JCNaR
Journal of Chemical Natural Resources
Journal homepage: https://jcnar.usu.ac.id

Screening of Phytochemicals and Determination of Total Phenolic and Flavonoid Contents in Different Plant Parts of *Kalanchoe ceratophylla* Haw.

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ARTICLE INFO
Article history:
Received 30 September 2025
Revised 20 November 2025
Accepted 24 November 2025
Available online 30 November 2025

E-ISSN: 2656-1492

How to cite:
Alyssa Zahra Br Karo, Sovia Lenny, Lamhot Parulian Manalu. Screening of Phytochemical and Determination of Total Phenolic and Flavonoid Contens in Different Plant Parts of *Kalanchoe ceratophylla* Haw. Journal of Chemical Natural Resources. 2025, 7(2):87-95.

https://doi.org/10.32734/jcnar.v7i2.22964

ABSTRACT
Kalanchoe ceratophylla Haw. is a medicinal plant traditionally used to address various health disorders. Its biological activities are believed to be associated with its secondary metabolites, particularly phenolics and flavonoids. Despite its traditional use, scientific information This study assesses the phytochemical composition alongside the total phenolic and flavonoid contents in the stem and leaf preparations of *Kalanchoe ceratophylla* Haw. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, and phenols in the stem extract, while the leaf extract also contained steroids. The total phenolic content was 7.43 mg GAE/100 g extract for stem and 7.11 mg GAE/100 g extract for leaves, whereas total flavonoids amounted to 0.324 mg QE/g extract (stem) and 1.621 mg QE/g extract (leaves). These findings highlight the potential use of *K. ceratophylla*, especially its leaves, for developing antioxidant-based herbal medicines.

Keyword: *Kalanchoe ceratophylla* Haw., Total Phenolic Content, Total Flavonoid Content, UV-Vis Spectrophotometry, *Folin-ciocalteu.*

ABSTRAK
Kalanchoe ceratophylla Haw. merupakan tanaman obat yang telah lama digunakan untuk mengatasi berbagai masalah kesehatan. Aktivitas biologisnya berkaitan erat dengan kandungan metabolit sekunder, khususnya fenolik dan flavonoid. Meskipun demikian, informasi ilmiah mengenai perbedaan kandungan senyawa pada batang dan daun masih terbatas. Penelitian ini bertujuan untuk mengevaluasi profil fitokimia serta kadar total fenolik dan flavonoid pada ekstrak batang dan daun *K. ceratophylla*. Hasil skrining fitokimia menunjukkan bahwa ekstrak batang mengandung alkaloid, flavonoid, tanin, dan fenol, sementara ekstrak daun juga mengandung steroid. Kandungan fenolik total pada batang dan daun masing-masing sebesar 7,43 mg GAE/100 g dan 7,11 mg GAE/100 g ekstrak, sedangkan kandungan flavonoid total yaitu 0,324 mg QE/g ekstrak pada batang dan 1,621 mg QE/g ekstrak pada daun. Temuan ini menunjukkan bahwa daun *K. ceratophylla* Haw berpotensi lebih besar sebagai sumber antioksidan alami dalam pengembangan obat herbal.

Kata Kunci: *Kalanchoe ceratophylla Haw.,* Kadar Fenolik Total, Kadar Flanonoid Total, *UV-Vis Spectrophotometry, Folin-ciocalteu.*

1. Introduction

Medicinal plants have historically provided essential therapeutic agents in both contemporary and traditional medicine, due to their varied secondary metabolites and extensive biological activity. The secondary metabolites are identified, including flavonoid and phenolic compounds that have potential in antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer properties [1][2]. In this study, the *Kalanchoe* genus; comprising approximately 35 genera and 1410 species, has been used in traditional medicine across different cultures due to its pharmacologically active constituents [3]

