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Analysis of Flavonoid and Tannin Contents in Pumpkin Seed (*Cucurbita moschata*) Extract

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Abstract

Pumpkin is one of the herbal plants that possesses various medicinal properties, including antidiabetic, antifungal, antibacterial, anti-inflammatory, and antioxidant activities, due to the presence of bioactive compounds in its seeds. This study aimed to identify the bioactive compounds present in pumpkin seed extract using qualitative and quantitative descriptive methods. Qualitative analysis was conducted through color reaction tests using several specific reagents to identify the presence of flavonoid and tannin compounds. Quantitative analysis was performed using the UV-Vis spectrophotometric method based on standard solution calibration curves to determine the concentration of each compound. The qualitative test results indicated that the pumpkin seed extract positively contained flavonoids and tannins, as evidenced by the formation of specific color changes in each test. The quantitative analysis showed that the average flavonoid content was 9.34 mg/g, while the average tannin content was 9.42 mg/g. Based on the results of this study, it can be concluded that pumpkin seed extract contains high levels of flavonoid and tannin compounds, indicating its potential as a natural source of antioxidants. This study is expected to serve as a basis for further development regarding the potential utilization of pumpkin seeds with pharmacological effects.

Keywords: Pumpkin seeds; Flavonoids; Tannins; Qualitative analysis; UV-Vis spectrophotometry.

INTRODUCTION

Plants are known to contain various chemical compounds, namely primary and secondary metabolites. Secondary metabolites are compounds produced through secondary metabolic processes whose distribution is not uniform among living organisms and are generally found in relatively small quantities. The presence of these metabolites in plants is influenced by various internal and external environmental factors. Secondary metabolites possess distinctive characteristics in each organism and are formed through specific biosynthetic pathways derived from primary metabolites such as carbohydrates, lipids, and amino acids that constitute proteins (Rahmat & Hairani, 2025). Secondary metabolites have beneficial roles as natural antioxidants capable of neutralizing free radicals. These compounds are produced by plants and include several groups such as alkaloids, flavonoids, phenolics, tannins, and saponins (Pangisian et al., 2022).

Pumpkin is a commonly found creeping plant belonging to the Cucurbitaceae family. It is considered a food source with high nutritional value, and its flesh is frequently utilized as a basic ingredient in various processed foods and beverages (Maria & Devi, 2019). Pumpkin seeds are one of the parts of this herbal plant that possess various beneficial properties, including antidiabetic, antifungal, antibacterial, anti-inflammatory, and antioxidant activities due to the presence of diverse bioactive compounds (Nuryanti et al., 2023). However, pumpkin seeds are often regarded as waste despite their potential for further utilization (Aditiya & Ismawati, 2023).

Pumpkin seeds are generally only used for simple processed products such as roasted seeds or snacks similar to sunflower seeds (Wildan et al., 2024). In Palu City, pumpkin seeds have not been optimally utilized because they are still considered waste products from fruit consumption, along with the limited public knowledge regarding the bioactive compounds contained in the seeds. In fact, pumpkin seeds contain various important nutrients such as proteins, fats, fiber, and carbohydrates. Pumpkin seed extract can also be utilized as an estrogenic therapy because it contains phytoestrogen compounds, which are components of the flavonoid group (Ramadhani et al., 2024). Furthermore, color reagent testing has shown that ethanol extract of pumpkin seeds contains bioactive compounds in the form of flavonoids and tannins (Pelu et al., 2020). Nevertheless, pumpkin seeds are still predominantly

utilized only as simple snack products such as roasted seeds (Wildan et al., 2024).

