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Original Article

In Vitro Antioxidant Activity of Extracts and Fractions from Common Bean (*Phaseolus vulgaris* L.) Pods and Leaves Using the DPPH Method

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Abstract
Phaseolus vulgaris L. is a legume rich in nutrients and containing a variety of chemical constituents. Although its seeds have been extensively studied, comparative information regarding the antioxidant potential of the pod and leaf particularly in various solvent fractions has not yet been established. The objective of this study was to determine the in vitro antioxidant activity of ethanol extracts, n-hexane fractions, ethyl acetate fractions, and aqueous fractions of the pods and leaves of common bean (*Phaseolus vulgaris* L.) using UV-Visible spectrophotometry with the 1,1-dipheny l-2-picrylhydrazyl (DPPH) method. Phytochemical screening results indicated that the pods and leaves simplicia of the common bean contain flavonoids, polyphenols, saponins, monoterpenoids, sesquiterpenoids, and quinones. The n-hexane fraction of common bean leaves had an EC_{50} value of 7.16 µg/mL, and the aqueous fraction of common bean pod had an EC_{50} value of 10.24 µg/mL, each exhibited the strongest free radical scavenging activity compared to the other extracts and fractions. These values were determined using quercetin as a reference substance, which had a concentration of 0.6321 µg/mL. This indicates that common bean pods and leaves have potential as natural sources of antioxidant compounds. Therefore, further research is needed to isolate the compounds that act as antioxidants in common bean pod and leaf extracts, as well as to study the in vivo antioxidant activity of common beans.

Keywords: DPPH; Extract; Fraction; Quercetin; Common bean.

INTRODUCTION

The use of plants as traditional medicine in Indonesia has been on the rise recently, and some natural ingredients are even being produced on a fairly large scale. Traditional medicine is considered to have relatively fewer side effects compared to chemically derived drugs. In addition, the raw materials for traditional medicine are easier to obtain. One plant that is useful for making herbal medicine is the common bean/green bean plant (Sukohar et al., 2024) (Campanelli et al., 2024) (Borges Martínez et al., 2022) (Suci et al., 2023).

Antioxidants are compounds that can neutralize free radicals or compounds that can delay, slow down and prevent oxidation processes in the body. Free radicals are unstable molecules formed as a result of chemical processes in the body and contain one or more unpaired electrons, so free radicals become very reactive and can cause damage or cell death (Jain & Priyadarsini, 2025) (Apriliyanti et al., 2026).

Natural antioxidants that can reduce free radicals are increasingly in demand because they are considered to have a better level of safety compared to synthetic antioxidants (Lu et al., 2021). Naturally, plants that contain antioxidants are scattered in various parts of the plant such as roots, stems, skin, twigs, leaves, flowers, pods and seeds. Generally, the class of antioxidants is included in the class of phenolic derivative compounds such as

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flavonoids, derivatives of hydroxycinnamic compounds, coumarins, and tocopherols (vitamin E) (Lu et al., 2021) (Honga et al., 2021). One of the plants that produce flavonoid, phenol and tannin compounds is the common bean. These compounds work as free radical catchers and prevent chain reactions (Suci et al., 2023).

Common bean is a crop that is widely grown in Indonesia, these were obtained from the village of Cihideung in the Lembang subdistrict of West Java. Common bean contains flavonoids, alkaloids, saponins, stigmasterin, trigonelin, amino acids, kholina, tannins, starch substances, vitamins and minerals. Based on a literature review, flavonoid compounds such as anthocyanins, quercetin glycosides, and tannins found in common bean pod and leaf exhibit significant antioxidant activity (Priyadarshini et al., 2024) (Ratyani et al., 2025) (Campanelli et al., 2024).

