



RESEARCH ARTICLE

REVISED Analyzing Aloe vera and Avocado Seed Extracts for Antioxidants, Saponins, Tannins, Flavonoids, and Alkaloids Using the UV-VIS Spectrophotometric Method

[version 2; peer review: 5 approved with reservations]

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Abstract**Background**

This study aimed to quantify saponins, alkaloids, flavonoids, and tannins and determine the IC₅₀ values of avocado seed and aloe vera extracts.

Methods

The materials investigated were 70% ethanol extracts derived from avocado seeds and aloe vera. Both samples underwent quantitative phytochemical analyses to ascertain the total component content, and antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique to evaluate % inhibition and IC₅₀ values. The absorbance of the samples was quantified using a UV-Vis spectrophotometer, which facilitated the calculation of the total chemical content and antioxidant activity.

Results

The avocado seed extract comprised saponins, alkaloids, flavonoids, and tannins at concentrations of 0.21%, 0.0232%, 19.94%, and 10.66%, respectively, with an IC₅₀ value of 135 µg/mL. Aloe vera extract contains saponins, alkaloids, flavonoids, and tannins at concentrations of 0.74%, 0.0313%, 0.99%, and 4.68%, respectively, with an IC₅₀ value

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of 4614 µg/mL.

Conclusion

Avocado seeds exhibited elevated flavonoid and tannin levels, whereas aloe vera demonstrated increased alkaloid and saponin concentrations. In the antioxidant activity assessment, avocado seeds demonstrated superior antioxidant efficacy.

Keywords

Antioxidant, Flavonoid, Aloe vera, Avocado seed, Quantitative phytochemistry



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REVISED Amendments from Version 1

An introduction (added an explanation about Aloe vera and Avocado seed), In methods, added part of Aloe vera and the total compound of alkaloid: 10000 based on laboratory standard, and in discussion, added the mechanism of antioxidant. And the limitations of this study.

Any further responses from the reviewers can be found at the end of the article

Introduction

Indonesia is a tropical nation characterized by a vast array of terrestrial flora. As many as 22,500 species have been recorded, around 1000 plants can be used as medicine,¹ such as avocado seeds and Aloe vera, which have long been known as plants believed to have high efficacy and lower side effects.^{2,3} Avocado seeds and aloe vera have the potential to serve as raw materials for pharmaceuticals devoid of chemical additives, as well as for food, beverages, and cosmetics. Avocado seeds have analgesic and anti-inflammatory properties in dentistry and may be used as a gel for the treatment of canker sores. Conversely, Aloe vera is currently applied to dentistry to promote wound healing, diminish inflammation, and alleviate discomfort.³

Avocado seeds and aloe vera possess the same secondary metabolite chemicals, namely tannins, saponins, flavonoids, and alkaloids.^{2,4} Tannins possess antioxidant, astringent, antidiarrheal, and antibacterial effects.⁵ Flavonoids are the predominant antioxidant and anti-inflammatory chemicals.⁶ The content of different compounds, namely saponins, has the potential to have antibacterial, antioxidant, anticancer, and anti-diabetics properties.⁷ The constituents of many substances, including saponins, have the potential for antibacterial, antioxidant, anticancer, and anti-diabetic properties.⁸ Both materials' secondary metabolite levels were determined using quantitative phytochemical tests. Avocado seed extract is abundant in polyphenols and flavonoids, which have potent antibacterial and antioxidant effects, especially against oral infections. Aloe vera is renowned for its anti-inflammatory and wound-healing properties, rendering it significant for mucosal tissue regeneration. This work tackles the gap by examining a dual-extract formulation that may provide a safer, more accessible alternative to synthetic drugs. This work is essential due to the increasing worry over antibiotic resistance and the desire for biocompatible, plant-based medicines in dental care.^{3,5,6}

Quantitative phytochemical analyses ascertain the quantities of secondary metabolites.⁹ The ethanol extract of avocado seeds has 0.435% alkaloids, 0.1084% flavonoids, 0.0309% phenols, and 0.2044% tannins, as determined using a spectrophotometer.¹⁰ A separate investigation employing UV-Vis spectrophotometry determined that avocado seed extract has 56.5063 µg/ml of alkaloids, 245.6875 µg/ml of flavonoids, 15.65 µg/ml of phenols, 27.03 µg/ml of tannins, and 22.73 µg/ml of saponins.¹¹ Aloe vera extract analyzed via UV-Vis spectrophotometry revealed concentrations of 4.65 ± 0.08 tannins, 1.43 ± 0.031 flavonoids, 60.85 ± 0.61 saponins, and 22.86 ± 0.15 alkaloids.¹² Aloe vera extract (100 g) had 31.067 g of alkaloids, 25.66 g of tannins, and 10.67 g of saponins. The ethanol extract of avocado seed showed a moderate antioxidant capacity (IC₅₀ 77.298 µg/mL). Still, the ethanol extract of Aloe vera displayed 70.7% antioxidant activity as assessed by the DPPH technique using a UV-Vis spectrophotometer.¹³ These compounds demonstrate considerable antioxidant capabilities, particularly tannins and flavonoids, which correlate with elevated antioxidant levels.¹⁴ The ethanol extract of avocado seeds had a moderate antioxidant capacity (IC₅₀ 77.298 µg/mL),¹⁵ but the ethanol extract of Aloe vera displayed 70.7% antioxidant activity as assessed by the DPPH technique.¹⁶

This study will investigate the extraction of avocado and aloe vera seeds by the maceration method utilizing 70% ethanol for 48 hours, distinguishing it from prior studies. This study compares quantitative phytochemical analyses using a UV-VIS spectrophotometer with antioxidant assessments employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, also utilizing a UV-VIS spectrophotometer to ascertain the antioxidant activity and IC₅₀ values in avocado seed extract and Aloe vera extract, thereby evaluating the compound levels and antioxidant activity in both extracts.