Flavonoids belong to the group of secondary metabolites and are classified as polyphenolic compounds (Qamarani & Aryani, 2023). These compounds naturally occur in various plant parts, including fruits, flowers, leaves, roots, and seeds (Hakim & Saputri, 2020). Flavonoids are known to exhibit diverse biological activities and provide significant pharmacological benefits because they can function as antioxidants, anticancer agents, and anti-inflammatory compounds. Consequently, flavonoids have attracted considerable attention from researchers in the search for more effective and sustainable therapeutic alternatives for managing and preventing inflammatory conditions (Hidayah et al., 2023). Based on their chemical structure and biosynthetic pathways, flavonoids are divided into several subclasses, namely flavones, flavonols, flavanones, flavanonols, flavanols (catechins), and anthocyanins (Panche et al., 2016).

Tannins are another group of polyphenolic compounds that differ from flavonoids but are also widely found in plants (Udayani et al., 2022). Tannins can be defined as polyphenolic compounds with relatively large molecular masses, often exceeding 1000 g/mol, and possessing the ability to interact and form complex bonds with proteins. These compounds have significant biological potential because they can precipitate proteins and bind metals, thereby exhibiting antioxidant potential in biological systems (Noer et al., 2018). The astringent properties of tannins can be utilized for treating diarrhea, stopping bleeding, and preventing inflammation, particularly in the oral mucosa. In addition, tannins can function as antidotes against heavy metal poisoning and harmful alkaloids. In plants, tannins contribute to the bitter and sour taste found in leaves and fruits, serving as a defense mechanism against herbivores (Hidjrawan, 2018). Tannins are generally classified into two groups, namely hydrolyzable tannins and condensed tannins (Pratama et al., 2019). Therefore, this study aimed to analyze the secondary metabolite compounds contained in pumpkin seeds, particularly flavonoids and tannins, which are known to possess various pharmacological benefits such as natural antioxidant, anti-inflammatory, and anticancer activities.

MATERIALS AND METHODS

Study Area

This study employed qualitative and quantitative descriptive approaches. The qualitative approach aimed to provide a systematic and accurate description of the presence of flavonoid and tannin compounds in pumpkin seed (*Cucurbita moschata*) extract. Meanwhile, the quantitative approach was used to determine the levels of flavonoids and tannins numerically based on laboratory measurement results. This study was conducted from January 23 to 26, 2026. The samples in the form of pumpkin seeds were obtained from Balaroa Village, specifically from the Inpres Market area, and were subsequently processed through drying, grinding, and extraction using ethanol solvent. The resulting extract was then qualitatively analyzed to identify the presence of flavonoid and tannin compounds and quantitatively analyzed to determine their concentrations using the UV-Vis spectrophotometric method. All analytical procedures were carried out at the Chemistry Laboratory, Faculty of Teacher Training and Education, Tadulako University.

