_2HPO_4 0,2 M (ml)
3.24	67.8	32.2
5.52	42.0	58.0
7.44	6.35	93.65

Chemical Stability Test of Acetone Extract from Avocado Seeds

Preparation for a Test Solution

An acetone extract of avocado seeds (100 mg) was accurately weighed and dissolved in a 10 mL volumetric flask using 96% ethanol to obtain a stock solution with a concentration of 10,000 µg/mL. An aliquot of 2 mL of this solution was transferred into a 50 mL volumetric flask and diluted to volume with citrate–phosphate buffer at pH 3.24, followed by homogenization to yield a final concentration of 400 µg/mL. The resulting solution was divided into ten vials, which were subsequently sealed and wrapped in aluminum foil to prevent light exposure. The same procedure was repeated using citrate–phosphate buffer solutions at pH 5.52 and 7.44.

Storage Temperature Orientation

One vial from each buffer system (pH 3.24, 5.52, and 7.44) was incubated in a water bath at temperatures of 40°C, 60°C, and 80°C for 2 hours. Visual observations of color changes were conducted following incubation. The appearance of a brown coloration was considered indicative of extract degradation, as excessive darkening may interfere with subsequent colorimetric determination of phenolic content. Based on these observations, an appropriate temperature for the stability test was selected.

Stability Test at 60°C

Three vials from each buffer system were incubated in a water bath maintained at 60°C for 2 hours. After incubation, all vials were removed and stored in a freezer until further analysis. In parallel, three additional vials from each buffer system were analyzed immediately to determine their total phenolic content.

Determination of Total Phenolic Content

i) Preparation of Gallic Acid Standard Solution

Gallic acid (10 mg) was accurately weighed and dissolved in methanol in a 100 mL volumetric flask, then adjusted to volume to obtain a stock solution. A series of standard solutions with concentrations of 10, 20, 40, 50, and 60 µg/mL were subsequently prepared by appropriate dilution.

ii) Preparation of Calibration Curve

An aliquot of 1 mL from each gallic acid standard solution was transferred into separate test tubes. To each tube, 5.0 mL of 7.5% (v/v) Folin–Ciocalteu reagent diluted with distilled water was added, and the mixture was allowed to stand for 8 minutes. Subsequently, 4.0 mL of 1% (w/v) NaOH solution was added, and the reaction mixture was incubated for 1 hour. Absorbance was measured at the maximum wavelength of 745 nm using a UV–Vis spectrophotometer. A blank solution was prepared following the same procedure, with methanol substituted for the standard solution. A calibration curve was constructed by plotting absorbance against gallic acid concentration, and the corresponding regression equation was obtained.[12]

iii) Total Phenolic Content Analysis (0 Hours)

An aliquot of 2.5 mL of the unheated test solution was transferred into a 5 mL volumetric flask and diluted to volume with citrate–phosphate buffer at pH 3.24, followed by homogenization. This procedure was performed in triplicate and repeated for test solutions prepared in citrate–phosphate buffers at pH 5.52 and 7.44. A 1 mL aliquot of each diluted test solution was transferred into separate test tubes, followed by the addition of 5.0 mL of 7.5% Folin–Ciocalteu reagent. After standing for 8 minutes, 4.0 mL of 1% NaOH solution was added, and the mixture was incubated for 1 hour. Absorbance was measured at 745 nm against a blank prepared using methanol.[12] Total phenolic content was calculated using the gallic acid calibration curve equation.

iv) Total Phenolic Content Analysis (After 2 Hours)

The three vials containing samples in citrate–phosphate buffer at pH 3.24 were removed from the freezer and allowed to equilibrate to room temperature. A 2.5 mL aliquot from each vial was transferred into a 5 mL volumetric flask, diluted to volume with the corresponding buffer, and homogenized. This procedure was repeated for samples prepared in citrate–phosphate buffers at pH 5.52 and 7.44. Each test solution (1 mL) was then treated with 5.0 mL of 7.5% Folin–Ciocalteu reagent and allowed to stand for 8 minutes, followed by the addition of 4.0 mL of 1% NaOH solution. After incubation for 1 hour, absorbance was measured at 745 nm using methanol as the blank.[12] Total phenolic content was calculated based on the gallic acid calibration curve.