Phenolic compounds are structurally diverse plant metabolites that play critical roles in defense mechanisms against environmental stresses, including pathogens, UV radiation, and drought [4]. They also function as hydrogen peroxide scavengers and are among the most abundant metabolites in higher plants, ranging from simple phenols to complex polymers like lignin. Beyond their roles in plants, many phenolic compounds exhibit antiviral, immunomodulatory, and anti-inflammatory activities [5]. Flavonoids, a major group within the phenolic class found abundantly in fruits, vegetables, teas, and medicinal herbs [6], demonstrate potent antioxidant properties by neutralising free radicals and safeguarding cells against oxidative harm and ageing [7]. Futhermore, these compounds are known to have strong antioxidant activity, thereby protecting the human body from chronic diseases [8].The chemicals have proven to have anti-inflammatory [9], anticancer [10], and cardioprotective effects [11], making them promising for therapeutic and preventive applications.

Previous studies on several *Kalanchoe* species, including *K. pinnata* and *K. tomentosa*, have demonstrated notable levels of phenolic and flavonoid compounds with high biological activity. It has also been reported that extraction methods and plant parts influence both total phenolic content (TPC) and total flavonoid content (TFC), alongside the resulting bioactivity [12] [13]. Despite this growing interest, *Kalanchoe ceratophylla* Haw. remains relatively underexplored compared to other species within the genus. Specifically, there is a lack of comparative data on the distribution of secondary metabolites between different plant parts, particularly using aqueous extraction methods that are both environmentally friendly and aligned with traditional preparation practices [2].

This study addresses this research gap by conducting a phytochemical screening and quantifying TPC and TFC in aqueous extracts of the leaves and stems of *K. ceratophylla*. Unlike earlier studies that focused on single plant parts or used organic solvents, this work provides a direct comparison of bioactive content between leaves and stems using water as the extraction medium. While the study does not claim absolute novelty in concept, it offers essential empirical evidence that contributes to a more comprehensive understanding of the phytochemical distribution within *K. ceratophylla*. This insight not only enhances the scientific foundation for its medicinal use but also supports future applications in pharmaceutical and nutraceutical formulations.

2. Material and Method

2.1. Equipments

The instruments used in this research were UV-Vis spectrophotometer (Shimadzu UV mini-1240), UV lamp ELISA reader (BioTek Synergy HTX Multi-Mode).

2.2. Materials

Fresh stems and leaves of *Kalanchoe ceratophylla* Haw. were collected in Depok District, West Java, Indonesia, at four months after planting. The plant specimens were taxonomically verified at the Herbarium Bandungense (FIPIA), Bandung, Indonesia. Chemicals used included methanol (99.8%), hydrochloric acid (HCl 1 M and 37%), ethyl acetate, sulfuric acid (H_2SO_4), n-hexane, magnesium powder, ferric chloride ($FeCl_3$ 5%), Mayer's reagent, Dragendorff's reagent, distilled water, and quercetin and gallic acid standards (Merck).

2.3. Preparation and Extraction Procedure

The collected plant samples were gently rinsed with flowing water to eliminate soil residues and subsequently dried with sterile tissue paper. The stems and leaves were separated, chopped, and homogenized using a blender. Extraction was performed by maceration using distilled water at a plant-to-solvent ratio of 1:5 (w/v) for 24 hs at room temperature with constant stirring at 200 rpm. The obtained extract was filtered using a muslin cloth, and the filtrate was evaporated using a blower oven to remove water. Next, 50 mg of the dried extract from both plant parts was dissolved in 1 mL of methanol (99.8%) to prepare stock solutions, which were stored at −20°C for further determination.

2.4. Preparation and Extraction Procedure

The presence of various classes of secondary metabolites was evaluated using standard qualitative methods:

- **Alkaloids:**
  0.5 g of extract was dissolved in 5 mL of HCl 2N and heated in a water bath. After cooling and filtration, Mayer's and Dragendorff's reagents were added. A white precipitate (Mayer's) or orange-red coloration (Dragendorff's) indicated a positive result.

