



Original article

Determination of Antioxidant Activity of Leaves and Flowers of *Faba bean*

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Abstract

Today, medicinal plants used in folk medicine are increasingly being researched and used in pharmaceutical, nutraceutical fields, and food. Despite its nutritional and medicinal properties, *Vicia faba* is a legume whose value is not fully understood. More research is needed on its multiple biological effects, such as antioxidant activity and other aspects. The aim of this study is to determine the antioxidant activity of extracts obtained from faba bean leaves and flowers. For this purpose, leaf and flower samples, which were dried in the open air and ground into fine powder, were extracted by steeping in boiling water for ten minutes. The total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity of the extracts were analyzed using spectrophotometric techniques. In addition, organic acid and phenolic compound contents were determined by high performance liquid chromatography technique. It was determined that the total phenolic, total flavonoid and antioxidant contents of faba bean flowers were higher than the leaves. The main phenolic compound in flowers and leaves is ellagic acid. In addition, cytotoxic effects of leaf and flower extracts were investigated by colorimetric test using CCK-8 (Cell Counting Kit-8) kit. The cytotoxic effects of leaf and flower extracts of faba bean were investigated by colorimetric test using CCK-8 (Cell Counting Kit-8) kit. No cytotoxic effect of faba bean extracts was observed. Faba bean is a good source of natural antioxidants and can be used to prevent harmful effects caused by free radicals. Therefore, this study shows that tea prepared from the leaves and flowers of faba bean may be a good choice for people with Parkinson's and those seeking health-promoting beverages.

Keywords: Antioxidant Activity, Flavonoid Compounds, Phenolic, *Faba Bean*.

Received: 31 August 2022 * **Accepted:** 19 September 2022 * **DOI:** <https://doi.org/10.29329/ijjaar.2022.475.11>

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INTRODUCTION

Oxidative damage caused by excessive free radicals is responsible for the pathogenesis of chronic diseases (Zhang et al, 2025). Natural products have been widely used in recent years for the prevention and treatment of chronic diseases such as cancer and cardiovascular disease, partly due to their strong antioxidant activities (Zhao et al, 2017; Fu, 2011).

Tea is the general name of the beverage made from the leaves, buds or flowers of the plant. It is the second most consumed beverage in the world (Rietveld and Wiseman, 2003). Various herbal teas are becoming more and more popular due to their functional properties. Various herbal teas have been found to have abundant natural antioxidants that can strengthen the antioxidant defense system and have the potential to prevent diseases caused by oxidative stress (Li et al, 2014; Jin et al, 2016).

Faba bean is of particular interest to researchers because of its nutritional and medicinal properties. It has the ability to reduce complications and progression of certain diseases. Also it plays an important role as an aid in the treatment of sequential diseases such as AIDS, hypertension, heart, kidney, liver and Parkinson's disease (Fernandes and Banerji, 1995, Jordinson, 1999; Hornykiewicz, 2002; Ye and Ng, 2002; Ellwood et al, 2008)

Faba bean blooms a lot due to its unlimited growth feature, but most of the blooms do not form fruits and fall off. In addition, faba bean is one of the earliest blooming plants in spring due to its low overall temperature demand. The flowers and leaves of faba bean are presented in Figure 1.



Figure 1. *Faba bean*

It has been determined that different parts of the broad bean plant containing L-Dopa in increasing proportions, including fruit, leaves and flowers. L-Dopa is one of the essential drugs declared by the World Health Organization (WHO) (Fu et al, 2018; Topal et al, 2020).

This study was originally designed to evaluate these spilled flowers and to gain added value. In this study, the consumption of leaves and flowers of faba bean plant as tea was investigated. For this purpose, total phenolic, flavonoid and antioxidant activities of broad bean leaf and flower tea were investigated using spectrophotometric techniques. Phenolic compounds and organic acids in herbal tea were identified and quantified by high performance liquid chromatography (HPLC). The cytotoxic effect of herbal tea was also investigated by colorimetric test using CCK-8 (Cell Counting Kit-8).