Stability Test of the DPPH Free Radical Scavenging Activity of Acetone Extract of Avocado Seeds

Preparation for a Test Solution

An acetone extract of avocado seeds (50 mg) was accurately weighed and dissolved in 96% ethanol in a 50 mL volumetric flask to obtain a stock solution with a concentration of 1,000 µg/mL. An aliquot of 2 mL of this solution was transferred into a 50 mL volumetric flask and diluted to volume with citrate–phosphate buffer at pH 3.24, yielding a final concentration of 40 µg/mL. The solution was divided into six vials, which were sealed and wrapped in aluminum foil. The same procedure was carried out using citrate–phosphate buffers at pH 5.52 and 7.44.

Stability Test at 60°C

Three vials from each buffer system were incubated in a water bath at 60°C for 2 hours. Following incubation, all vials were stored in a freezer until analysis. Simultaneously, the remaining three vials were analyzed immediately to determine their DPPH free radical scavenging activity.

Determination of DPPH free radical scavenging Activity

i) Preparation of 0.4 mM DPPH Solution

DPPH powder (4 mg; molecular weight 394.32 g/mol) was accurately weighed and dissolved in analytical-grade methanol (p.a.) in a 25 mL volumetric flask. The solution was adjusted to volume with methanol p.a. and homogenized.

ii) Determination of Maximum Wavelength of DPPH Solution

A 2 ml aliquot of 0.4 mM DPPH solution was pipetted into a 10 ml volumetric flask, diluted to the volume mark with methanol p.a., homogenized, and left for 30 minutes at room temperature in a dark place. The absorbance was measured in the wavelength range of 400–800 nm using a UV-Vis spectrophotometer. The maximum wavelength was determined from the obtained spectrum.

A 2 mL aliquot of the 0.4 mM DPPH solution was transferred into a 10 mL volumetric flask, diluted to volume with methanol p.a., and homogenized. The solution was incubated for 30 minutes at room temperature in the dark. Absorbance was measured over a wavelength range of 400–800 nm using a UV–Vis spectrophotometer, the maximum wavelength was identified from the resulting absorption spectrum, and the absorbance measured at this wavelength was designated as the control absorbance.

iii) DPPH free radical scavenging Activity Test of 0-Hour Test Solution

An aliquot of 0.1 mL of the unheated test solution was transferred into a 10 mL volumetric flask, mixed with 2 mL of 0.4 mM DPPH solution, diluted to volume with methanol p.a., and homogenized. The mixture was incubated for 30 minutes at room temperature in the dark, after which absorbance was measured at the maximum wavelength (517 nm) using methanol p.a. as the blank. The procedure was conducted in triplicate. A control solution was prepared following the same procedure, with methanol p.a. substituted for the sample solution.

iv) DPPH free radical scavenging Activity Test of 2-Hour Test Solution

The three frozen vials were thawed at room temperature. A 0.1 mL aliquot from each vial was transferred into a 10 mL volumetric flask, mixed with 2 mL of 0.4 mM DPPH solution, diluted to volume with methanol p.a., and homogenized. The mixture was incubated for 30 minutes in the dark at room temperature, and absorbance was measured at 517 nm using methanol p.a. as the blank.

v) Calculation of DPPH free radical scavenging Activity

The DPPH free radical scavenging activity of the extract was expressed as the percentage of DPPH inhibition and calculated using the following equation (1).[13]

$$\% \text{ DPPH inhibition} = \frac{\text{Control Absorbance-Sample Absorbance}}{\text{Control Absorbance}} \times 100\% \quad \text{...} (1)$$

Data Analysis

The obtained data were presented descriptively in the form of tables and graphical representations. Differences in total phenolic content and DPPH free radical scavenging activity before and after thermal treatment were statistically analyzed using a paired t-test at a 95% confidence level.