- **Flavonoids:**
  0.5 g of extract was dissolved in 2 mL of ethanol 70%, and little quantity of magnesium powder and three drops of strong hydrochloric acid were introduced. The emergence of orange to red hues signified the presence of flavonoids.
- **Steroids:**
  0.5 g of extract was mixed with chloroform and then combined with 10 drops of acetic anhydride and 3 drops of concentrated H_2SO_4. A color change to blue-green indicated steroids.
- **Tannins:**
  0.5 g of extract was dissolved in distilled water and reacted with 3 drops of $FeCl_3$. The appearance of a blue-black or greenish-blue color indicated tannins.
- **Phenolics:**
  Extracts (1 mL) were reacted with 2-3 drops of 5% $FeCl_3$. A dark blue or green color indicated phenolic compounds.

Negative controls for each test consisted of solvent without plant extract, while positive controls used standards for each metabolite class**.**

2.5. Determination of Total Phenolic Contents (TPC)

The TPC of *Kalanchoe ceratophylla* Haw. extract was assessed using the Folin–Ciocalteu reagent with gallic acid as the standard. In summary, 0.5 mL of extract or standard was combined with Folin–Ciocalteu reagent and sodium carbonate, incubated for 30 minutes at room temperature, and absorbance was recorded at 734 nm using a spectrophotometer. A standard calibration curve of gallic acid (10–200 ppm) was used to calculate total phenolic content, expressed as mg gallic acid equivalents (GAE) per gram of extract. All measurements were conducted in triplicate (n = 4).

2.6. Determination of Total Flavonoid Contents (TFC)

The TPC was assessed utilising the aluminium chloride colorimetric technique, employing quercetin as the standard reference. In brief, 1 mL of extract or quercetin standard was combined with 1 mL of $AlCl_3$ 2% and 1 mL of potassium acetate 120 mM, incubated for 30 minutes, and the absorbance was recorded at 400–450 nm using an ELISA reader. A quercetin calibration curve (2–12 ppm) was employed to assess the flavonoid content, reported as mg quercetin equivalent per gram of extract (mg QE/g). All analyses were conducted in triplicate (n = 4)**.**

2.6. Statistical Analysis

All the obtained data were analysed using one-way ANOVA to evaluate treatment effects. Mean separations were carried out utilising the least significant difference test at a 95% confidence level ($p < 0.05$). The findings are displayed as mean ± standard deviation (SD) from three independent replicates (n = 4).

3. Result and Discussion

3.1. Extraction

The extraction yield of *Kalanchoe ceratophylla* varied slightly between the leaf and stem samples, with the leaf extract producing a higher yield (2.79%) than the stem extract (2.40%) using aqueous maceration (Table 1). This difference may reflect the higher concentration of water-soluble secondary metabolites in the leaves, which are known to accumulate greater levels of phenolics, flavonoids, and other hydrophilic compounds compared to structural tissues like stems. Similar trends have been reported in other medicinal plants, where leaves consistently show greater extractive capacity due to their higher metabolic activity and surface area, as demonstrated in *Cassia alata* and *Olea europaea* [14] Given that extraction yield is strongly influenced by solvent polarity, tissue composition, and metabolite distribution, the present findings suggest that the leaves of *K. ceratophylla* may be a more efficient target for extracting bioactive, water-soluble compounds, aligning with previous studies emphasizing the influence of plant part selection on extract composition and extraction efficiency [15][16].

Table 1. Yield of *Kalanchoe ceratophylla* Haw. extract.