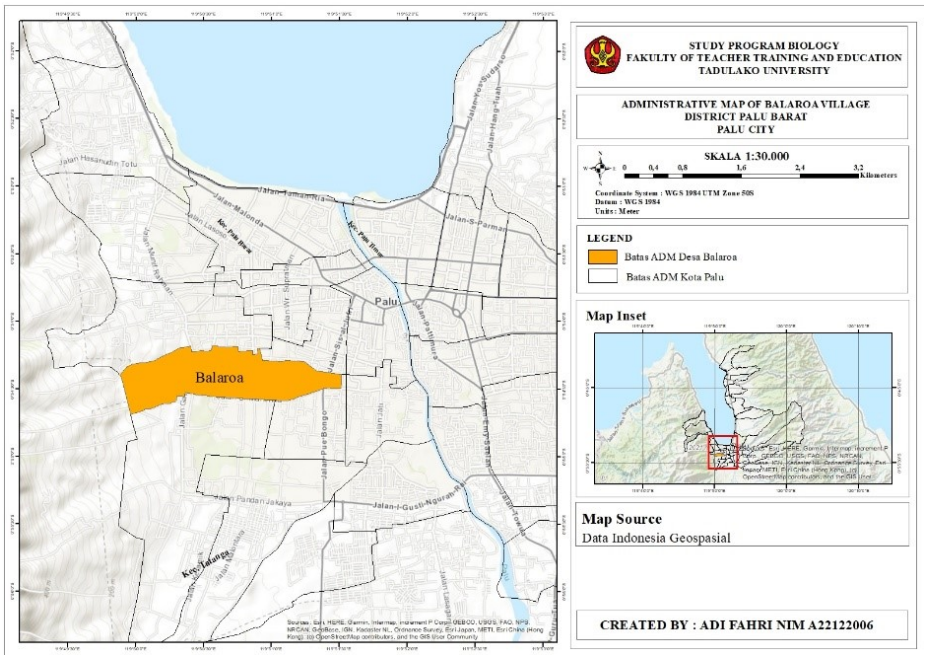

Figure 1. Sampling location of pumpkin seeds: West Palu District, Palu, Central Sulawesi, Indonesia.

Procedures

The tools and materials used in this study included equipment for the extraction process, namely a blender, sieve, Erlenmeyer flask, beaker glass, stirring rod, filter paper, and evaporation apparatus. The equipment used for the identification of bioactive compounds included test tubes and droppers. Instruments used to measure the content of bioactive compounds included cuvettes and a UV-Vis spectrophotometer. The materials used in the extraction process were ethanol and pumpkin seed samples. The reagents used for the identification of bioactive compounds were sodium hydroxide (NaOH), hydrochloric acid (HCl), magnesium, gelatin reagent, and 1% $FeCl_3$. The materials used for determining the levels of bioactive compounds included ethanol, potassium acetate, Folin–Denis reagent, tannic acid, sodium carbonate (Na_2CO_3), quercetin, aluminum chloride ($AlCl_3$), aluminum foil, filter paper, and tissue paper.

Sample Preparation

At the preparation stage, the pumpkin seeds were separated from the flesh and washed thoroughly. Subsequently, 1 kg of seeds was dried by air-drying indoors until completely dry. The dried samples were then ground using a blender and sieved to obtain fine pumpkin seed powder ready for extraction (Hartati & Noer, 2020). The preparation of pumpkin seed (*Cucurbita moschata*) extract was carried out using the maceration method. A total of 25 g of powdered pumpkin seeds was weighed and soaked in 250 mL of 30% ethanol for 3 × 24 hours with controlled stirring, followed by filtration. The residue remaining after filtration was remacerated twice until the filtrate was obtained. The filtrate was then evaporated using a rotary vacuum evaporator to obtain a concentrated pumpkin seed extract (Arifah et al., 2023).

Qualitative Test

Qualitative phytochemical testing was conducted to determine the presence of flavonoid and tannin compounds. The reagents used for flavonoid testing included NaOH. A total of 2 mL of pumpkin seed extract was placed into a test tube, followed by the addition of NaOH reagent. The mixture was shaken and allowed to stand. The formation of a yellow color indicated a positive result for flavonoids. Another flavonoid test was carried out using HCl and magnesium reagents. A total of 2 mL of the extract was mixed with a small amount of magnesium powder and 3–4 drops of concentrated HCl. The solution was then gently shaken and observed for color changes. The presence of flavonoid compounds was indicated by the appearance of red, orange, or yellow coloration in the solution. Furthermore, tannin content was tested using 1% $FeCl_3$ reagent. A total of 2 mL of pumpkin seed extract was heated in a test tube for approximately 5 minutes, followed by the addition of 1% $FeCl_3$ solution. The formation of a greenish-brown color or brown precipitate indicated the presence of tannins (Nuryanti et al., 2023).