RESULT AND DISCUSSION

Preparation of Acetone Extract of Avocado Seeds

Phenolic compounds are secondary metabolites that degrade easily when exposed to heat. Therefore, maceration is the preferred method for extracting these compounds, as heat exposure can reduce their antioxidant activity.[12,13,14] Acetone is the optimal solvent for extraction, yielding an extract with the highest antioxidant capacity compared to other solvents such as water, ethanol, and ethyl acetate.[15] The extraction process involved 740 g of dried avocado seed powder with acetone at a 1:10 ratio (w/v). The resulting extract weighed 41.103 g, yielding an extraction efficiency of 5.55%. The extract appeared as a thick, brown-colored substance with a characteristic odor.

Storage Temperature Orientation

After storage at 40°C, 60°C, and 80°C for 2 hours, a color change was observed, particularly in the solution stored at 80°C, indicating compound degradation (**Figure 1**). Excessive color intensity can interfere with the quantification of active compounds using colorimetric methods due to wavelength interference.[16]

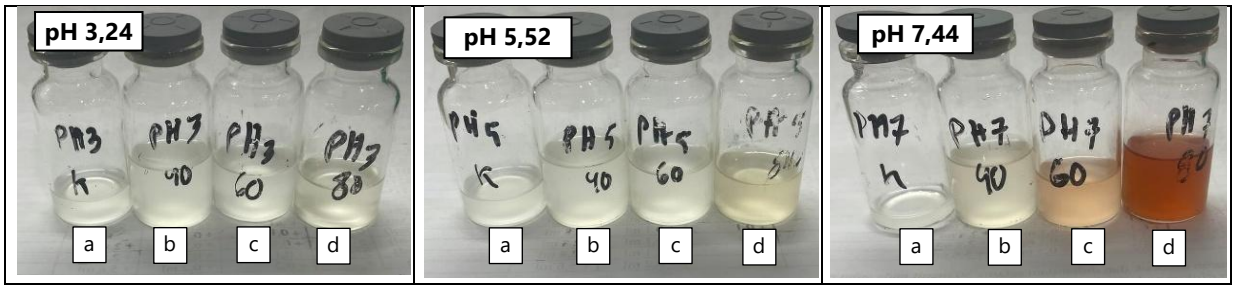

Figure 1. Extract Solution in Phosphate-Citrate Buffer After Storage: (a) 0 Hours at Room Temperature, (b) 2 Hours at 40°C, (c) 2 Hours at 60°C, (d) 2 Hours at 80°C.

Based on these findings, a storage temperature of 60°C was selected for the stability test. At this temperature, sufficient degradation occurred after 2 hours without excessive color intensity, minimizing interference in quantification using the colorimetric method.

Chemical Stability Test of Acetone Extract of Avocado Seeds

The total phenolic content was determined using a calibration curve of gallic acid, which serves as the reference standard for expressing total phenolic content as gallic acid equivalents (GAE).[17,18] The calibration curve (**Figure 2**) was expressed as the equation $y = 0.01x + 0.0204$, with a coefficient of determination (R^2) of 0.9984 and a correlation coefficient (R) of 0.9992, indicating excellent linearity (R > 0.99).[19,20]

