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Total Flavonoid and Tannin Content in Pumpkin Stem Extract (*Cucurbita moschata*)

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Abstract

Cucurbita moschata is a medicinal plant that possesses various pharmacological properties, including antidiabetic, antifungal, antibacterial, anti-inflammatory, and antioxidant activities, which are associated with its bioactive compound content. One plant part that has been relatively underutilized is the pumpkin stem, although it has the potential to contain secondary metabolites such as flavonoids and tannins. This study aimed to identify and determine the levels of bioactive compounds in pumpkin stem extract based on stem maturity stages, namely young stems and mature stems, using both qualitative and quantitative methods. Qualitative analysis was conducted through phytochemical screening using color reaction methods with specific reagents to detect flavonoids and tannins. Quantitative analysis was performed using UV-Vis spectrophotometry based on standard calibration curves. The results showed that pumpkin stem extract tested positive for both flavonoids and tannins. Quantitative analysis indicated that the flavonoid content in young stems was 7.22, which was higher than in mature stems (3.00). In contrast, tannin content was higher in mature stems (9.03) compared to young stems (5.02). These differences indicate that flavonoids are more dominant in young tissues with active metabolic activity, whereas tannins tend to accumulate in mature tissues as part of plant defense mechanisms. Based on these findings, it can be concluded that pumpkin stem extract contains flavonoid and tannin compounds with varying levels depending on stem maturity, suggesting its potential as a natural source of antioxidants.

Keywords: Pumpkin Stem (*Cucurbita moschata*); Flavonoids; Tannins; Quantitative Analysis; UV-Vis Spectrophotometry.

INTRODUCTION

Cucurbita moschata is a horticultural crop with high nutritional value and is easily cultivated under various environmental conditions. This plant has a wide adaptive range, enabling it to grow from lowlands to highlands and under moderate rainfall conditions (Zufahmi et al., 2015). This adaptability makes pumpkin a promising agricultural commodity for further development, both as a food source and as raw material in the health sector. In addition, the plant is relatively easy to cultivate and has high productivity, making it widely utilized by local communities. To date, the utilization of pumpkin has mainly focused on its fruit, which is used as a food source due to its high nutritional content, such as vitamins, minerals, and fiber (Arifin & Ibrahim, 2018)

Other parts of the plant, however, such as stems, leaves, and seeds, have not been optimally utilized and are often considered agricultural waste. In fact, several studies have shown that vegetative parts of plants may also contain bioactive compounds with economic and pharmacological value. Therefore, efforts are needed to explore the potential of underutilized plant parts, particularly the pumpkin stem (Mawardika et al., 2023). Physiologically, the stem plays an important role in transporting water and nutrients from the roots to the leaves, as well as distributing photosynthetic products throughout the plant. In addition, the stem also serves as a site for the synthesis of various secondary metabolites that function in plant defense mechanisms against environmental stress, such as pathogen attacks, herbivory, and abiotic stress conditions (Supriyanto et al., 2018)

The presence of these secondary metabolites makes plant stems a potential source of bioactive compounds with various biological activities. One important group of secondary metabolites is flavonoids. Flavonoids are polyphenolic compounds widely distributed in plant tissues and possess complex chemical structures. These compounds are known to exhibit various biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects (Noviyanti et al., 2020). The antioxidant activity of flavonoids plays a role in scavenging free radicals that can cause cellular damage, thereby potentially preventing various degenerative diseases. In addition, flavonoids also contribute to plant

protection against ultraviolet radiation and pathogen attacks (Astryna et al., 2024). Thus, the presence of flavonoids in a plant is an important indicator of its pharmacological potential.

In addition to flavonoids, tannins are also phenolic secondary metabolites commonly found in plants. Tannins have the ability to bind proteins, thereby functioning as a natural defense mechanism against herbivores and pathogens (Sutomo et al., 2026). Chemically, tannins are classified into two main groups, namely hydrolyzable tannins and condensed tannins, with condensed tannins being the most commonly found type in plants (Bachtiar et al., 2023). Tannins are also known to exhibit various biological activities such as antioxidant, antidiarrheal, and anti-inflammatory effects. These properties make tannins important compounds with potential applications in the pharmaceutical and health sectors. Although various studies have investigated the bioactive compound content of pumpkin, most have focused on the fruit, seeds, or leaves. Information regarding the flavonoid and tannin content in the stem of *Cucurbita moschata* remains very limited (Supriyanto et al., 2018).