In this study, antioxidant activity of extracts and fractions of common bean pod and leaf (*Phaseolus vulgaris* L) was tested to prove the presence of antioxidant activity (Ouedraogo et al., 2023) (Rodríguez Madrera et al., 2021). Antioxidant activity was determined using UV Visible spectrophotometry with determination of EC_{50} value by colorimetric method using 1,1-dipheny l-2-picrylhydrazyl (DPPH) reagent. DPPH is a stable free radical compound due to the presence of N groups that can be reduced by antioxidant compounds, purple in color, and in the form of prism shaped crystals (Hernandez et al., 2021) (Bean & Obtained, 2022) (Siswoyo et al., 2021). Because of its stability, DPPH is often used as a proxy in free radical scavenging assay activity (Suci et al., 2023). Free radical scavenging assay activity is done by calculating the EC_{50} value, which is the effective concentration of the test solution required to reduce 50% of the absorbance of the blank solution measured at a wavelength of 516 - 517 nm (Susmayanti & Rahmadani, 2023).

METHODS

Equipment

Desiccator, oven (Memmert), mortar and pestle, analytical balance (Sortorius BL 2105), watch glass and cover slip, chromatography paper, dropper pipette, volumetric pipette, test tubes and test tube racks, evaporating dish, spray bottle, porcelain dish, stirring rod, measuring cylinders, volumetric flasks, metal spatulas, beakers, Erlenmeyer flasks, wooden clamps, funnels, tripods, Bunsen burners, furnaces (Naberthem-Germany), UV-Vis spectrophotometer (Shimadzu UV-1601 PC).

Materials

Common bean (*Phaseolus vulgaris* L) pod and leaf were obtained from Cihideung Village, Lembang District, West Java, distilled water, 95% ethanol, ethyl acetate, n-hexane, chloroform, 1,1-dipheny l-2-picrylhydrazine (DPPH), methanol, quercetin, ferric chloride, bismuth nitrate, nitric acid, potassium iodide, mercuric chloride, dilute ammonia, magnesium powder, amyl alcohol, 5% potassium hydroxide, gelatin, ether, Dragendorff's reagent, Mayer's reagent, and drop plates.

Methods

A total of 250 g of each crude drug was macerated for 24 hours in 95% ethanol; the mixture was stirred several times during the first 6 hours, then left undisturbed for 18 hours, the filtrate was filtered, and the residue was macerated again until the solvent ran clear. The collected filtrate was used as the total extract. The extract was evaporated in a rotary evaporator to produce a concentrated extract, then further evaporated until a thick extract formed,

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after which the extract was weighed. A total of 20 grams of the ethanol extract from the common bean pod and leaf was taken and dissolved in 100 mL of water, it was then subjected to liquid-liquid extraction (LLE) with n-hexane until the solvent became clear, yielding an aqueous fraction and an n-hexane fraction. The aqueous fraction was then re-extracted with 100 mL of ethyl acetate several times until the solvent became clear, resulting in three fractions: the aqueous fraction, the n-hexane fraction, and the ethyl acetate fraction.

Phytochemical screening of common bean pod and leaf simplicia includes examination of the content of alkaloids, polyphenolics, tannins, flavonoids, monoterpenoids, sesquiterpenoids, quinones, saponins, streroid and triterpenoids (Lu et al., 2021) (Analysis et al., 2024) (Salas Salazar et al., 2025) (Shi et al., 2022).

The DPPH assay to assess free radical scavenging assay activity was performed on the ethanol extract, n-hexane fraction, water fraction, and ethyl acetate fraction, which were spotted onto a spot plate (Jain & Priyadarsini et al., 2025) (Salas-Salazar et al., 2025). The spot test plates were sprayed with 0.2% DPPH in methanol. Extracts and fractions that act as DPPH free radical scavenging assay activity will turn yellow within 30 minutes. (Florvil et al., 2022) (Rodríguez Madrera et al., 2021). Tests of the DPPH free radical scavenging assay activity were conducted on the ethanol extract, n-hexane fraction, water fraction, and ethyl acetate fraction as follows: (Hernandez et al., 2021) (Bean & Obtained, 2022).