Plant Part	Colour	Sample Weight (g)	% Yield of Extract
Stem	yellowish-green	24.04	2.40
Leaf	yellowish-green	27.97	2.79

3.2. Phytochemical Screening Test

The phytochemical screening (Table 2) revealed that both the stem and leaf extracts of *Kalanchoe ceratophylla* Haw. contained phenolics, flavonoids, tannins, and alkaloids, although their distribution differed slightly between plant parts. Alkaloids were detected only in the stem, while steroids were identified exclusively in the leaf extract, indicating a tissue-specific allocation of certain metabolite classes. The universal presence of phenolics and tannins in both samples supports the established role of these compounds in plant defense and oxidative stress regulation. These results are consistent with findings from recent studies, where metabolites such as phenolics, flavonoids, and tannins were reported to vary across roots, stems, and leaves depending on their physiological function, extraction method, and solvent choice [17] [18] [15]The observed variation between leaf and stem metabolite profiles aligns with the broader understanding that secondary metabolite biosynthesis is influenced by organ specialization and environmental adaptation [16]. Therefore, the presence of diverse phytochemicals in both extracts highlights the pharmacological potential of *K. ceratophylla* and supports further quantitative and biological evaluation of its different plant parts.

Table 2. Identification of secondary metabolite compounds results

Secondary Metabolites	Sample Section	Indicator	Result
Alkaloid	Stem	Mayer	+
		Dragendorff	+
	Leaf	Mayer	-
		Dragendorff	-
Flavonoid	Stem	Mg- HCl	+
	Leaf	Mg- HCl	+
Steroid	Stem	$(CH_3CO)_2O + H_2SO_4$	-
	Leaf	$(CH_3CO)_2O + H_2SO_4$	+
Tannin	Stem	$FeCl_3$	+
	Leaf	$FeCl_3$	+
Phenolic	Stem	$FeCl_3$	+
	Leaf	$FeCl_3$	+

Description:
(+) = indicates presence of compound
(-) = indicates absence of compound

3.3. *Determination of Total Phenolic Contents (TPC)*

The total phenolic contents of the purified extract of *Kalanchoe ceratophylla* Haw. was analyzed using the Folin-Ciocalteu technique, and the standard used was gallic acid [19]. The Folin-Ciocalteu method is the most prevalent technique employed in laboratories globally for quantifying total polyphenol content due to its efficiency and cost-effectiveness [20]. In this study, the Folin–Ciocalteu (F–C) assay was applied to determine the total phenolic content (TPC) of *Kalanchoe ceratophylla* extracts. This method is based on an electron-transfer reaction in which phenolic compounds act as reducing agents in alkaline conditions, donating electrons to the phosphomolybdic–phosphotungstic complexes present in the F–C reagent. This reduction converts Mo(VI) and W(VI) to their lower oxidation states, resulting in the formation of a blue-colored complex, the intensity of which is proportional to the total reducing capacity of the sample. While originally developed as a "total phenolic" assay, recent studies have highlighted that the F–C reagent also reacts with non-phenolic reducing substances [21]. Consequently, the values obtained reflect the overall reducing potential rather than absolute phenolic concentrations. Nonetheless, when consistently applied across samples, the assay remains a reliable approach for comparing phenolic richness between different plant parts or treatments, as demonstrated in this work. Similar observations have been made by [22], who also emphasized that while the details of the reaction mechanism are not entirely understood, the reduction of molybdenum (Mo^{6+}) to Mo^{5+} and the formation of phenolate radicals represent key steps in the colorimetric response.