Plant stems as vegetative organs have the potential to serve as sources of valuable bioactive compounds, particularly when evaluated based on tissue maturity levels, such as young and mature stems. Differences in maturity levels may influence the content of secondary metabolites produced, as plant metabolic activity tends to vary across different growth stages. Therefore, research on the identification and quantification of flavonoids and tannins in pumpkin stems is important to be conducted. The results of this study are expected to provide scientific information regarding the potential of pumpkin stems as a source of bioactive compounds, as well as serve as a basis for the development of agricultural waste utilization into value-added products, particularly in the field of phytopharmaceuticals and health (Hidayah, 2016). This study aimed to analyze the flavonoid and tannin content both qualitatively and quantitatively in pumpkin stem extract using the UV-Vis spectrophotometry method.

MATERIALS AND METHODS

Study Area

This study was conducted at the Chemistry Laboratory, Faculty of Teacher Training and Education (FKIP), Universitas Tadulako, from February 2026 until completion. The samples used were pumpkin stems (*Cucurbita moschata*) collected from North Laemanta Village, Kasimbar District, and subsequently transported to the Chemistry Education Laboratory for analysis of their bioactive compound content.

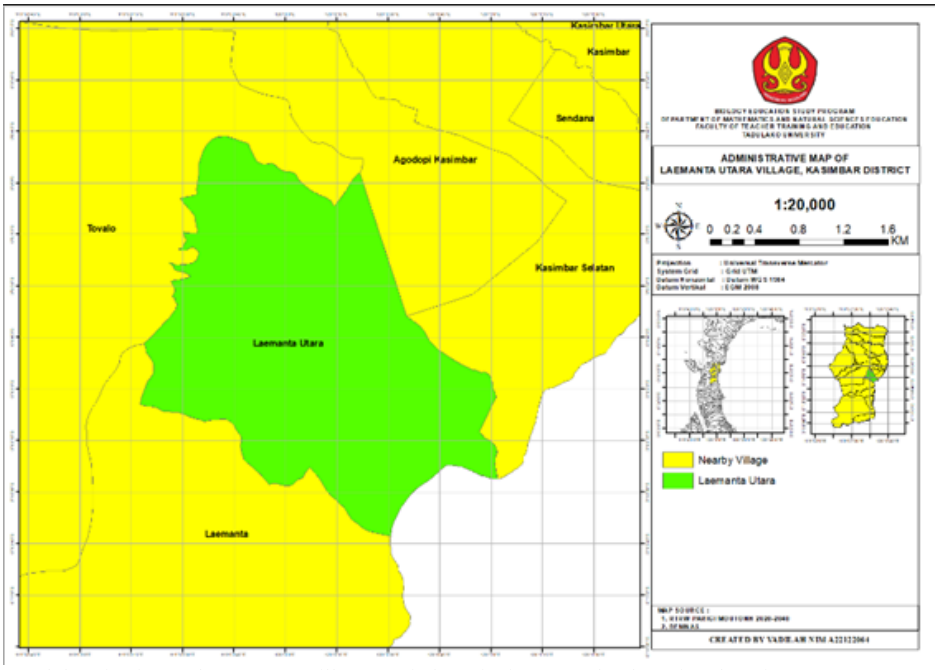

Figure 1. Map of the research location in North Laemanta Village, Kasimbar District, Central Sulawesi, Indonesia.

Procedures

Sample Preparation

Sample preparation began with separating pumpkin stems from leaves, followed by washing thoroughly, air-drying, and then grinding and sieving to obtain a fine powder. Extraction was carried out by macerating 500 g of powdered sample in 1500 mL of 30% ethanol for 3 × 24 hours. The mixture was then filtered to separate the filtrate and residue. The maceration process was repeated twice, and the combined filtrates were evaporated to

obtain a thick extract. In a separate procedure, 25 g of powdered pumpkin stem was soaked in 250 mL of 30% ethanol for 3 × 24 hours and then filtered. The residue was macerated twice to maximize extraction yield. The combined filtrates were concentrated using a rotary vacuum evaporator to obtain a crude extract of pumpkin stem (Herman et al., 2025).