Quantitative Test

Quantitative analysis was carried out to determine the levels of flavonoids and tannins using UV-Vis spectrophotometry. Prior to flavonoid measurement, a quercetin standard solution and a series of standard concentrations were prepared by weighing 10 mg of quercetin standard, which was then transferred into a 10 mL volumetric flask and diluted with ethanol to the calibration mark to obtain a 100 ppm stock solution (Elvira et al., 2024). Subsequently, standard solutions with concentrations of 5, 10, 15, 20, and 25 mg/L were prepared. One milliliter of each standard solution was pipetted and mixed with 1.5 mL of 70% ethanol, 0.1 mL of 10% aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was then incubated for 30 minutes. Absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer, and a calibration curve was subsequently constructed (Ningrum et al., 2022). For the sample solution analysis, 10 mg of filtrate was weighed and placed into an Erlenmeyer flask, followed by the addition of 10 mL ethanol. One milliliter of the sample solution was pipetted and mixed with 1.5 mL of 70% ethanol, 0.1 mL of 10% aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated for 30 minutes, and the absorbance was measured at the maximum wavelength of 445 nm using a UV-Vis spectrophotometer (Ningrum et al., 2022).

The determination of tannin content began with the preparation of a stock standard solution and a series of concentrations. A total of 10 mg of tannic acid was weighed and transferred into a 10 mL volumetric flask, then dissolved with ethanol up to the calibration mark to obtain a 100 ppm solution. One milliliter of the tannic acid solution was pipetted into another 10 mL volumetric flask and diluted with ethanol to the calibration mark to produce a 100 ppm stock standard solution (Pratama et al., 2019). The stock solution was then pipetted in volumes of 1, 2, 3, 4, and 5 mL into separate 10 mL volumetric flasks and diluted with distilled water to obtain concentrations of 10, 20, 30, 40, and 50 ppm, respectively (Wahid, 2020). From each concentration, 1 mL was pipetted and mixed with 0.5 mL of Folin–Ciocalteu reagent, shaken, and allowed to stand for 8 minutes. Subsequently, 2 mL of 20% Na_2CO_3 solution was added, vortexed for 10 minutes, and allowed to stand for 2 hours at room temperature. The absorbance was then measured at the maximum wavelength of 742.5 nm (Pratama et al., 2019). For the sample solution analysis, 10 mg of filtrate was weighed and transferred into an Erlenmeyer flask, then dissolved in 10 mL ethanol. One milliliter of the extract solution was pipetted into a test tube and mixed with 0.5 mL of Folin reagent, shaken,

and allowed to stand for 8 minutes. Subsequently, 2 mL of 20% Na_2CO_3 was added to the mixture, and the solution was allowed to stand for 2 hours at room temperature. The absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength of 742.5 nm (Pratama et al., 2019).

Analisis Analysis

Qualitative Analysis

The determination of bioactive compounds in pumpkin seed extract was carried out using several reagents to detect the presence of flavonoids and tannins. Flavonoid testing was conducted using NaOH reagent as well as concentrated HCl and magnesium, while tannin testing was performed using 1% $FeCl_3$ reagent.

Quantitative Analysis

Quantitative analysis was conducted to determine the levels of flavonoid and tannin compounds in pumpkin seed extract using UV-Vis spectrophotometry. The analysis results were obtained based on the following formula for calculating bioactive compound content:

$$Compound\ levels = \frac{C\ x\ V\ x\ FP}{m}$$

$$Average\ compound\ content = \frac{C(1) + C(2)}{2}$$

Description:

C = compound concentration
V = volume of extract used
FP = dilution factor
m = weight of the sample used

RESULT AND DISCUSSION

Phytochemical Screening Test

Phytochemical screening is a simple test used to identify the potential bioactive compounds of plants as medicinal materials, which is indicated by the formation of color changes or precipitates in the sample after being reacted with several specific reagents. In this study, phytochemical screening was conducted to identify the presence of flavonoid and tannin compounds in pumpkin seed (*Cucurbita moschata*) extract. Different reagents were used for each test, where NaOH and HCl were utilized to detect flavonoid compounds, while 1% $FeCl_3$ reagent was used to detect tannin compounds in the sample (Table 1).

Table 1. Phytochemical screening.