Methods**Ethics statement**

Ethics clearance certificate number: 2.057/XI/HREC/2023. This in vitro study will be conducted at the Integrated Laboratory UPT Universitas Gadjah Mada Yogyakarta (Integrated Research and Testing Laboratory) for phytochemical and antioxidant testing, and the Plant Systematics Laboratory of the Faculty of Biology UGM for determination purposes.

Plant determination (materials)

The avocado seeds used were taken from a resident garden in Kepurun Village, Manisrenggo, Klaten. The Aloe vera was taken from a resident's garden on Kalimantan Street, Purwosari Sinduadi, Mlati District, Sleman. The aloe vera used is the

peel/inner leaf and gel/inner pulp from Malang. The determination was made at the Plant Systematics Laboratory, Faculty of Biology, UGM, using certificate number 00569/S. Tb./II/2024 for Aloe vera, and 00568/S. Tb./II/2024 for the avocado seeds.

Extraction of Avocado seed and Aloe vera

Avocado seeds and Aloe vera (peel/inner leaf and gel/inner pulp) were washed, cleaned, air-dried, ground into powdered simplicia by grinding, and then sieved. The simplicity of both ingredients was then extracted using the maceration technique. Maceration was performed by soaking the simplicia in 70% ethanol solvent (1:10) for 2×24 h with occasional stirring. The filtered filtrate was concentrated using a rotary evaporator.

Phytochemical quantitative test

1. Saponin test

A standard curve was established using a saponin scale of 10 mg of sample, specifically avocado seed extract and aloe vera extract, adding 5 ml of water to each extract. The mixture was vortexed for 5 min, and 50 μ L of anisaldehyde was added. It was then shaken and allowed to stand for 10 minutes. Introduce 2 ml of 50% sulfuric acid and subject the mixture to heating in a water bath at 60°C for 10 minutes. Water was added to a 10 ml volume in a measuring flask, with the standard concentrations beginning at 200, 100, 50, 25, 12.5, and 6.25 μ L. Absorbance was measured at a wavelength of 435 nm, and the standard used was Quillaja saponin.

To quantify total saponins, avocado seed extract and aloe vera extract samples were weighed to approximately 100 mg. Then, 2 ml of 25% H_2SO_4 was introduced, and the mixture was autoclaved for 120 min at 110°C, followed by extraction with ether and subsequent drying of the filtrate. Add 1 ml of water and extract by vortexing for 5 minutes. Then, 50 μ L of anisaldehyde was added, mixed thoroughly, and allowed to stand for 10 min. Introduce 2 ml of 50% sulfuric acid and subject the mixture to heating in a water bath at 60°C for 10 minutes. Ten milliliters of water were diluted using a volumetric flask, and the absorbance was measured at a wavelength of 435 nm.

2. Alkaloid test

A meticulous weighing of 10 milligrams of quinine standard and 5 milliliters of 2N hydrochloric acid produced the standard curve. The solution was passed through a separating funnel thrice with 10 mL of chloroform each time, and the chloroform phase was then discarded. 0.1 N NaOH was added to the mixture. Then, 5 ml of BCG Solution and 5 ml of Phosphate Buffer were added. Following 15 min of magnetic stirring at 500 rpm, 5 mL of chloroform was added to the solution for extraction. Chloroform was used for extraction in two separate instances. The chloroform phase was removed by evaporation using nitrogen gas, and 10 mL of chloroform was added. The absorbance was measured at 470 nm.

The sample was measured to within ± 100 mg to determine the total alkaloid content. Then, 5 mL of 2N HCl was added and mixed by shaking. The mixture was then rinsed thrice with 10 mL of chloroform in a separating funnel. After removing the chloroform phase, the solution was acidified with 0.1 N NaOH solution. Then, 5 ml of BCG Solution and 5 ml of Phosphate Buffer were added. After 5 ml of chloroform was added to the solution, it was swirled magnetically at 500 rpm for 15 min. Chloroform was used for extraction in two separate instances. Chloroform (5 mL) was added after the chloroform phase was recovered and evaporated using nitrogen gas. Absorbance was measured at 470 nm, and the standard used was Quillaja bark.

3. Flavonoid test

A quercetin standard (10.0 mg) and 0.3 ml of 5% sodium nitrite were utilized to construct the standard curve. Subsequently, 0.6 ml of 10% aluminum chloride was added after 5 minutes and allowed to stand for another 5 minutes. Subsequently, 2 ml of 1 M sodium hydroxide was introduced. About 10 ml of distilled water was introduced into the measuring flask. The solution was placed in a cuvette, and absorbance was measured at a wavelength of 510 nm.