Qualitative Analysis

Qualitative analysis was conducted using phytochemical screening to detect secondary metabolites, specifically flavonoids and tannins, in pumpkin stem extract. This method is based on specific color changes after the addition of certain reagents, providing a preliminary indication of the presence of target compounds. Flavonoid testing was performed using sodium hydroxide (NaOH). Approximately 2 mL of extract was placed in a test tube, followed by several drops of NaOH. The mixture was shaken until homogeneous and allowed to stand for a few moments. A yellow coloration indicated a positive result for flavonoids (Ipandi et al., 2016). This color change occurs due to the reaction between flavonoid phenolic groups and strong bases. A confirmatory test (Shinoda test) was also performed by adding a small amount of magnesium powder and 3–4 drops of concentrated hydrochloric acid (HCl) to 2 mL of extract. The mixture was gently shaken, and the formation of red, orange, or yellow coloration indicated the presence of flavonoids due to the reduction of carbonyl groups in the flavonoid structure.

Tannin analysis was performed using 1% $FeCl_3$ reagent. Approximately 2 mL of extract was heated for about 5 minutes to accelerate the reaction, followed by the addition of a few drops of $FeCl_3$ solution. The formation of a greenish-brown color or brown precipitate indicated the presence of tannins due to complex formation between Fe^{3+} ions and phenolic groups in tannins.

Quantitative Analysis

Quantitative analysis of flavonoid and tannin contents was performed using UV-Vis spectrophotometry. A quercetin standard solution was prepared by dissolving 10 mg of quercetin in a 10 mL volumetric flask with ethanol to obtain a 100 ppm stock solution. A series of standard solutions (5, 10, 15, 20, and 25 mg/L) were prepared. From each standard solution, 1 mL was pipetted and mixed with 1.5 mL of 70% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated for 30 minutes. Absorbance was measured at the maximum wavelength obtained using a UV-Vis spectrophotometer, and a calibration curve was constructed. For sample analysis, 10 mg of pumpkin stem extract was dissolved in 10 mL ethanol. Then, 1 mL of this solution was treated in the same way as the standards and incubated for 30 minutes. Absorbance was measured at 445 nm using a UV-Vis spectrophotometer.

Tannin analysis began with the preparation of a tannic acid stock solution by dissolving 10 mg of tannic acid in 10 mL ethanol to obtain a 100 ppm solution. From this stock, serial dilutions (10, 20, 30, 40, and 50 ppm) were prepared. From each concentration, 1 mL was pipetted, then mixed with 0.5 mL Folin–Denis reagent and allowed to stand for 8 minutes. Subsequently, 2 mL of 20% Na_2CO_3 solution was added, vortexed for 10 minutes, and incubated at room temperature for 2 hours. Absorbance was measured at 742.5 nm using a UV-Vis spectrophotometer (Rahmawati, 2020). For sample analysis, 10 mg of extract was dissolved in 10 mL ethanol. Then, 1 mL of the solution was mixed with 0.5 mL Folin–Denis reagent, incubated for 8 minutes, followed by addition of 2 mL of 20% Na_2CO_3. The mixture was incubated for 2 hours at room temperature, and absorbance was measured at 742.5 nm using a UV-Vis spectrophotometer (Utami et al., 2025).

Data 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 performed using NaOH reagent, concentrated HCl in combination with magnesium powder (Shinoda test), while tannin testing was conducted using 1% $FeCl_3$ reagent.

Quantitative Analysis

Quantitative analysis to determine the levels of flavonoids and tannins in pumpkin seed extract was conducted using UV-Vis spectrophotometry. The results of the analysis are presented as follows:

$$\text{Compound content} = \frac{C \times V \times FP}{m}$$

$$Average\ compound\ content\ = \frac{C(1) + C2}{2}$$

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

RESULTS AND DISCUSSION

Determination of Flavonoid Content in Young Pumpkin Stem Extract

Based on the results of flavonoid content analysis in young pumpkin stems using UV-Vis spectrophotometry conducted in triplicate, different absorbance values were obtained for each measurement. In the first trial, an absorbance value of 0.564 was recorded, while the

second and third trials yielded absorbance values of 0.542 and 0.537, respectively. The corresponding sample concentrations (ppm) obtained were 7.35, 7.21, and 7.09 (Table 1).

Table 1. Determination of flavonoid content in young pumpkin stem extract.