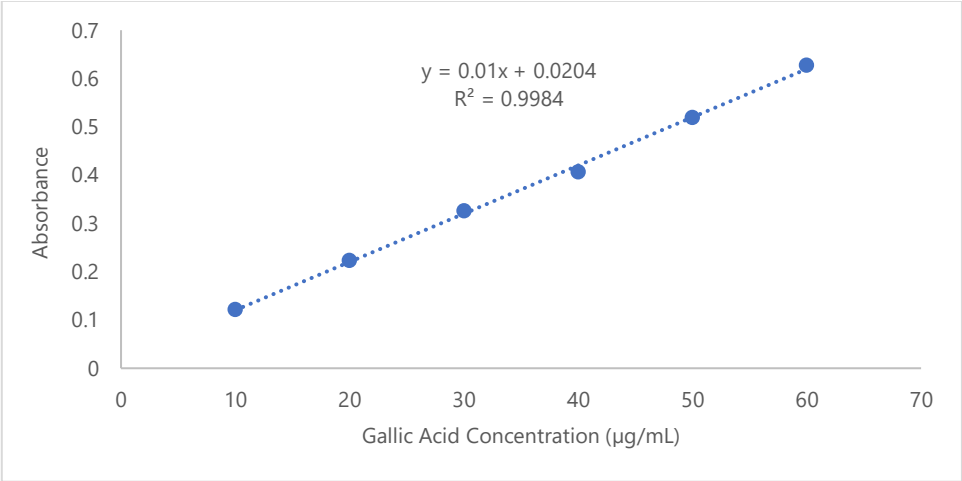

Figure 2. Standard Calibration Curve of Gallic Acid

Following 2 hours of storage in a water bath at 60°C, the total phenolic content of the acetone extract dissolved in phosphate-citrate buffer at pH 3.24, 5.52, and 7.44 decreased (**Table 2**). The reductions were as follows: pH 3.24: 5.76% (from 101.853 ± 1.361 to 95.987 ± 1.286 µg GAE/mL); pH 5.52: 3.89% (from 111.520 ± 1.587 to 107.187 ± 0.987 µg GAE/mL); pH 7.44: 22.08% (from 130.120 ± 3.020 to 101.387 ± 4.909 µg GAE/mL) (**Figure 3**).

Table 2. Chemical Stability and DPPH Free Radical Scavenging Stability of Acetone Extract of Avocado Seeds

Parameter		pH 3.24	Normality test (p-value)	pH 5.52	Normality test (p-value)	pH 7.44	Normality test (p-value)
Total Phenolic Content (mg GAE/g)	0 hour	100.32 102.32 102.92	0.424	110.92 110.32 113.32	0.363	130.52 132.92 126.92	0.780
	2 hours	96.52 94.52 96.92	0.298	106.72 108.32 106.52	0.194	106.12 101.72 96.32	0.888
DPPH Free Radical Scavenging Activity (% DPPH Inhibition)	0 hour	13.5 11.1 11.9	0.637	13.6 13.1 12.2	0.688	13.0 12.8 16.1	0.103
	2 hours	10.6 8.1 8.7	0.443	13.3 12.7 12.2	0.900	5.3 6.6 6.5	0.132

The stability of polyphenols in sweet potato leaves is highest at pH 5–7, with extreme acidic or alkaline conditions significantly affecting stability.[21] The total phenolic content of *Terminalia nigrovenulosa* extract at pH 4 showed no significant change after heating at 100°C for 20 minutes but decreased significantly at pH 7.[22] Solvent pH is a key factor affecting polyphenol stability, along with light, temperature, oxygen, metal ions, enzymes, and proteins.[23] Under strongly acidic or alkaline conditions, phenolic compounds undergo hydroxylation, leading to a decrease in total phenolic content.[24] In alkaline solutions, polyphenols tend to undergo degradation, isomerization/dimerization, and oxidation.[25,26]