Fifty milligrams of the sample were precisely measured to determine total flavonoids, followed by adding 0.3 milliliters of 5% sodium nitrite. After 5 minutes, 0.6 ml of 10% aluminum chloride was added and allowed to stand for another 5 minutes, followed by 2 ml of 1 M sodium hydroxide. Distilled water was added to a volume of up to 10 ml using the measuring flask and diluted as necessary. The solution was placed in a cuvette, and the absorbance was recorded at a wavelength of 510 nm.

4. Tannin test

The Tannic Acid standard was accurately weighed to construct the standard curve. Subsequently, 10 mL of Folin Ciocalteu reagent was added, vortexed, and allowed to stand for 5 minutes. Prepare a 20% Sodium Carbonate solution and adjust the final volume to 100 mL. Following a 30-minute incubation at room temperature, absorbance was assessed at 760 nm.

The sample was weighed to an accuracy of ± 100 mg to assess the total tannin content. Perform extraction using 10 mL of diethyl ether for 20 hours, followed by filtration. The residual diethyl ether was evaporated, and distilled water was added to the sample to achieve a final volume of 10 mL. Measure 1 mL of the sample solution, incorporate 0.1 mL of Folin Ciocalteu reagent, vortex the mixture, and allow it to stand for 5 minutes. Introduce 2 mL of 20% Sodium Carbonate, vortex the solution, and allow it to stand for 5 minutes. Aquadest was introduced into a 10 mL volume and subsequently diluted by a factor of 10. Following a 30-minute incubation at room temperature, the absorbance was recorded at 760 nm.

To obtain a linear regression equation and correlation coefficient, the absorbance results were used to create a standard curve (flavonoids, tannins, alkaloids, and saponins). A regression equation was employed to compute the chemical content utilizing the following formula:

$$\text{Total compound (\%b/b)} = \frac{\text{Reading result (ppm)} \times \text{Solution Vol.add (ml)} \times \text{fp}}{\text{Sample Weight (g)}} : 10000$$

5. Antioxidant test

A stock solution was made by weighing 500 mg of the extract and diluting it in 20 mL of 70% ethanol. The mixture was transferred to a 50 mL volumetric flask, ethanol was poured to the mark, and subjected to ultrasonic treatment for 10 minutes. The solution was filtered until a transparent filtrate was acquired. The stock solution underwent dilution. Prepare a 0.4 mM DPPH solution using 15.7 mg of DPPH and ethanol (96%) to the mark in a 100 mL volumetric flask. The solution underwent ultrasonication for 5 minutes until DPPH was fully dissolved. A sample including 50 μ L of extract, 1.0 mL of 0.4 mM DPPH, and 3,950 mL of ethanol was vortexed in a microplate and incubated for 30 minutes in a dark environment. Absorbance was quantified at a wavelength of 515 nm. Development of standard curves and linear regression formulae. The antioxidant activity was determined using the following formula:

$$\% \text{Antioxidant} : \frac{\text{Abs.Control} - \text{Abs.Sample}}{\text{Abs.Control}} \times 100\%$$

The IC₅₀ value was determined utilizing the formula: (x) IC₅₀ = (50 – b) : a (a = intersep; b = koefisiensi beta).

Results

Quantitative measurement of saponins was conducted utilizing Quillaja bark standards at a wavelength of 435 nm, with the linear equation $Y = 9.44528e-004x - 0.00332132$ and a correlation coefficient (r^2) of 0.99974 (Figure 1). The subsequent equation was employed to ascertain the concentrations of saponin components, whereas the r-value indicates the correlation between the concentration of the standard solution and the absorbance. An R value around 1 is considered favorable as it implies a direct proportionality or linearity between the concentration of the standard solution and the absorbance. The overall saponin test findings indicated that the average saponin content in Aloe vera extract surpassed that of avocado seeds. The findings revealed that the mean saponin concentration in the Aloe vera extract sample was 0.74%, whereas that of the avocado seed extract was 0.21%.

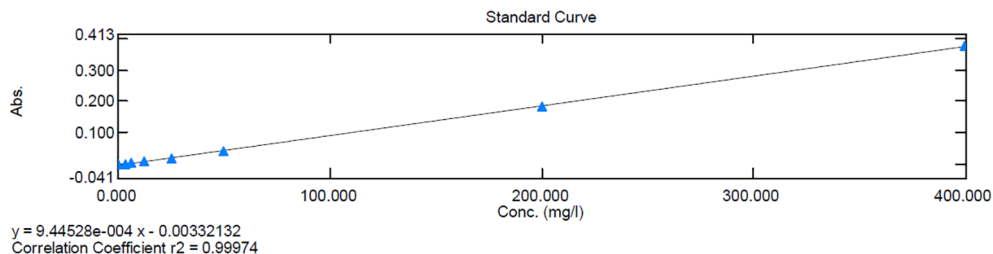


Figure 1. Curve of Saponin.