Component	Reagent	Results	Conclusion
Flavonoid	NaOH	Yellow 	+
	HCl & Magnesium	Yellow 	+
Tannin	$FeCl_3$	Brown 	+

Determination of Flavonoid Content in Pumpkin Seed Extract

Based on testing the flavonoid levels in the samples using UV-Vis spectrophotometry with two repetitions, the first experiment obtained an absorbance of 0.453 and the second repetition obtained 0.408, resulting in sample concentrations/ppm of 9.93 and 8.76, respectively. Data analysis was then performed to calculate the total flavonoid levels (Table 2).

Table 2. Determination of flavonoid content in pumpkin seed extract.

Sample	Absorbance	Concentration/ppm (mg/1)	Extract Volume (ml)	Extract Weight (mg)	Diluting Factor (FP)	Flavonoid Levels	Average
1	0.453	9.93	0.01	0.1	10	9.93	9.34
2	0.408	8.76	0.01	0.1	10	8.76	

Determination of Tannin Content in Pumpkin Seed Extract

Based on the determination of tannin content in the sample using UV-Vis spectrophotometry with two replications, absorbance values of 0.141 and 0.143 were obtained in the first and second measurements, respectively. The resulting sample concentrations were 18.63 ppm and 19.06 ppm. Subsequently, data analysis was performed to calculate the total tannin content (Table 3).

Table 3. Determination of tannin content in pumpkin seed extract.

Sample	Absorbance	Concentration/ppm (mg/1)	Extract Volume (ml)	Extract Weight (mg)	Diluting Factor (FP)	Flavonoid Levels	Average
1	0.141	18.63	0.01	0.1	5	9.31	9.42
2	0.143	19.06	0.01	0.1	5	9.53	

Discussion

Based on the phytochemical screening conducted to detect flavonoid and tannin compounds in pumpkin seed extract, the test using NaOH reagent showed a color change to yellowish, indicating that the sample positively contained flavonoid compounds (Nurjannah et al., 2022). The appearance of this yellow coloration occurred due to the reaction between flavonoid compounds and alkaline reagents, which caused changes in the chromophore structure of the flavonoids present in the extract. In addition, the phytochemical analysis using concentrated HCl and magnesium reagents also demonstrated a positive reaction, as indicated by the formation of a yellow coloration in the solution after the reagents were added. This reaction is commonly associated with the reduction process of flavonoid compounds, especially flavones and flavonols, which produce characteristic color changes during qualitative analysis. Furthermore, the addition of 1% $FeCl_3$ reagent to the pumpkin seed extract produced a brownish coloration, which indicated the presence of tannin compounds in the sample. The formation of this color occurs because tannins are polyphenolic compounds capable of forming complexes with ferric ions. The positive results obtained from all phytochemical tests confirmed that pumpkin seed extract contains secondary metabolites in the form of flavonoids and tannins, both of which are known to possess important biological and pharmacological activities, particularly as natural antioxidants and anti-inflammatory agents (Pelu et al., 2020).

The determination of flavonoid and tannin levels in pumpkin seed extract was carried out using UV-Vis spectrophotometry, which is widely used for quantitative analysis because of its sensitivity, accuracy, and ability to measure compounds based on their absorbance at specific wavelengths. In the flavonoid measurement, which was conducted in two replications using a sample volume of 0.01 mL and an extract weight of 0.1 mg with a dilution factor of 10 at a wavelength of 445 nm, absorbance values of 0.453 and 0.408 were obtained. Based on the calibration curve of quercetin standard solution, these absorbance values corresponded to concentrations of 9.93 and 8.76, respectively, resulting in flavonoid contents of 9.93 and 8.76. The average flavonoid content obtained from both replications was therefore calculated to be 9.34%. The relatively consistent absorbance values between replications indicate that the analytical method used in this study had good repeatability and reliability. The presence of flavonoids in relatively high amounts suggests that pumpkin seeds possess significant antioxidant potential because flavonoid compounds are known to donate hydrogen atoms or electrons that can neutralize free radicals. In addition, flavonoids also contribute to various pharmacological activities, such as anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects, making pumpkin seed extract a promising source of natural bioactive compounds for further pharmaceutical and functional food development (Nurjannah et al., 2022).