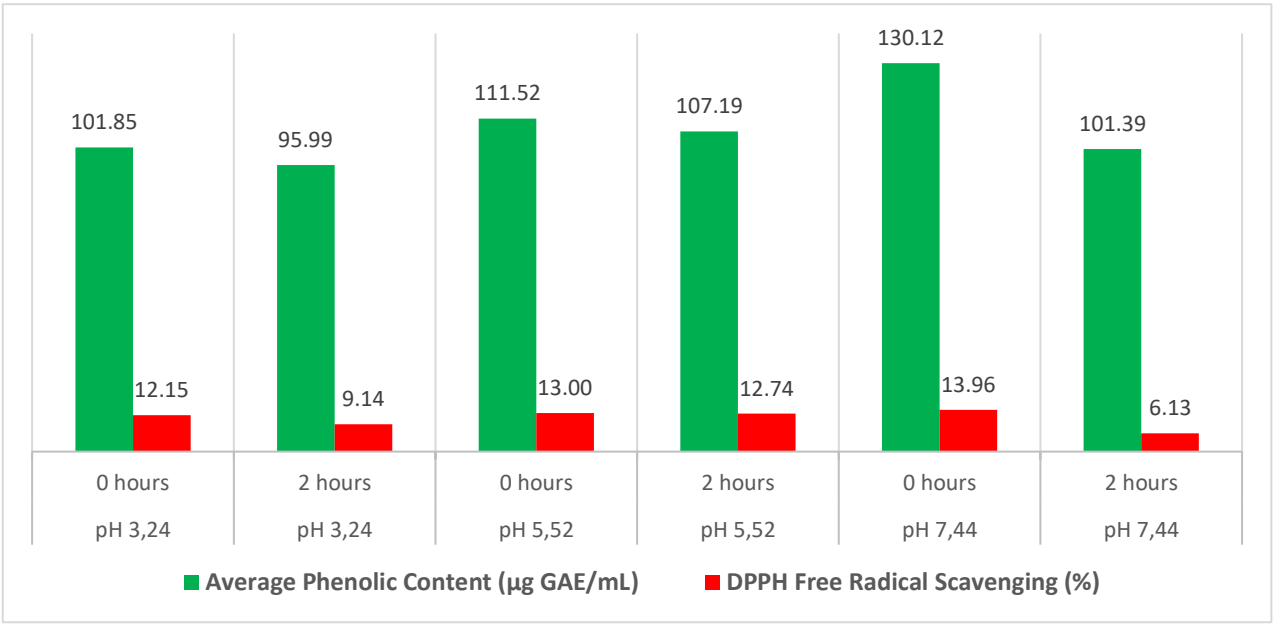

Figure 3. Mean Total Phenolic Content and DPPH Free Radical Scavenging Activity of Acetone Extract Solution of Avocado Seeds

The total phenolic content data before and after 2 hours of heating followed a normal distribution ($p > 0.05$, **Table 2**). A paired t-test was performed to assess the significance of changes in total phenolic content before and after heating. The results (**Table 3**) showed no significant difference at pH 5.52 ($p > 0.05$), whereas significant differences were observed at pH 3.24 and pH 7.44 ($p < 0.05$). These findings indicate that the acetone extract of avocado seeds in phosphate-citrate buffer at pH 5.52 exhibited the highest stability compared to pH 3.24 and pH 7.44. Higher total phenolic content corresponds to greater phenolic compound stability.[24]

Table 3. Paired t-Test Results for Total Phenolic Content and DPPH Free Radical Scavenging Activity

Data	Mean Difference (SD)	CI 95%		p-value
TPC pH 3.24	5.87 (2.00)	0.89	10.84	0.037
TPC pH 5.52	4.33 (2.40)	-1.64	10.30	0.089
TPC pH 7.44	28.73 (3.76)	19.38	38.09	0.006
DPPH FRS pH 3.24	3.03 (0.15)	2.65	3.41	0.001
DPPH FRS pH 5.52	0.23 (0.21)	-0.28	0.75	0.192
DPPH FRS pH 7.44	7.83 (1.70)	3.60	12.07	0.015

Note:
TPC: Total phenolic content of the solution in buffer with a specific pH; DPPH FRS: DPPH free radical scavenging activity of the solution in buffer with a specific pH.