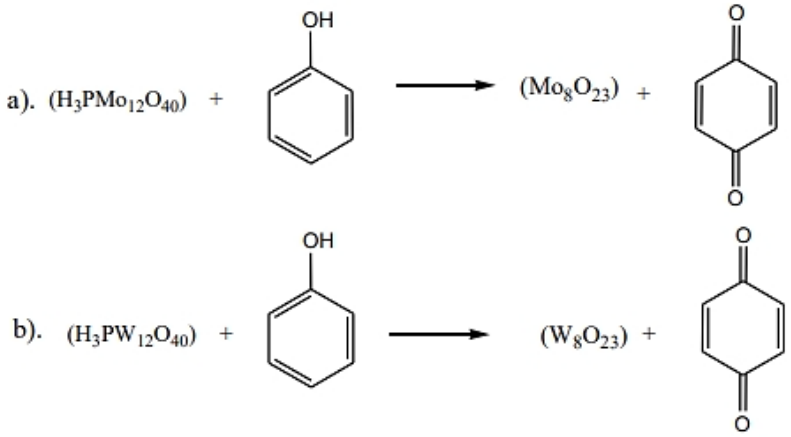

Figure 1. The interaction of the *Folin-Ciocalteu* reagent with phenolic compounds can be delineated as follows: a). the reaction of phosphomolybdic acid with phenolic compounds; b). the reaction of phosphotungstic acid with phenolic compounds.

The total phenolic content (TPC) in *Kalanchoe ceratophylla* was quantified using gallic acid as a standard, with concentrations ranging from 10 to 160 ppm. Gallic acid was chosen due to its wide distribution in plants and its stable three-hydroxyl structure, making it a suitable reference for phenolic quantification. The findings regarding the absorbance, as depicted in the standard curve of gallic acid, are illustrated in Figure 2.

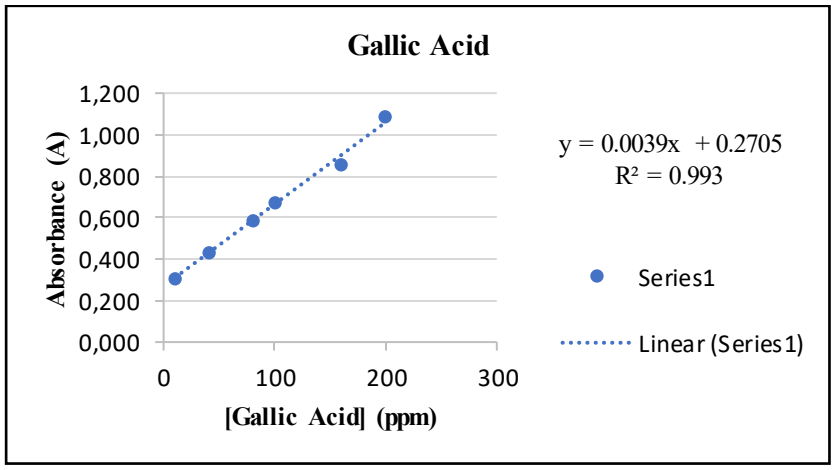

Figure 2. Gallic acid standard solution curve

The calibration curve displayed strong linearity with an R^2 value of 0.993, indicating a reliable correlation between absorbance and phenolic concentration. Based on this calibration, the TPC of *K. ceratophylla* extracts was determined to range from 7.11 mg GAE/100 g in leaves to 7.43 mg GAE/100 g in stems, following four repetitions for each sample to ensure data reliability. These results confirm that both leaves and stems contain appreciable amounts of phenolic compounds, which are recognized as key bioactive metabolites contributing to antioxidant capacity and potential therapeutic effects. The absorbance measurements can be seen in Table 3.

The observed differences between plant organs may reflect variations in biosynthesis and accumulation of phenolics, as leaves are photosynthetically active tissues often exposed to environmental stressors, while stems may accumulate slightly higher phenolics due to structural or metabolic factors. Furthermore, the TPC levels in *K. ceratophylla* are consistent with reports in other *Kalanchoe* species, such as *K. pinnata*, which exhibit substantial phenolic contents associated with antioxidant and cytotoxic activities. The findings underscore the role of phenolic compounds in mediating the biological activities of *Kalanchoe* species, supporting their traditional medicinal use and highlighting the importance of organ-specific analyses for accurate phytochemical assessment [13].