Meanwhile, the tannin content determination, which was also performed using UV-Vis spectrophotometry, showed that pumpkin seed extract contained relatively high levels of tannin compounds. The analysis was conducted in two replications using a sample volume of 0.01 mL and an extract weight of 0.1 mg with a dilution factor of 5 at a maximum wavelength of 742.5 nm. The absorbance values obtained were 0.141 and 0.143, corresponding to concentrations of 18.63 and 19.06, respectively. These concentrations resulted in tannin contents of 9.31 and 9.53, with an average tannin content of 9.42% in pumpkin seed extract. The small difference between the two measurements indicates that the analytical procedure produced stable and reproducible

results. The results also demonstrated that the tannin content was slightly higher than the flavonoid content in pumpkin seed (*Cucurbita moschata*) extract. Tannins are known as polyphenolic compounds that possess strong antioxidant activity due to their ability to bind free radicals and inhibit oxidative reactions. Moreover, tannins also exhibit antimicrobial, anti-inflammatory, and metal-chelating activities, which contribute to their pharmacological importance. The relatively high tannin content found in pumpkin seed extract further supports the potential utilization of pumpkin seeds as a natural source of bioactive compounds that may be beneficial for maintaining health and preventing oxidative stress-related diseases (Nuryanti et al., 2023).

Previous studies have analyzed the flavonoid content in ethanol extract of pumpkin leaves, and the results obtained using a standard quercetin calibration curve showed that the total flavonoid content in pumpkin leaf extract was 7.26 mg/g (Hari et al., 2025). Compared with the results of the present study, the flavonoid content in pumpkin seed extract was found to be higher than that in pumpkin leaves. This difference indicates that different plant parts may accumulate secondary metabolites in varying concentrations depending on their physiological functions and metabolic activities. Seeds generally serve as storage organs containing reserve compounds that support germination and early plant development, which may explain the higher accumulation of flavonoid compounds. In addition, differences in flavonoid content can also be influenced by several factors, including the type of solvent used during extraction, extraction duration, extraction method, environmental conditions, and plant age. Ethanol, which was used in this study, is known to be effective for extracting polar and semi-polar compounds such as flavonoids and tannins. Meanwhile, direct comparisons for tannin content could not yet be conducted because previous studies mainly focused on flavonoid analysis. Nevertheless, the findings of this study provide important preliminary scientific data regarding the tannin content of pumpkin seeds and contribute to the broader understanding of secondary metabolite compounds in plants (Rahmat & Hairani, 2025).

The presence of flavonoid and tannin compounds in pumpkin seed (*Cucurbita moschata*) extract indicates that this plant, especially its seeds, possesses significant potential as a natural source of antioxidants and other beneficial bioactive compounds. In many communities, pumpkin seeds are often discarded as waste after the fruit is consumed, despite their valuable phytochemical content and nutritional properties. The relatively high levels of flavonoids and tannins identified in this study suggest that pumpkin seeds may provide various pharmacological benefits, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Antioxidants play an important role in protecting the body from oxidative stress caused by free radicals, which are associated with aging and the development of chronic diseases such as cancer, cardiovascular disorders, and diabetes. In addition, tannins and flavonoids are also known to contribute to immune system enhancement and cellular protection. Therefore, the findings of this study not only provide additional scientific information regarding the phytochemical composition of pumpkin seeds but also highlight the potential utilization of pumpkin seed extract in the development of pharmaceutical products, herbal medicines, dietary supplements, and functional foods. The optimal utilization of pumpkin seeds could also increase the economic value of agricultural waste and support sustainable use of natural resources (Hidjrawan, 2018).

CONCLUSIONS

Based on the results of this study, it can be concluded that pumpkin seed (*Cucurbita moschata*) extract contains flavonoid compounds at a level of 9.34 mg/g and tannin compounds at a level of 9.42 mg/g, which are considered relatively high concentrations in a plant part.

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Competing Interests: The authors declare that there are no competing interests.

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