MATERIALS and METHODS

Folin–Ciocalteu reagent was purchased from Merck Company. Glacial acetic acid ($\geq 99\%$) and acetonitrile (HPLC grade, $\geq 99\%$) were purchased from Sigma Chemical Co. Pure gallic acid, catechin, quercetin, syringic acid, protocatechic acid, vanillic acid, coumaric acid, caffeic acid, and chlorogenic acid standards were purchased from Sigma Chemical Co. Faba bean used in this study was grown in the fields of Uşak University Faculty of Agriculture and Natural Sciences in Turkey. Different organs of the plant were separated and dried in a dark room for 15 days. The dried material was ground to fine powder in a mortar.

Sample preparation

Faba bean tea is obtained from the leaves and flowers of the faba bean plant collected from Uşak (Turkey). Teas were made using an aqueous extraction process to imitate home brewing settings for a cup of tea. The samples (10 g) were poured with 1000 mL of boiling water and stirred for 10 minutes. The extracts were filtered through cotton, cooled at room temperature, diluted to 100 ml with deionized water and used for spectrophotometric and chromatographic analyses.

Total phenolic content (TPC)

TPC was determined using the Folin–Ciocalteu method (Petrović et al, 2022). Twenty microliters of the extract were mixed with 100 μL of Folin–Ciocalteu reagent, followed by 300 μL of sodium carbonate solution (7.5%). The solutions were left at room temperature for 5 min, after which 50 μL of Folin–Ciocalteu reagent was added and vortexed well. After incubation for 30 min, the absorbance value was measured by spectrophotometer at 760 nm (Shimadzu UV-1800 spectrophotometer, Japan). The TPC was expressed as mg gallic acid equivalent (GAE) per g dried plant.

Total flavonoid content (TFC)

TFC was determined using the Aluminum chloride colorimetric method (Lopes et al, 2022). 0.5 mL of extract, 4.5 mL of deionized water, 0.3 mL of sodium nitrite solution (5%) were placed in a 10 mL volumetric flask. After 5 minutes, 3 mL of aluminum chloride solution (10%) was added. After 6 minutes, 2 mL of NaOH (4%) solution was added and then the total volume was made up to 10 mL with

deionized water. The solution was then thoroughly mixed and the absorbance at 510 nm versus blank was measured using a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). Total flavonoid content was expressed as mg quercetin equivalent (QE) per 1 g dried plant.

Antioxidant activity

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazil) radical method with slight modifications (Ebrahimzadeh et al. 2008). A stock reagent solution (6×10^{-5} M) was prepared by dissolving 0.0024 g of DPPH in 100 mL of methanol. A DPPH working reagent solution (40 mg L^{-1}) was prepared by diluting with methanol from stock reagent solution. It was mixed with 300 μL of extract and 5700 μL of DPPH working solution in a 10 mL tube. The reaction mixture was thoroughly vortexed and incubated in a dark cabinet at room temperature for 60 minutes. The absorbance of the reaction mixture was measured at 517 nm using a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). A control solution containing no extract was prepared and its absorbance was measured at 517 nm. Antioxidant activity was calculated as follows:

$$\text{Antioxidant activity (\%)} = (\text{AC(O)}_{517} - \text{AA(t)}_{517}) / \text{AC(O)}_{517} \times 100$$

where AC(O)_{517} is the absorbance of the control at $t = 0$ min and AA(t)_{517} is the absorbance of the antioxidant at $t = 1$ h.

Organic Acid Content

Tea samples were filtered using a $0.45 \mu\text{m}$ membrane filter. Filtered tea samples were analyzed using an Agilent 1260 liquid chromatographic system equipped with a UV detector, quaternary pump, autosampler and Chemstation software to determine organic acids. An ACE-C18 column (4 mm x 150 mm, $5 \mu\text{m}$) was used. As the mobile phase, an aqueous solution of 10 mM potassium phosphate adjusted to pH 2.2 with ortho-phosphoric acid was used at a flow rate of 1 mL min^{-1} . The detector was set to a wavelength of 245 nm (Fu et al., 2015).