Stability of DPPH free radical scavenging Activity of Acetone Extract of Avocado Seeds

The percentage of DPPH free radical scavenging indicates the antioxidant activity of the acetone extract solution. After heating at 60°C for 2 hours, a reduction in DPPH free radical scavenging activity was observed in phosphate-citrate buffer at pH 3.24, 5.52, and 7.44 (**Table 2**). The percentage decrease in DPPH free radical scavenging activity was as follows: pH 3.24: 24.78% (from 12.17 ± 1.22% to 9.13 ± 1.31%); pH 5.52: 1.96% (from 12.97 ± 0.71% to 12.73 ± 0.55%); pH 7.44: 56.09% (from 13.97 ± 1.85% to 6.13 ± 0.72%) (**Figure 3**). The greatest reduction in DPPH free radical scavenging activity was observed in the pH 7.44 buffer, likely due to the decrease in total phenolic content. Organoleptically, the solution at pH 7.44 displayed the darkest color intensity after 2 hours of heating, compared to other pH conditions.

The DPPH free radical scavenging activity of polyphenols in sweet potato leaves is optimal at pH 5–7.[27] The DPPH scavenging ability of *Terminalia nigrovenulosa* extract at pH 4 showed no significant change after heating at 100°C for 20 minutes, while a significant reduction was observed at pH 7. Extracts with higher total phenolic content generally exhibit stronger antioxidant activity.[28] The antioxidant mechanism of phenolic compounds includes hydrogen atom transfer, single-electron transfer, proton loss followed by electron transfer, and transition metal chelation. Phenolic compounds donate hydrogen atoms to radicals, forming stable molecules and antioxidant-free radicals. The antioxidant potential of phenolic compounds is influenced by their structure, particularly the benzene ring and the number and position of hydroxyl groups. Proton loss followed by electron transfer is considered the most relevant antioxidant mechanism, as phenolic compounds donate protons to free radicals, forming anions, which then donate electrons to generate stable molecules.[29]

The DPPH free radical scavenging activity data before and after 2 hours of heating followed a normal distribution ($p > 0.05$, **Table 2**). A paired t-test was conducted to determine the significance of changes in DPPH free radical scavenging activity. The results (**Table 3**) indicated a significant difference in the DPPH free radical scavenging activity of the extract solution at pH 3.24 and pH 7.44 before and after heating at 60°C for 2 hours ($p < 0.05$). However, no significant difference was observed at pH 5.52 ($p > 0.05$). These findings suggest that the acetone extract of avocado seeds in phosphate-citrate buffer at pH 5.52 exhibited the highest stability compared to solutions at pH 3.24 and 7.44.

The main limitations of this study were the wide buffer pH interval and the absence of analysis of extract degradation products that may interfere with the determination of total phenolic content and DPPH free radical scavenging activity. Therefore, further studies employing narrower buffer pH intervals and appropriate analytical methods are required to obtain a more accurate pH stability value and to ensure that extract degradation products do not interfere with the determination of total phenolic content and DPPH free radical scavenging activity.

CONCLUSION

After the avocado seed acetone extract solution was stored at 60°C for 2 hours, the total phenolic content in solutions with pH 3.24, 5.52, and 7.44 decreased by 5.76%, 3.89%, and 22.08%, respectively. Meanwhile, the DPPH free radical scavenging activity of solutions with pH 3.24, 5.52, and 7.44 decreased by 24.78%, 1.96%, and 56.09%, respectively. Solutions with pH 3.24 and 7.44 significantly affected the chemical stability and DPPH free radical scavenging stability of the avocado seed acetone extract solution. However, no significant effect was observed at pH 5.52. Therefore, in the development of formulations containing acetone extract of avocado seeds, buffering at a pH close to 5.22 is required.

FUNDING

This work was supported by Faculty of Pharmacy Universitas Mahasaraswati Denpasar, Grant No. 1516.4/E.003/FF-UNMAS/XII/2023.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the laboratory staff and the grant team of the Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, for their assistance and support in the conduct of this study.