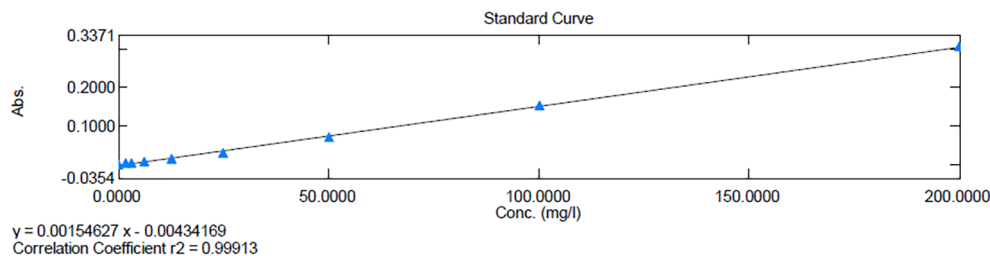


Figure 2. Curve of Alkaloid.

The quantitative measurement of alkaloids was conducted utilizing the Quillaja bark standard at a wavelength of 470 nm, with the linear equation $Y = 0.00154627x - 0.00434169$ and a correlation coefficient (r^2) of 0.99913 (Figure 2). The overall alkaloid test findings indicated that the average alkaloid level in Aloe vera extract surpassed that of avocado seeds. The findings revealed that the mean concentration in avocado seeds was 0.0232%, whereas that in Aloe vera was 0.0313%.

The quantitative analysis of flavonoids was conducted utilizing the quercetin standard at a wavelength of 510 nm, resulting in a linear equation of $Y = 0.00569497x - 0.00473317$, with a correlation coefficient (r^2) of 0.99979 (Figure 3). The total flavonoid test results indicated that the average flavonoid content in Aloe vera extract was lower than in avocado seeds. The observation results revealed that the average content of avocado seeds was 19.44%, while in Aloe vera, it was 0.99%.

The quantitative analysis of tannin was conducted utilizing the Tannin Acid standard at a wavelength of 760 nm, with a linear equation represented as $Y = 0.019469x + 9.88071e-004$, and a correlation coefficient (r^2) of 0.99994 (Figure 4). The total tannin test results on avocado and Aloe vera seeds indicated that the average tannin content in Aloe vera extract was less than that found in avocado seeds. The observation results revealed that the average content of avocado seed extract was 10.66%, while Aloe vera extract was measured at 4.68%.

The test results for Aloe vera extract samples (Figure 5) indicated an IC₅₀ value of 4614 µg/mL, categorizing it as inactive. Examinations of avocado seeds (Figure 6) revealed an IC₅₀ value of 135 µg/mL, categorizing it as moderate.

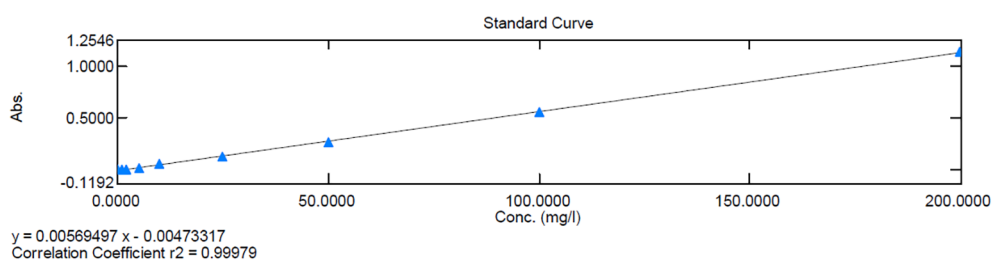


Figure 3. Curve of Flavonoid.

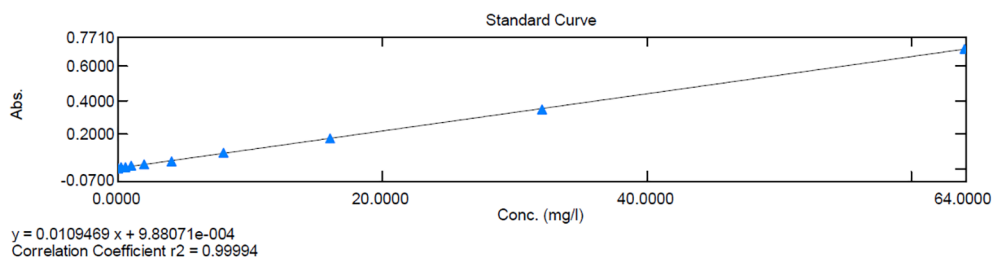


Figure 4. Curve of Tanin.

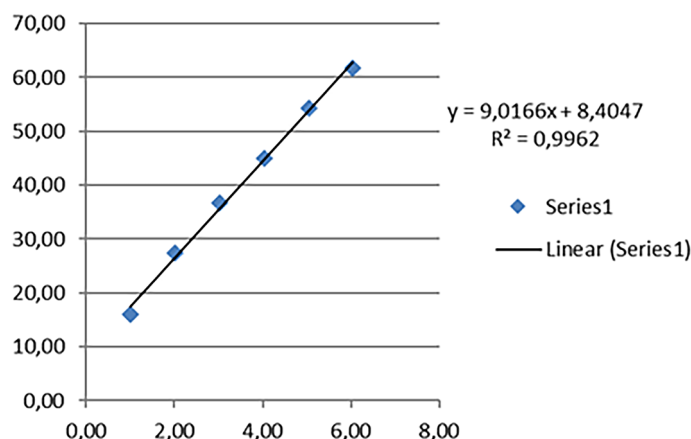


Figure 5. Antioxidant from Aloe vera.