Table 3. Determination of total phenolic content of sample

Plant Part	Replication	Absorbance	Absorbance Average	Total Phenolic Content (mgGAE/100 g extract)
Stem	1	0.415	0.5995	7.43
	2	0.925		
	3	0.463		
	4	0.595		
Leaf	1	0.481	0.5665	7.11
	2	0.670		
	3	0.572		
	4	0.543		

3.4. *Determination of Total Flavonoid Contents (TFC)*

The aluminium chloride assay, which serves as a prominent illustration within the realm of spectrophotometric analyses, is predicated upon the interaction between said compound and flavonoids, typically occurring at an acidic pH. The foundational methodology was pioneered by Christ and Müller in 1960 and was suggested for the quantitative evaluation of flavonoid derivatives [23]. The total flavonoid content in *Kalanchoe ceratophylla* extracts was determined using the aluminium chloride ($AlCl_3$) colorimetric assay (Figure 3), which relies on the formation of stable complexes between flavonoids and Al^{3+} ions through interactions with functional groups such as the 3-hydroxyl and 4-keto group on the C-ring or ortho-dihydroxyl structures on the B-ring, as found in quercetin. This assay generates a bathochromic shift detectable via spectrophotometry and is particularly effective for estimating flavonoid concentrations in plant extracts. The higher TFC observed in the leaf extract compared to the stem likely reflects tissue-specific biosynthesis, as leaves are more exposed to environmental stressors and typically accumulate more phenolic compounds for protection. This analytical approach is consistent with recent studies on flavonoid metal chelation mechanisms, such as those described by [24], and provides reliable data for comparative phytochemical evaluation.

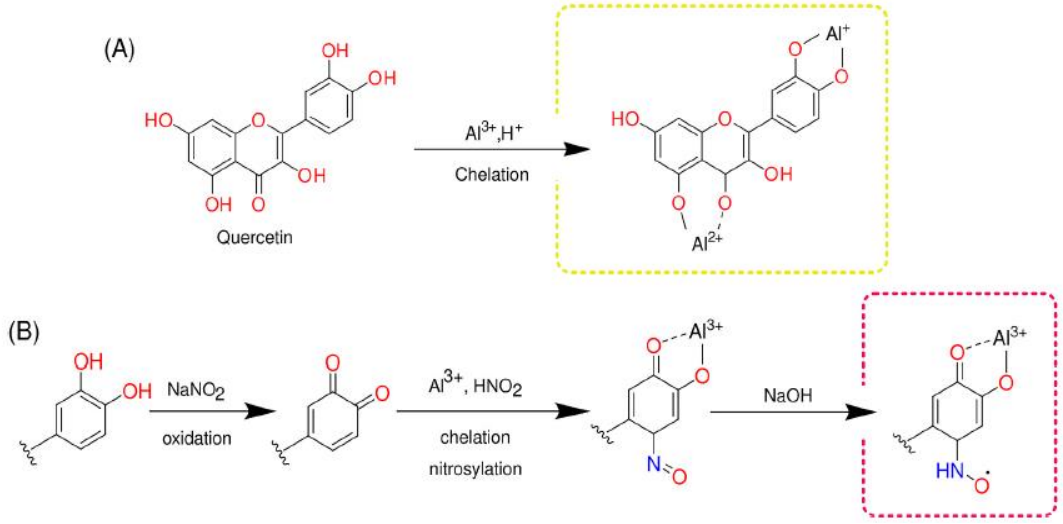

Figure 3. The complexation reaction of $AlCl_3$ and the flavonoid backbone

The quantification of total flavonoid content (TFC) in this study was conducted using quercetin as the standard, with absorbance measurements recorded at 440 nm. The calibration curve generated from quercetin standards showed a high degree of linearity, as reflected by the regression equation $y = 0.0105x + 0.0298$ and a correlation coefficient of $R^2 = 0.9958$., as shown in Figure 4. A correlation coefficient value close to 1 indicates that the regression relationship is linear. This regression equation was subsequently used to calculate TFC values for the stem and leaf extracts.