GENERATIVE AI DISCLOSURE STATEMENT

The authors confirm that no Generative Artificial Intelligence (AI) was utilized in the drafting, data analysis, or preparation of this manuscript.

AUTHOR CONTRIBUTION STATEMENT

I Gede Made Suradnyana: conceptualization, methodology, conduct the study, writing original draft preparation; **Debby Juliadi:** writing-editing; **Ni Nyoman Yudianti Mendra:** writing-editing; **Ni Made Dharma Shantini Suena:** data curation and validation, writing-reviewing.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

REFERENCE

1. Kurniawan K, Rahmat A. Determination of antioxidant activity, total phenolic, and total flavonoid contents from avocado seeds (*Persea americana*). *IOP Conf Ser Earth Environ Sci*. 2023;1201(1). doi:10.1088/1755-1315/1201/1/012098
2. Rivai H, Putri YT, Rusdi R. Qualitative and quantitative analysis of the chemical content of hexane, acetone, ethanol and water extract from avocado seeds (*Persea americana* Mill.). *Sch Int J Tradit Complement Med*. 2019;2(3):25-31.
3. Suradnyana IGM, Mendra NNY, Juliadi D, Suena NMDS. Formulation and Stability Test of Avocado Seed Acetone Extract Cream. *J Ilm Medicam*. 2024;10(2):97-108. doi:10.36733/medicamento.v10i2.8728
4. Setyawan HY, Sukardi S, Puriwangi CA. Phytochemicals properties of avocado seed: A review. In: *IOP Conference Series: Earth and Environmental Science*. Vol 733. IOP Publishing; 2021:1-6.
5. Silva GG da, Pimenta LPS, Melo JOF, Mendonça H de OP, Augusti R, Takahashi JA. Phytochemicals of avocado residues as potential Acetylcholinesterase Inhibitors, Antioxidants, and Neuroprotective Agents. *Molecules*. 2022;27(6):1-18. doi:https://doi.org/10.3390/molecules27061892
6. Tan CX, Chin R, Tan ST, Tan SS. Phytochemicals and Antioxidant Activity of Ultrasound-Assisted Avocado Seed Extract. *Malaysian J Anal Sci*. 2022;26(3):439-446.
7. Gao Y, Xia W, Shao P, Wu W, Chen H, Fang X, Mu H, Xiao J, Gao H. Impact of thermal processing on dietary flavonoids. *Curr Opin Food Sci*. 2022;48:1-8. doi:10.1016/j.cofs.2022.100915
8. Chaaban H, Ioannou I, Chebil L, Slimane M, Gérardin C, Paris C, Charbonnel C, Chekir L, Ghoul M. Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *J Food Process Preserv*. 2017;41(5):1-12. doi:10.1111/jfpp.13203
9. Lu Q, Li L, Xue S, Yang D, Wang S. Stability of flavonoid, carotenoid, soluble sugar and Vitamin C in 'Cara Cara' Juice during storage. *Foods*. 2019;8(9):1-15. doi:10.3390/FOODS8090417
10. Esparza I, Cimminelli MJ, Moler JA, Jiménez-Moreno N, Ancín-Azpilicueta C. Stability of phenolic compounds in grape stem extracts. *Antioxidants*. 2020;9(8):1-14. doi:10.3390/antiox9080720
11. Kemit N, Dewa Gde Mayun Permana dan Pande Ketut Diah Kencana Program Pascasarjana I, Studi Ilmu dan Teknologi Pangan P, Teknologi Pertanian F. Flavonoid Stability of Avocado Leaf (*Persea americana* Mill.) Extract on pH and Temperature Treatment. *Media Ilm Teknol Pangan (Scientific J Food Technol*. 2019;6(1):34-42.
12. DepkesRI. *Farmakope Herbal Indonesia*. II. Departemen Kesehatan Republik Indonesia; 2017.
13. Lee J, Park G, Chang YH. Nutraceuticals and antioxidant properties of Lonicera japonica Thunb. as affected by heating time. *Int J Food Prop*. 2019;22(1):630-645. doi:10.1080/10942912.2019.1599389
14. Krungkri W, Areekul V. Effect of Heating Condition and pH on Stability of Total Phenolic Content and Antioxidant Activities of Samui (*Micromelum minutum*) Extract. In: *The 16th ASEAN Food Conference (16th AFC 2019) - Outlook and Opportunities of Food Technology and Culinary for Tourism Industry*. Science and Technology Publications; 2020:126-132. doi:10.5220/0009980801260132
15. Ioannou I, Chekir L, Ghoul M. Effect of heat treatment and light exposure on the antioxidant activity of flavonoids.