a. Preparation of the reagent solution: The reagent is a DPPH solution with a concentration of 40 µg/mL in methanol. The reagent solution is used to measure the free radical scavenging assay activity of various concentrations and fractions, which are measured after 30 minutes. To prepare the reagent solution, weigh 10 mg of DPPH and dissolve it in methanol in a 10 mL volumetric flask, then shake until homogeneous. Pipette 2 mL of this solution into a 50 mL volumetric flask, then add methanol up to the mark.
b. Determination of the DPPH Maximum Wavelength. The absorbance of 3 mL of the DPPH reagent solution was measured at wavelengths ranging from 400 to 800 nm using a methanol blank.
c. Preparation of a series of solutions containing the ethanol extract, n-hexane fraction, water fraction, and ethyl acetate fraction. Test solutions were prepared by weighing 50 mg of the concentrated extract and the fractions, dissolving them in methanol in a 50 mL volumetric flask, and then shaking until homogeneous. From these stock solutions, several test solution series were prepared with concentrations ranging from 50 µg/mL to 400 µg/mL (Mubarok & Fikroh, 2024) (Samukha et al., 2024).

RESULTS

The common bean was collected from Cihideung Village, Lembang District, West Java, and identified at the Faculty of Life Sciences (SITH) at ITB under reference number 2390/K01.14.2/PP.2.4/2009. The results of the analysis are shown in Table 1 below.

Table 1. Plant identification results

No	Plants	Type	Tribe
1	Common bean	*Phaseolus vulgaris* L	*Papilionaceae*

Phytochemical screening results indicate that common bean pod simplicia contain alkaloids not detected, Meanwhile,

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the pods and leaves simplicia of common beans contain polyphenols, tannins, flavonoids, monoterpenes, sesquiterpenes, saponins, and quinones, whereas steroids and triterpenoids were not detected. As shown in Table 2 and Table 3.

Table 2. Phytochemical screening of common bean pod simplisia

Class of compound	Reagent	Result
Alkaloid	a. Dragendorff	Cloudy brown (+)
	b. Mayer	Yellow to white precipitate (+)
Polyphenol	Iron(III)chloride	Blue-black (+)
Tannin	Gelatin 1%	White precipitate (+)
Flavonoid	Mg powder, HCl, dan alcohol amil	Yellow to red (+)
Monoterpenoid and seskuiterpen	Vanillin-sulfuric acid 10%	Purplish green (+)
Saponin	HCl	Stable foam (+)
Quinone	KOH 5%	Yellow (+)
Steroid dan Triterpenoid	Liebermann-Burchard	No characteristic color change (-)

Notes: (+) = indicates the presence of the analyzed substance; (-) = indicates the absence of the analyzed substance.

Table 3. Phytochemical screening of common bean leaf simplisia

Class of compound	Reagent	Result
Alkaloid	a. Dragendorff	(-)
	b. Mayer	(-)
Polyphenol	Iron(III)chloride	Blue-black (+)
Tannin	Gelatin 1%	White precipitate (+)
Flavonoid	Mg powder, HCl, dan alcohol amil	Yellow to red (+)
Monoterpenoid and seskuiterpen	Vanillin-sulfuric acid 10%	Purplish green (+)
Saponin	HCl	Stable foam (+)
Quinone	KOH 5%	Yellow (+)
Steroid dan Triterpenoid	Liebermann-Burchard	No characteristic color change (-)

Note: (+) = indicates the presence of the analyzed substance; (-) = indicates the absence of the analyzed substance.

The concentrated common bean pod extract obtained weighed 61.76 grams with a yield of 24.704%, and the concentrated common bean leaf extract obtained weighed 47.05 grams with a yield of 18.82%. The n-hexane fraction from the common bean pods was 1.40 grams with a yield of 7%, the n-hexane fraction from the leaf was 0.75 grams with a yield of 3.75%; and the ethyl acetate fraction from the common bean pods was 4.42 grams with a yield of 22.10%. From the leaf, an ethyl acetate fraction of 1.16 grams was obtained with a yield of 5.89%; the water fraction from the pods was 8.56 grams with a yield of 42.80%; and the water fraction from the leaf was 7.10 grams with a yield of 35.50%.