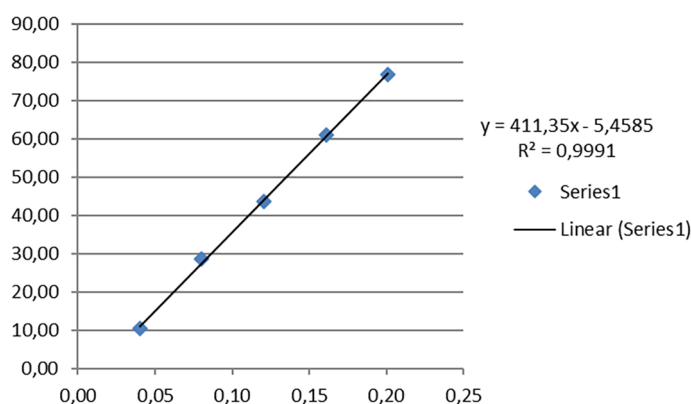


Figure 6. Antioxidant from Avocado seed.

Discussion

The saponin concentration in *A. vera* surpasses that of avocado seeds, as the highest levels of saponin in plants are located in the fruit and leaves, particularly in mature leaves^{17,18}; this aligns with prior research indicating that saponin synthesis transpires in the leaves.¹⁹ Saponin demonstrates foaming properties upon interaction with water.¹⁹ The composition and concentration of saponins are significantly affected by environmental conditions and extraction methods.^{20–22} Saponins work by exhibiting antibacterial action through the denaturation of proteins. The active compound saponin, akin to a detergent, can diminish the surface tension of bacterial cell walls, hence compromising the permeability of the bacterial membrane.²³

Aloe vera extract contained more alkaloid compounds. Plant type can also influence the difference, and environmental factors such as soil pH approaching neutral (5.79-6.97) can affect alkaloid production. The extraction process also affected the amount of alkaloid compound withdrawal.²⁴ The condition of the planting and maintenance soil is also thought to affect the quality of the avocado and Aloe vera plants used, so soil that uses nitrogen fertilizer can increase the production of alkaloids.²⁵ Alkaloids have basic properties and contain one or more nitrogen atoms in a cyclic system combination. This compound is likely to be present in small quantities and must be separated from a complex mixture of compounds. The alkaloid compounds were the lowest compared to the other compounds.^{26,27} Alkaloid compounds have non-polar properties; therefore, they are less suitable for extraction with polar compounds, such as 70% ethanol. Alkaloids play a role in plant metabolism and control plant development. Most alkaloid compounds originate from plants, especially Angiosperms.²⁸

Flavonoid content is higher in plants grown in lowlands, which is closely related to temperature and soil nutrient availability.^{29,30} The avocado and Aloe vera seeds used in this study were collected from different areas. The avocado seeds used in this study were obtained from lowland plantations with higher soil calcium content. This can affect

flavonoid content. The significant difference in flavonoid content was also caused by the fact that the types and varieties of plants used were not uniform. Flavonoids are primary antioxidants that provide hydrogen atoms to the free radicals. The flavonoid levels in plants affect the plant's antioxidant activity. Flavonoids are polar compounds that can dissolve in polar solvents, such as ethanol, methanol, butanol, acetone, and dimethylformamide. Water-soluble flavonoids are included in the polyphenol family. Flavonoids are also bound to glycosides. Hence, the mixture of the above solvents with water is a suitable solvent for flavonoid glycosides. In contrast, aglycones such as flavones, flavonols, and flavanones are more easily dissolved in chloroform and ether solvents.^{30–32}

The tannin content in avocado seeds was higher than in aloe vera seeds. Tannin compounds can be found in dicotyledonous plants' stems, skin, flowers, seeds, leaves, and cell walls or vacuoles. Generally, tannin compounds are more commonly found in dicotyledonous plants or flowering plants with dicotyledonous seeds, especially in angiosperm plants, such as plants with closed seeds, such as avocado seeds. During the maturity phase, fruit seeds experience a significant increase in tannin levels.³² Avocado is a dicotyledonous angiosperm plant that is thought to have a high tannin content, especially in seeds. Simultaneously, Aloe vera is included in monocotyledonous angiosperm plants.^{33–35} In addition, tannin has a molecular weight consisting of hydroxyl groups and several other groups, such as carboxyl and complex organic substances, which are difficult to separate and crystallize. Chemically, tannins are divided into condensed and hydrolyzed tannins. The type of tannin commonly found in plants is hydrolyzed tannin, usually marked by a color change from greyish-blue to blackish when given specific reagents. Tannins possess a sour and astringent flavor and create colloids upon dissolution in water. Tannin's structure comprises two aromatic rings connected by three carbon atoms.^{35–37}