Processes. 2020;8(9):2-17. doi:10.3390/pr8091078
16. Folasade OA, Aderibigbe Olaide R, Olufemi TA. Antioxidant Properties of *Persea americana* M. Seed as Affected by Different Extraction Solvent. *Orig Res Artic J Adv Food Sci Technol*. 2016;3(2):101-106. doi:10.13140/RG.2.1.1714.2165
17. Shrestha YK, Shrestha SK. *Advances in Colorimetry*. (Samanta AK, ed.).; 2024. doi:10.5772/intechopen.108083
18. Ratnasari FA, Wulandari L, Kristiningrum N. Determination of Total Phenolic in Leave Extracts Using Spectroscopy NIR and Chemometric. *e-Journal Pustaka Kesehat*. 2016;4(2):235-240.
19. Hapsari AM, Masfria M, Dalimunthe A. Total Phenol Content Determination in Tempuyung (*Shoncusarvensis* L.) Ethanol Extract. *Talent Conf Ser Trop Med*. 2018;1(1):284-290. doi:10.32734/tm.v1i1.75
20. Hulupi M, Keryanti K, Rahmawati KA, Dewi WT, Abdilah F. Validation of Methylene Blue Analysis Method in Wastewater Samples by Uv-Vis Spectrophotometry. *Equilib J Chem Eng*. 2023;7(2):101. doi:10.20961/equilibrium.v7i2.75807
21. Raikar PR, Dandagi PM, Kazi T. Development and Validation of Novel RP-HPLC Method for the Simultaneous Estimation of Capecitabine and Thymoquinone in the Biodegradable Nanoparticles using Full Factorial Design. *J Chromatogr Sci*. 2023;61(8):773-783. doi:10.1093/chromsci/bmad021
22. Sun HN, Mu TH, Xi LS. Effect of pH, heat, and light treatments on the antioxidant activity of sweet potato leaf polyphenols. *Int J Food Prop*. 2017;20(2):318-332. doi:10.1080/10942912.2016.1160410
23. Nguyen QV. Stability to Heat, Hydrogen Peroxide at Different pH Values and Antioxidant Activity of *Terminalia nigrovenulosa* Extract. *Asian J Biotechnol Bioresour Technol*. 2017;1(3):1-9. doi:10.9734/ajb2t/2017/35822
24. Xiao J. Recent advances on the stability of dietary polyphenols. *eFood*. 2022;3(3):1-7. doi:10.1002/efd2.21
25. Wang D, Liu J, Qiu S, Wang J, Song G, Chu B, Li L, Xiao G, Gong J, Zheng F. Ultrasonic degradation kinetics and isomerization of 3- and 4-O-caffeoylquinic acid at various pH: The protective effects of ascorbic acid and epigallocatechin gallate on their stability. *Ultrason Sonochem*. 2021;80. doi:10.1016/j.ultsonch.2021.105812
26. Zeb A. Concept, mechanism, and applications of phenolic antioxidants in foods. *J Food Biochem*. 2020;44(9):1-22. doi:10.1111/jfbc.13394