Phenolic Compounds

Tea samples were analyzed using an Agilent 1260 liquid chromatographic system equipped with a UV detector, quadruple pump, autosampler and Chemstation software to determine organic acids. Mobile phase A was ultrapure water containing 0.1% by volume acetic acid, and mobile phase B was acetonitrile. An ACE-C18 (4.6 mm x 150 mm, $5 \mu\text{m}$) column was used for chromatographic separation. The mobile phase flow rate was kept constant at 1.0 mL min^{-1} . The column temperature was kept constant at 25°C . The injection volume was 10 μL . The following gradient conditions were used during the analysis: 0.00–3.25 min, 8–10% B; 3.25–8.00 min, 10–12% B; 8.00–15.00 min, 12–25% B; 15.00–15.80 min, 25–30% B; 15.80–25.00 min, 30–90% W; 25.00–25.40 min, 90–100% B; 25.40–30.00 min, 100% B. Detection wavelengths were chosen considering the wavelengths at which the phenolic

compounds to be analyzed had maximum absorption. According to this; Vanilic acid was detected at 225 nm, syringic acid, protocatechic acid and gallic acid at 280 nm, coumaric acid at 305 nm, caffeic acid and chlorogenic acid at 330 nm (Wen et al, 2005).

Cytotoxicity Analysis Method

Sterilization Before Cell Culture

All plastic materials and ready-made sterile media used in cell culture were obtained from commercial companies. Cell viability test was performed on lysate and medium samples prepared from cells obtained after incubations, using the methods described below (Li et. Al, 2017; Gu et al, 2016; Li et al, 2016; Zhu et al, 2016; Min et al, 2018).

Cell culture studies

Medium with RPMI 1640 as the main ingredient was used in the culture of A549 cells, and it contains 1% penicillin/streptomycin, and 10% fetal bovine serum (FBS). Cell culture was carried out by incubating under sterile conditions in T25 and T75 flasks containing sterile medium, in a carbon dioxide incubator providing CO₂ (5%) and 37 °C temperature conditions. Passaging was performed when the cells being replicated reached a density that covered approximately 85% of the culture flask. Cells were used in experiments when they reached sufficient numbers.

CCK 8 Cell Viability Assay

The CCK-8 (Cell Counting Kit-8) is a colorimetric test kit used to measure cell proliferation and cytotoxicity. For (ABP Bioscience lot: AB1714A2), cells were seeded in 90 µl at 5x10⁵ cells/ml in RPMI medium containing 10% FBS and 1% penicillin/streptomycin on a 96-well plate and incubated in a 37°C incubator in 5% CO₂ media. After 24 hours, the wells were divided into the following groups and 10 µl applications were made (Table 3.3). Three repetitions of each group were performed. After the applications were made, they were left to incubate for 48 hours. Then, 10 µl of CCK 8 solution was added to them. It was again incubated for 2 hours. At the end of the incubation period, shaking was done for 10 seconds and measurements were made with a plate reader at 450 nm wavelength.

RESULTS and DISCUSSION

Total phenolic content (TPC)

TPC values of different parts of plant material are presented in Table 1 as mg gallic acid equivalent (GAE) per g dried plant. TPC values of leaves of faba bean was determined as 65.50±3.21. This result corresponds to a high TPC value in faba bean leaves and is in agreement with the reports of Duan S.C et al. We did not find any literature on TPC values of flowers of faba bean. It was expected that the TPC value of the flowers of the plant was higher than the TPC value of the leaves. The TPC value of the flowers of the plant was determined as 93.20±4. This value corresponds to a result close to

or even higher than the TPC value of many medicinal plants. TPC and TFC are essential indices of antioxidant capacity for any product that is meant to be a natural source of antioxidants in functional foods.