The antioxidant activity was evaluated utilizing the DPPH assay. Before assessing the sample concentrations by UV-Vis spectrophotometry, the DPPH solution was analyzed to ascertain the optimal wavelength, ensuring that the sample sensitivity was maximized at the highest absorbance value. A potent antioxidant possesses an IC₅₀ value under 50 µg/mL, a powerful antioxidant has an IC₅₀ value ranging from 50 to 100 µg/mL, a moderate antioxidant exhibits an IC₅₀ value between 101 and 250 µg/mL, a weak antioxidant shows an IC₅₀ value from 250 to 500 µg/mL, and an inactive antioxidant has an IC₅₀ value exceeding 500 µg/mL.^{37,38} The analysis of the DPPH solution indicated an optimal wavelength of 515.5 nm for DPPH. This wavelength was employed to assess the antioxidant activity of the samples. Sample testing was conducted by reacting the test solution with the DPPH solution, followed by a 30-minute incubation in a dark environment. The incubation period is ideal for the DPPH technique, facilitating effective binding between antioxidant molecules and DPPH radicals. The comparator employed in this investigation was Vitamin C. Vitamin C comprises powerful natural antioxidant molecules. Vitamin C serves as a reliable comparator with well-defined features; hence, the vitamin C antioxidant test findings can assist in assessing the quality and efficacy of antioxidant components in the extract under examination.^{38,39}

The avocado seed extract in this study was tested with three repetitions or taking from the mother liquor, and obtained an average percentage inhibition result of 44.12% with an IC₅₀ value of 135 µg/mL, classified as moderate. Aloe vera extract was tested with three repetitions or taken from the mother liquor, and obtained an average percentage inhibition result of 40.17% with an IC₅₀ value of 4614 µg/mL, classified as inactive, but still has no potential as an antioxidant. The levels or concentrations used in Aloe vera were higher than those in avocado seeds, allowing the IC₅₀ value to be obtained. The requirement for calculating the IC₅₀ value is when there is an intersection between the inhibition value and a level above 50. If both levels are the same, the inhibition value obtained will not be more than 50%; therefore, the IC₅₀ value in Aloe vera cannot be determined.³⁹

IC₅₀ denotes the concentration of antioxidant chemicals required to neutralize 50% of free radicals. A lower IC₅₀ value indicates greater antioxidant activity.^{40,41} The difference in results obtained in A. vera and avocado seed extracts can occur due to several factors, such as plant factors themselves, plant quality, planting methods, the environment around the planting, and contamination by other substances. The difference in results between avocado seeds and Aloe vera can be associated with tannins and flavonoids in both extracts. The levels of flavonoids and tannins obtained in avocado seed extract were much higher, at 10.66% and 19.44%, respectively, while in Aloe vera, the flavonoid and tannin contents were only 4.68% and 0.99%, respectively. The difference in the content of these two compounds can affect the antioxidant activity of a plant. Previous studies stated that the phenolic content, such as flavonoids and tannins, strongly influences a plant's antioxidant activity.^{41–43} The comparison of flavonoid and tannin levels reveals that Aloe vera contains 4.68% tannins and 0.99% flavonoids, whereas avocado seeds exhibit significantly higher concentrations of 10.66% tannins and 19.44% flavonoids. Thus, the levels of both compounds are markedly greater in avocado seeds compared to Aloe vera. Both compounds are significant contributors to antioxidant activity.

This study is limited to identifying the highest antioxidant activity, and further research is needed to optimize dosage and assess in vivo toxicity.

Conclusion

The levels of flavonoids and tannins in the ethanol extract of avocado seeds were higher than those in *A. vera*. The levels of saponin and alkaloid compounds were higher in *Aloe vera*.

The antioxidant activity of the ethanol extract from avocado seeds exceeded that of *Aloe vera*. The inhibition percentage in avocado seeds was 44.12%, corresponding to an IC₅₀ value of 135 µg/mL, indicating moderate activity. In contrast, *Aloe vera* exhibited an inhibition percentage of 40.17% with an IC₅₀ value of 4164 µg/mL, suggesting inactivity.

Ethics and consent

Ethical approval and consent were not required.

Data availability

Extended data

Harvard Dataverse: "Analyzing *Aloe vera* and Avocado Seed Extracts for Antioxidants, Saponins, Tannins, Flavonoids, and Alkaloids Using the UV-VIS Spectrophotometric Method", <https://doi.org/10.7910/DVN/MQPRDT>.⁴⁴

This project contains the following underlying data:

Figure 1: Curve Saponin, Figure 2: Curve of Alkaloid, Figure 3: Curve of Flavonoid, Figure 4: Curve of Tanin, Figure 5: Antioxidant Avocado Seed, Figure 6: Antioxidant Avocado Seed, Result of Antioxidant, Sample for Curve Saponin, Tanin, Flavonoid, and Table flavonoid, alkaloid, tanin and saponin.

License: Data is available under the terms of the [CC0 1.0 Universal](#).

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Open Peer Review

Current Peer Review Status: ? ? ? ? ?