Sample	Absonance	Concentration (Cq) (mg/L)	Extract Volume (v) (L)	Extract Weight (m) (g)	Dilution Factor (FP)	Total Flavonoids	Average
BLK 1	0.564	8.8215314	0.01	0.3	25	7.35	7.22
BLK 2	0.542	8.6557273	0.01	0.3	25	7.21	
BLK 3	0.573	8.5144127	0.01	0.3	25	7.09	

Determination of Flavonoid Content in Mature Pumpkin Stem Extract

Based on the results of flavonoid content analysis in mature pumpkin stems using UV-Vis spectrophotometry conducted in triplicate, different absorbance values were obtained for each measurement. In the first trial, an absorbance value of 0.264 was recorded, while the second and third trials yielded absorbance values of 0.272 and 0.303, respectively. The corresponding sample concentrations (ppm) obtained were 2.96, 3.01, and 3.05 (Table 2).

Table 2. Determination of flavonoid content in mature pumpkin stem extract.

Sample	Absonance	Concentration (Cq) (mg/L)	Extract Volume (v) (L)	Extract Weight (m) (g)	Dilution Factor (FP)	Total Flavonoids	Average
BLK 1	0.264	3.5507132	0.01	0.3	25	2.96	3.00
BLK 2	0.272	3.6075051	0.01	0.3	25	3.01	
BLK 3	0.303	3.6561349	0.01	0.3	25	3.05	

Determination of Tannin Content in Young Pumpkin Stem Extract

Based on the results of tannin content analysis in young pumpkin stems using UV-Vis spectrophotometry conducted in triplicate, different absorbance values were obtained for each measurement. In the first trial, an absorbance value of 0.056 was recorded, while the second and third trials yielded absorbance values of 0.054 and 0.057, respectively. The corresponding sample concentrations (ppm) obtained were 5.04, 4.93, and 5.08 (Table 3).

Table 3. Determination of tannin content in young pumpkin stem extract.

Sample	Absonance	Concentration (Cq) (mg/L)	Extract Volume (v) (L)	Extract Weight (m) (g)	Dilution Factor (FP)	Total Flavonoids	Average
BLK 1	0.056	6.0533333	0.01	0.3	25	5.04	5.02
BLK 2	0.054	5.9244449	0.01	0.3	25	4.93	
BLK 3	0.057	6.1088888	0.01	0.3	25	5.08	

Determination of Tannin Content in Mature Pumpkin Stem Extract

Based on the results of tannin content analysis in mature pumpkin stems using UV-Vis spectrophotometry conducted in triplicate, different absorbance values were obtained for each measurement. In the first trial, an absorbance value of 0.091 was recorded, while the second and third trials yielded absorbance values of 0.089 and 0.093, respectively. The corresponding sample concentrations (ppm) obtained were 9.05, 8.94, and 9.11 (Table 4).

Table 4. Determination of tannin content in mature pumpkin stem extract.

Sample	Absonance	Concentration (Cq) (mg/L)	Extract Volume (v) (L)	Extract Weight (m) (g)	Dilution Factor (FP)	Total Flavonoids	Average
BLK 1	0.091	10.8555555	0.01	0,3	25	9.05	9.03
BLK 2	0.089	10.7266666	0.01	0,3	25	8.94	
BLK 3	0.093	10.9344444	0.01	0,3	25	9.11	

Phytochemical Screening Results for Flavonoid Test in Pumpkin Stem

The flavonoid test on young pumpkin stems was conducted in two replications. Flavonoid detection was performed using NaOH reagent as well as HCl combined with magnesium powder. In both sample types (young and mature stems), a yellow coloration was observed after the addition of the reagents. This yellow color change indicates a positive result (+) for the presence of flavonoid compounds (Table 5).

Table 5. Phytochemical screening results for flavonoid test in pumpkin stem.

Sample	Reagent	Result	Conclusion
BLK.1	NaOH	Yellow	+
BLK.2	HCL and Magnesium	Yellow	+

Phytochemical Screening Results for Tannin Test in Pumpkin Stem

The tannin test was performed once using 1% $FeCl_3$ reagent. In both samples (young and mature stems), a brown coloration was observed after the addition of the reagent. This color change indicates a positive result (+) for the presence of tannins. The reaction between $FeCl_3$ and phenolic compounds such as tannins forms a brown to dark complex, which serves as an indicator of tannin presence in the samples (Tables 6).