The screening results show that common bean leaf exhibit DPPH free radical scavenging assay activity, as indicated by the appearance of a yellow color. The results are shown in figure 1 and figure 1 and figure 2 below:

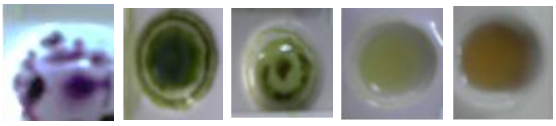

Figure 1. Screening of free radical scavenging assay activity in ethanol extracts and fractions of common bean pod.

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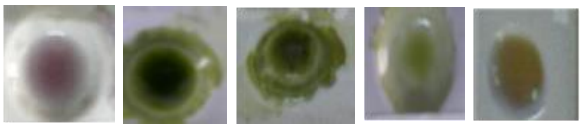

Figure 2. Screening of free radical scavenging assay activity.
Notes:
1 and 6: DPPH reagent solution (blank)
2 and 7: ethyl acetate fraction with antioxidant activity
3 and 8: n-hexane fraction with antioxidant activity
4 and 9: aqueous fraction with antioxidant activity
5 and 10: ethanol extract with antioxidant activity

The reagent solution is a DPPH solution with a concentration of 40 µg/mL in methanol. The reagent solution is used to measure the free radical scavenging activity at various concentrations, and the fractions are measured after 30 minutes. A reagent solution with a concentration of 40 µg/mL was prepared.

This test was conducted by measuring the decrease in DPPH absorption at the maximum wavelength. The reagent solution exhibited an absorbance of 1.163 at a maximum wavelength of 517.80 nm. The absorbance curve of the reagent solution is shown in Figure 3.

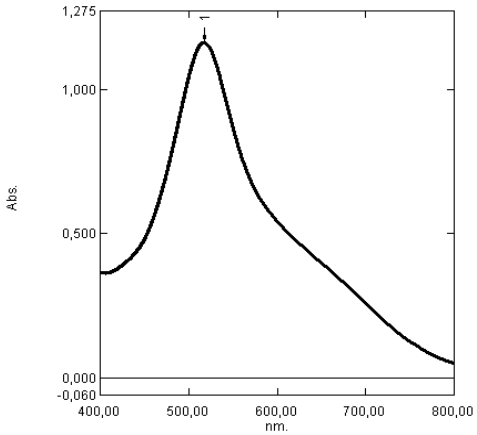

Figure 3. Absorbance curve of a 40 µg/mL DPPH reagent solution.
Notes: λ max 517.8 nm with a maximum absorbance of 1.163.

The test solutions consisted of an ethanol extract, an n-hexane fraction, an aqueous fraction, and an ethyl acetate fraction. From these stock solutions, several series of test solutions were prepared with concentrations ranging from 50 µg/mL to 400 µg/mL. Based on the results of antioxidant activity tests on extracts and fractions of common bean pods and leafs, the n-hexane fraction of common bean leafs, with an EC_{50} value of 7.16 µg/mL, and the water fraction of common bean pods, with an EC_{50} value of 10.24 µg/mL, exhibited the strongest free radical scavenging assay activity. A regression curve showing the percentage inhibition of the n-hexane fraction of common bean leaf is provided, along with a table of test results for the free radical scavenging assay activity of common bean pods and leafs extracts and fractions.

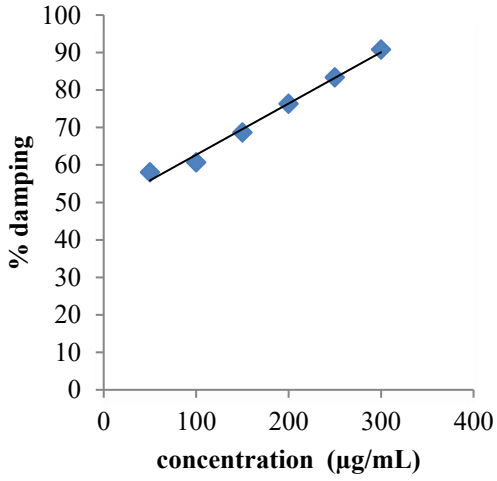

Figure 4. Regression curve of the percentage (%) of n-hexane fraction soaking from common bean leaf
Note: Regression equation: y = 0.136 x + 49.02
Correlation coefficient: r = 0.993.