Table 4. Total phenolic contents of faba bean

Sample	Unit	TPC ± S.D.
Flower	mg. GAE/ g d.w.	93.20 ± 4.12
Leaf	mg. GAE/ g d.w.	65.50 ± 3.21

Total flavonoid content (TFC)

TFC values of different parts of plant material are presented in Table 2 as mg quercetin equivalent (QE) per g dried plant. As can be seen from the Table 2, the highest TFC value was observed in flowers with 55.30±3.86. TFC values of leaves were determined as 37.44±3.12. As can be seen from the results, the plant has a higher total phenolic content than the total flavonoid contents. Commonly found in plants, flavonoids play an important role in reproduction, insect resistance, disease prevention, and defense against environmental changes (Mathesius, 2018). This may explain why a higher TFC was observed in flowers than in leaves.

Table 2. Total flavonoid contents of faba bean

Sample	Unit	TPC ± S.D.
Flower	mg. QUE/g D.M.	55.30±2.86
Leaf	mg. QUE/g D.M.	37.44±2.12

Antioxidant activity

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazil) radical method. Antioxidant activity of different parts of plant material are presented in Table 3 as inhibition %.

Table 3. Antioxidant activity of faba bean

Sample	Unit	A.A. ± S.D.
Flower	Inhibition %	86.53±0.67
Leaf	Inhibition %	75.13±2.03

Ascorbic acid solution ($500 \mu\text{g mL}^{-1}$) was used as the control solution in the antioxidant test. The capacity of ascorbic acid solution to inhibit DPPH radical was determined as 95%. As can be seen from the Table 3, % inhibition value was observed in flowers with 86.53 ± 0.67 . The percentage inhibition values of leaves and fruits were determined as 75.13 ± 2.03 and 69.78 ± 2.61 , respectively. This shows that the antioxidant activity of faba bean flowers are higher than the antioxidant activity of other organs. Determining the antioxidant activity results as high confirms that the total phenolic content is high.

Organic Acid Analysis

Organic acid contents of faba bean organs are presented in Table 4. In general, it was determined that the organic acid content of the leaves was higher than the organic acid content of the flowers. The dominant organic acid in leaves and flowers is oxalic acid.

Table 4. Organic Acids of faba bean

Sample	Oxalic acid mg/g d.w.	Tartaric acid mg/g d.w.	Malic acid mg/g d.w.	Acetic Acid mg/g d.w.	Citric Acid mg/g d.w.
Flower	1.70	1.30	1.50	0.13	0.55
Leaf	2.40	1.60	1.80	0.22	0.75

Phenolic Compounds

Phenolic compounds of faba bean organs are presented in Table 5. In general, it was determined that the phenolic compounds content of the flowers was higher than the phenolic compounds content of the leaves. The dominant the phenolic compounds in leaves and flowers is ellagic acid.

Table 5. Phenolic compounds of faba bean

Sample	Coumaric acid mg/g d.w.	Ferrulic acid mg/g d.w.	Ellagic acid mg/g d.w.	Quercetin mg/g d.w.
Flower	0.24	1.05	1.22	0.15
Leaf	0.21	0.68	1.07	0.11

Cytotoxicity Analysis

In addition, cytotoxic effects of leaf and flower extracts were investigated by colorimetric test using CCK-8 (Cell Counting Kit-8) kit. The cytotoxic effects of leaf and flower extracts of faba bean were investigated by colorimetric test using CCK-8 (Cell Counting Kit-8) kit. No cytotoxic effect of faba bean extracts was observed. Faba bean is a good source of natural antioxidants and can be used to prevent harmful effects caused by free radicals.

CONCLUSION

This study contributes to the determination of phenolic, flavonoid, and antioxidant content in leaves and flowers of faba bean. A strong association was discovered between phenolic content, flavonoid content, and antioxidant activity. Phenolic content can be utilized as a key marker of antioxidant strength. The tea made from the plant had no harmful effects. As a result of this research, tea made from the leaves and blossoms of faba bean may be an excellent alternative for Parkinson's sufferers and others looking for health-promoting drinks.

Additional Statement

Author contribution rates: The authors contributed equally to the study.

The text confirming adherence to the ethical standards for research and publication: IJIAAR's research and publication ethics principles were followed throughout the article's process.

Conflict of interest declaration: There is no potential conflict of interest in this study.

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