Version 2

Reviewer Report 05 January 2026

<https://doi.org/10.5256/f1000research.189646.r439209>

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Zehra Tekin

Adiyaman Üniversitesi, Adiyaman, Turkey

Is the study design appropriate and is the work technically sound? (In this study, only the DPPH method was used to determine antioxidant activity. However, we cannot conclude that antioxidant activity is strong or weak solely based on the free radical scavenging site. Therefore, it could be investigated using at least one other method based on different mechanisms)

Are sufficient details of methods and analysis provided to allow replication by others? Are all the source data underlying the results available to ensure full reproducibility?

No references were given when describing the analyses performed. Were the methods developed by the authors, or was the attribution simply forgotten? Furthermore, when the flavonoid test was described, was sodium nitrite used only to create the calibration curve? Was it not used to determine the amount of flavonoid components in the plant extracts? There is confusion. The method should be detailed and verified for the readers.

Are the conclusions drawn adequately supported by the results?

General information was provided during the discussion. The results of the study were not supported by previously published studies.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antioxidant activity and phytochemical analysis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 26 December 2025

<https://doi.org/10.5256/f1000research.189646.r432122>

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Thi-Van Nguyen

PHENIKAA University, Hanoi, Vietnam

The manuscript presents a clear and well-structured comparison of phytochemical contents and antioxidant activity in avocado seed and Aloe vera extracts. From a methodological and presentation standpoint, the study is informative and easy to follow. I appreciate the authors' effort in organizing their data and providing supporting figures.

To further strengthen the clarity and reproducibility of the work, I offer a few friendly suggestions:

Points to consider improving

- The description of the Aloe vera material is not entirely consistent across sections; aligning this information would help readers better understand the experimental setup.
- The alkaloid assay seems to reference two different standards (quinine and Quillaja bark). Clarifying which one was actually used would avoid confusion.
- Some procedural steps and analytical methods would benefit from added references for transparency.
- Figures 5 and 6 could be improved by adding axis labels, similar to the earlier figures.
- A few formulas and steps could be clarified to make it easier for others to reproduce the procedures.

Overall, the study presents valuable comparative data, and the main findings are clearly supported. With these refinements, the manuscript will be even more accessible and robust for readers.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Data Analysis; Research Methodology Evaluation; Scientific Computing; Data Integrity Assessment; Information Technology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 25 November 2025

<https://doi.org/10.5256/f1000research.189646.r434304>

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Ahmad Royani

National Research and Innovation Agency–BRIN, Banten, Indonesia

Abstract:

- IC₅₀ of Aloe vera (4614 µg/mL) clearly indicates *extremely weak* antioxidant activity, yet the abstract does not comment on its significance.
- The author should explain in the abstract the underlying mechanisms or contributing compounds for their claims of “superior antioxidant efficacy”.

Introduction:

- The same numerical values (e.g., the IC₅₀ of avocado seeds) appear multiple times with different citations; this may confuse readers.
- Some statements contain outdated or non-standard referencing styles (the citations are not

consistent)

- The gap and novelty: The authors claim that their novelty, only in “maceration for 48 hours using 70% ethanol,” is not scientifically strong because many studies already use similar extraction approaches.
- Some claims (e.g., dual-extract formulations, antibiotic resistance concerns) are not directly connected to the study and appear speculative.

Methods:

- The key steps should be summarized with references.
- The author should provide replicates, statistical tests, and error analysis for quantitative measurements.

Results:

- The author should provide tables to summarize results; readers must extract values from the paragraphs manually.
- The author should provide statistical measures (mean, SD, n-values), compromising reproducibility.
- The reported flavonoid content of avocado seeds (19.44%) seems unusually high; it is likely a dry-weight calculation issue.
- IC₅₀ of Aloe vera (4614 µg/mL) is extremely high, suggesting methodological or concentration-range issues; this should have been flagged in the results section.
- Some numerical values contradict earlier introductory references.

Discussion:

- Many explanations are speculative and not supported by experimental data from this study. (e.g., soil calcium content, fertilization practices, and environmental factors, it is not measured).
- The discussion does not address methodological limitations, particularly for standard deviations, means, etc.
- The author should provide a critical analysis of the extremely weak antioxidant activity of Aloe vera extract, rather than treating it merely as a descriptive outcome.
- The author should provide a comparison of the results with previously published numerical values, even though many are mentioned in the introduction.
- Does not discuss whether the values obtained align with typical ranges for these plants.

Conclusion:

- The author should mention the implications, limitations, or recommendations for future work.
- The final statement (“inactive but still has potential”) contradicts the IC₅₀ definition of “inactive.”

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: extraction plant and isolation of bioactive compounds for green corrosion inhibitor.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 22 July 2025

<https://doi.org/10.5256/f1000research.172498.r391024>

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Mona Luciana Gălăţanu

Titu Maiorescu University, Bucharest, Romania

The paper presents a comparative study of phytochemical quantification of the predominant compounds between two plant species, as well as their antioxidant capacity. Few aspects must be clarified:

1. Which organ is used from Aloe vera, as it is not specified in the text of Plant determination.
2. In the methods, at Saponin test, it is not written which standard substance was used to realize the standard curve.
3. Also, at the Alkaloid test, in Methods, quinine is mentioned as standard, while at Results, Quillaja bark appear to be the standard used. Which one was in fact used for obtaining the calibration curve?
4. In Discussion, it worth to be mentioned that both alkaloids and steroid saponins can determine a high risk of toxicity, that must be assessed through different studies. It is important also, to notice, that avocado seeds have these compounds in smaller doses, which make them safer for consumption.
5. Few typos in the text must be corrected.