Table 6. Phytochemical screening results for tannin test in pumpkin stem.

Sample	Reagent	Result	Conclusion
BLK.1	FeCl3 1%	Brown	+
BLK.2	FeCl3 1%	Brown	+

Discussion

Cucurbita moschata is a horticultural plant species belonging to the family Cucurbitaceae, widely known as pumpkin. This plant is an annual creeping herb with a soft stem covered with fine hairs and tendrils that function as climbing organs. Morphologically, it has broad leaves with a rough surface, bright yellow flowers, and fruits rich in nutrients such as carbohydrates, vitamin A, and fiber (Zufahmi et al., 2015). However, its utilization is still predominantly focused on the fruit, while the stem remains underutilized and is often considered agricultural waste, despite its potential to contain secondary metabolites such as flavonoids and tannins. *Cucurbita moschata* has broad adaptive capabilities and the potential to produce various bioactive compounds throughout its plant parts, including the stem (Raharjo et al., 2023). Flavonoids and tannins are phenolic compounds that play important roles in plant defense systems and exhibit strong antioxidant activity. Therefore, the identification of these compounds in pumpkin stems is important to evaluate their potential as natural bioactive sources (Arifin & Ibrahim, 2018).

This study was conducted through several stages, including sample preparation, extraction, qualitative analysis, and quantitative analysis. The pumpkin stem samples were first cleaned, dried, and ground into powdered simplicia. Extraction was then carried out using the maceration method with 30% ethanol for 3 × 24 hours. Ethanol was selected as the solvent due to its ability to dissolve polar compounds such as flavonoids and tannins. The resulting filtrate was then concentrated using a rotary evaporator to obtain a thick extract for further analysis. Based on the qualitative test results, the pumpkin stem extract showed positive results for flavonoids and tannins. In the flavonoid test, the addition of NaOH reagent produced a yellow color change, while the addition of HCl resulted in a pink coloration. These color changes indicate the presence of flavonoids reacting with acidic and basic reagents through their phenolic groups (Ipandi et al., 2016). Meanwhile, in the tannin test, the addition of gelatin produced a precipitate, and the addition of $FeCl_3$ reagent resulted in a brown coloration, indicating the presence of tannins due to their ability to form complexes with proteins and metal ions (Hidayah, 2016).

The results indicate that both young and mature pumpkin stems contain flavonoids and tannins. However, differences in color intensity observed in the qualitative tests suggest variations in the concentration of active compounds between the two samples. This finding is further supported by quantitative analysis using UV-Vis spectrophotometry. The quantitative results showed that flavonoid content in young stems was higher than in mature stems. The flavonoid content in young stems was 7.22, whereas in mature stems it was 3.00. The higher flavonoid content in young stems is associated with more active secondary metabolic processes in developing tissues. At this stage, plants tend to produce protective compounds such as flavonoids to protect tissues from environmental stress, including ultraviolet radiation and pathogen attacks.

Tannin content exhibited a different pattern, with higher levels observed in mature stems compared to

young stems. The tannin content in mature stems was 9.03, while in young stems it was 5.02. This indicates that tannin accumulation increases with plant tissue age. Tannins function as defensive compounds capable of binding proteins and inhibiting microbial growth; thus, they tend to accumulate in older tissues as an adaptive response to environmental conditions. In addition, the increase in tannin content is also related to the accumulation of secondary metabolites during plant development. Flavonoid quantification was performed at the maximum wavelength using quercetin as a standard through complex formation with $AlCl_3$, whereas tannin quantification was conducted using tannic acid as a standard through complex formation with specific reagents. The absorbance values obtained were then used to calculate compound concentrations based on linear regression equations. A higher absorbance value indicates a higher concentration of compounds present in the sample (Noviyanti et al., 2020).

CONCLUSIONS

Pumpkin stem (*Cucurbita moschata*) extract contains bioactive compounds, namely flavonoids and tannins, based on both qualitative and quantitative analyses. Flavonoid content was higher in young stems, whereas tannin content was higher in mature stems. Stem maturity influences secondary metabolite composition, where flavonoids are more dominant in young tissues with active metabolic processes, while tannins tend to accumulate in older tissues as a protective mechanism.

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

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