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Table 4. Test Results on the free radical scavenging assay activity of common bean leaf extracts and fractions

Materials tested	EC_{50} (µg/mL)	r/R^2
Extract ethanol	28.04	0.997
Fraction n-hexane	7.16	0.993
Quercetin	0.6321	0.9995
Ethyl acetate Fraction	8.41	0.998
Water fraction	11.32	0.999

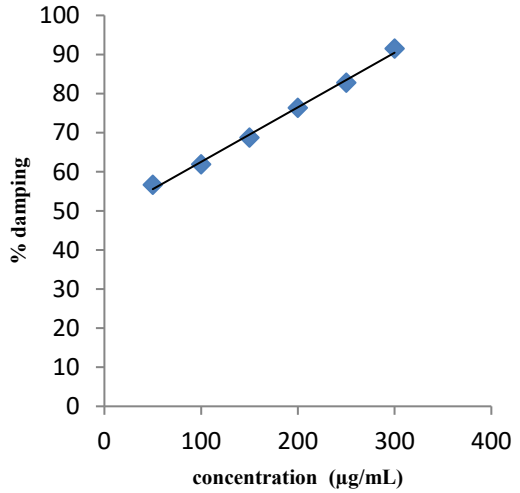

Figure 5. Regression curve for the percentage (%) of water fraction extraction from common bean pod
Note: Regression equation: y = 0,139 x + 48,57
Correlation coefficient: r = 0.993.

Table 5. Test Results on the free radical scavenging assay activity of common bean pod extracts and fractions

Materials tested	EC50 (µg/mL)	r/R^2
Extract ethanol	23,18	0,998
Fraction n-hexane	13,84	0,998
Quercetin	0,6321	0,9995
Ethyl acetate Fraction	13,16	0,998
Water fraction	10,24	0,997

The quercetin reference solution has antioxidant activity at a concentration of 0.6321 µg/mL, which is one of the requirements for comparing the test samples.

DISCUSSION

Determination was conducted at the Herbarium of the ITB School of Life Sciences and Technology. The results of the identification confirmed that the plant used in this study is common bean. The purpose of identifying the plant was to verify the species to be used in the study.

Phytochemical screening is conducted to determine the content of secondary metabolites in crude drug materials that are associated with their pharmacological properties or activities.

The presence of alkaloids is indicated by the formation of a white precipitate when reacted with Mayer's reagent and a brown orange precipitate when reacted with Dragendorff's reagent. The principle for identifying alkaloids in crude drug materials is based on their ability to form insoluble complexes with certain heavy metal reagents, such as Mayer's reagent (containing potassium iodide and mercury (II) chloride) and Dragendorff's reagent (containing bismuth nitrate and potassium iodide). The crude drug material must first be alkalized with ammonia to liberate the free alkaloids. Since free alkaloids are poorly soluble in water, chloroform is used as a solvent, and hydrochloric acid is then added to form soluble alkaloid salts.

The presence of polyphenolic compounds is indicated by a color change from yellow to blue-black; this is caused by a reaction between the phenolic groups and a solution of iron (III) chloride. In the crude drug preparations of common bean pods and leaves, flavonoids are present, as indicated by the formation of a yellow color in the amyl alcohol layer after reaction with magnesium powder and 2 N hydrochloric acid. The color formed is due to the reduction of the carbonyl group to an alcohol group, forming a colored hydroxy

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compound, and this color can be extracted by amyl alcohol. The crude drug of common bean pods and leaves contains tannins, as indicated by the formation of a white precipitate after the addition of 1% gelatin. This is caused by the formation of a water insoluble copolymer. The raw materials of common bean pod and leaf contain monoterpenoid-sesquiterpenoid compounds, as indicated by their ability to produce colors namely blue green and red upon the addition of a 10% vanillin solution in sulfuric acid, these two compounds are components of essential oils. The presence of saponins is indicated by their ability to form stable foam upon shaking and to remain stable upon the addition of a small amount of hydrochloric acid. In common bean leaves, foam formation is more stable than in common bean pods. The presence of quinone compounds is indicated by the formation of a blackish yellow color after the addition of a 5% KOH solution, this is due to the ability to form colored salts between hydroquinone and a strong alkali solution (5% KOH).