References

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: pharmacognosy, taxonomy, analytical chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 26 Nov 2025

Nur Ariska Nugrahani

The paper presents a comparative study of phytochemical quantification of the predominant compounds between two plant species, as well as their antioxidant capacity. Few aspects must be clarified:

1. Which organ is used from Aloe vera, as it is not specified in the text of Plant determination.

In this section of Plant determination (materials) in my paper:

Response: The avocado seeds used were taken from a resident garden in Kepurun Village, Manisrenggo, Klaten. The Aloe vera was taken from a resident's garden on Kalimantan Street, Purwosari Sinduadi, Mlati District, Sleman. The aloe vera used is the peel/inner leaf and gel/inner pulp from Malang. The determination was made at the Plant Systematics Laboratory, Faculty of Biology, UGM, using certificate number 00569/S. Tb./II/2024 for Aloe vera, and 00568/S. Tb./II/2024 for the avocado seeds.

2. In the methods, at Saponin test, it is not written which standard substance was used to

realize the standard curve.

Response: A standard curve was established using a saponin scale of 10 mg of sample, specifically avocado seed extract and aloe vera extract, adding 5 ml of water to each extract. The mixture was vortexed for 5 min, and 50 µL of anisaldehyde was added. It was then shaken and allowed to stand for 10 minutes. Introduce 2 ml of 50% sulfuric acid and subject the mixture to heating in a water bath at 60°C for 10 minutes. Water was added to a 10 ml volume in a measuring flask, with the standard concentrations beginning at 200, 100, 50, 25, 12.5, and 6.25 µl. Absorbance was measured at a wavelength of 435 nm, and the standard used was Quillaja saponin.

3. Also, at the Alkaloid test, in Methods, quinine is mentioned as standard, while at Results, Quillaja bark appear to be the standard used. Which one was in fact used for obtaining the calibration curve?

Response: The sample was measured to within ± 100 mg to determine the total alkaloid content. Then, 5 mL of 2N HCl was added and mixed by shaking. The mixture was then rinsed thrice with 10 mL of chloroform in a separating funnel. After removing the chloroform phase, the solution was acidified with 0.1 N NaOH solution. Then, 5 ml of BCG Solution and 5 ml of Phosphate Buffer were added. After 5 ml of chloroform was added to the solution, it was swirled magnetically at 500 rpm for 15 min. Chloroform was used for extraction in two separate instances. Chloroform (5 mL) was added after the chloroform phase was recovered and evaporated using nitrogen gas. Absorbance was measured at 470 nm, and the standard used was Quillaja bark.

4. In Discussion, it worth to be mentioned that both alkaloids and steroid saponins can determine a high risk of toxicity, that must be assessed through different studies. It is important also, to notice, that avocado seeds have these compounds in smaller doses, which make them safer for consumption.

Response: This study is limited to identifying the highest antioxidant activity, and further research is needed to optimize dosage and assess in vivo toxicity.

5. Few typos in the text must be corrected.

Response: We appreciate the reviewer's comment. The typographical errors have been carefully checked and corrected throughout the manuscript.

Competing Interests: "The authors declare that they have no competing interests that could influence the validity or importance of this article or the peer review report."

Reviewer Report 28 March 2025

<https://doi.org/10.5256/f1000research.172498.r356891>

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**Didi Nurhadi Illian**

Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia

This manuscript describes the quantification of the levels of saponins, alkaloids, flavonoids, and tannins from both extracts of avocado seed and aloe vera. In addition, the IC_{50} values were determined. It is such an interesting manuscript to be reviewed. The paper presents some intriguing results, but it requires some modifications for publication acceptance.

Major Points

- Please check again for grammatical errors and typos, or consult with a proofreader!

Introduction

- Please mention more clear in this section the author's reason for using both samples of avocado seed and aloe vera for this study! The research gap is not so clear and difficult to recognize; please mention it to emphasize a sense of urgency to perform this investigation.

Methods

- Please use a common format for all the chemicals and instruments. Also, mention the manufacturer and country in this section!
- Sub-section **Extraction of Avocado Seed and Aloe Vera:**

Please check the sentence structure of "Avocado and **aloe vera seeds** were washed..." Did you really use aloe vera seeds? Please mention which part of the aloe vera was used in this study!

- Sub-section **Phytochemical Quantitative Test:**

Please check the formula of Total Compound (%b/b); is that ":10000" or "×10000"?

- Not a single reference was embedded for all the procedures of this study, from the extraction procedure and phytochemical analysis to the antioxidant activity test. Please mention the references for all methods that the authors used in each procedure!

Results

- Figures 5 and 6 are not well presented; what values represent the x and y axes? Please complete and clarify! See Figures 1 to 4!

Discussion

- Please add further discussion (in more detail or molecularly) on how the mechanism of action of the secondary metabolite compounds detected in both samples in facilitating the antioxidant activity obtained to strengthen your results! Simplifying, how can they become significant contributors to antioxidant activity?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacology, Toxicology, Immunology, Cancer Signaling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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