The extract was prepared by maceration at room temperature using ethanol as the solvent, with the process repeated until the solvent became clear. This was done because the compounds contained in the crude drug are soluble in ethanol, and also because ethanol is a universal solvent. This extraction method is carried out at room temperature because the components contained in the common bean pods and leaves crude drugs are not heat-stable, thus, if extraction were performed using heat, the substances contained within would decompose, particularly in the common bean pod crude drug, as common beans cannot withstand excessively high temperatures, which could reduce their nutrient content. After maceration, the filtrate was filtered to obtain the ethanol extract, which was then concentrated using a rotary evaporator. It was subsequently heated in a water bath until a concentrated common bean pod extract weighing 61.76 g was obtained, with a yield of 24.704% based on the weight of 250 g of common bean pod powder. Meanwhile, the ethanol extract obtained from common bean leaf weighed 47.05 g, with a yield of 18.82% based on the weight of 250 g of common bean leaf powder.

Next, fractionation was performed to separate the compounds contained in the extract based on their distribution ratios in two immiscible solvents. Fractionation was carried out via liquid-liquid extraction using n-hexane, ethyl acetate, and water as solvents. Before fractionation, the extract was first dissolved in water and then fractionated using n-hexane and ethyl acetate. n-Hexane was used to extract nonpolar compounds, while ethyl acetate was used to extract semipolar compounds, and water was used to extract polar compounds.

In the screening of antioxidant activity using a drop plate, the ethyl acetate fraction of common bean pod tested positive for antioxidants, as indicated by the appearance of a yellow color on the drop plate within no more than 30 minutes. This free radical scavenging activity is attributed to the presence of flavonoids and polyphenols in the extracts and fractions derived from common bean leaf and pod. Antioxidant activity testing was performed using UV-Vis spectrophotometry on the extracts and fractions, based on their ability to scavenge DPPH free radicals. This test was determined by measuring the decrease in DPPH absorption at a maximum wavelength of 517.80 nm after reaction with test solutions of ethanol extract, n-hexane fraction, water fraction, and ethyl acetate fraction at various concentrations.

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The strength of antioxidant activity is expressed by the EC_{50} value, which is the effective concentration of the test solution required to reduce the absorbance intensity by 50% compared to the reagent solution. EC_{50} was determined from a linear regression equation between the percentage of absorbance reduction and the concentration of the test solution. The relationship between the test solution concentration and the percentage (%) of quenching is directly proportional, that is, the higher the test solution concentration, the higher the percentage of quenching. In this study, a color change in the reagent solution occurred after the addition of the test solution from purple to yellow due to a reaction between DPPH and compounds that act as free radical scavengers or antioxidants. Here, H^+ represents a hydrogen atom containing one proton and one electron derived from the free radical scavenger compound, which reacts with DPPH to form the stable compound DPP-hydrazine. Meanwhile, the free radical scavenger that loses H^+ becomes a new radical, but this radical is less reactive.

Based on the results of antioxidant activity tests on extracts and fractions of common bean pods and leafs, the n-hexane fraction of common bean leafs, with an EC_{50} value of 7.16 µg/mL, and the water fraction of common bean pods, with an EC_{50} value of 10.24 µg/mL, exhibited the strongest free radical scavenging assay activity, these values were the lowest compared to those of the other fractions and extracts.

The quercetin reference solution serves as a standard with antioxidant activity, since quercetin is a pure, isolated compound. Quercetin is a flavonoid belonging to the flavonol group and is used as an antioxidant reference because the aqueous fraction of the pod and the n-hexane fraction of the common bean leafs showed antioxidant potential due to their flavonoid content. Quercetin has a concentration of 0.6321 µg/mL, which meets one of the requirements for a reference substance it must be derived from a compound in the same group.

CONCLUSION

The water extract from the pod and the n-hexane extract from the common bean leafs have potential as natural sources of antioxidant compounds, as assessed using a quercetin solution as a reference standard with known antioxidant activity.

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.

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