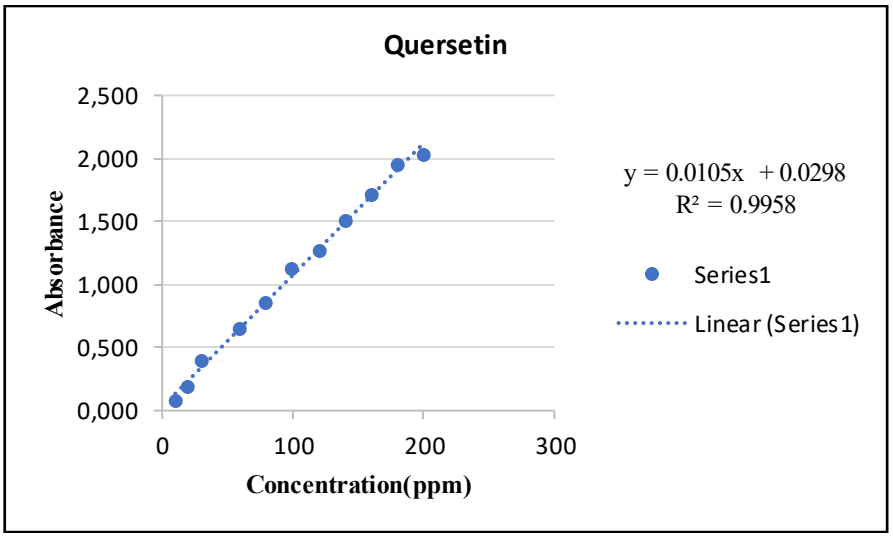

Figure 4. Quercetin standard solution curve

The results indicated that the leaf extract contained a higher concentration of flavonoids (1.62 mg QE/g) compared to the stem extract (0.32 mg QE/g), suggesting a richer accumulation of flavonoid metabolites in the leaf tissue. This trend aligns with findings reported by [13], where the leaf extract of *Kalanchoe ceratophylla* exhibited higher TFC values than the stem across both hot air drying (10.04 µg QE/g vs. 2.47 µg QE/g) and freeze-drying methods (12.27 µg QE/g vs. 3.49 µg QE/g). The consistently greater TFC in leaf tissues likely reflects inherent physiological and biochemical differences between plant organs, as leaves are the primary site of photosynthesis and synthesize larger quantities of flavonoids involved in photoprotection and oxidative stress responses.

Comparable trends were also observed in *Kalanchoe tomentosa*, where the methanol extract yielded a notably high TFC value of 72.46 mg QE/g, and contained quercetin and kaempferol based glycosides, both recognized for their antioxidant relevance. The extract demonstrated moderate to high activity in DPPH, ABTS, and H_2O_2 assays (50-300 µg/mL), and in silico analysis indicated possible interactions of these flavonoids with NAM/NAG peptide pathways important from gram positive bacteria [12]. These findings support our observation that leaf tissues generally accumulate more flavonoids than stems across *Kalanchoe* species, suggesting that the higher TFC in our leaf samples aligns with established phytochemical patterns and may contribute to their stronger antioxidant-associated properties.

Table 4. Determination of Total Flavonoid Content of sample

Sample Section	Replication	Absorbance	Absorbance Average	Total Flavonoid Content (mg QE/g)
Stem	1	0.158	0.169	0.32
	2	0.173		
	3	0.165		
	4	0.180		
Leaf	1	0.751	0.790	1.62
	2	0.772		
	3	0.836		
	4	0.801		

4. Conclusion

This study demonstrated that the aqueous extracts of both stem and leaf parts of *Kalanchoe ceratophylla* Haw. contain notable levels of water-soluble secondary metabolites, with the leaf yielding slightly more extract (2.79%) than the stem (2.40%). Phytochemical screening revealed that both parts are rich in flavonoids, phenolics, and tannins, although there were distinct differences in the presence of specific metabolite groups. Steroids were only detected in the leaves, while alkaloids were exclusive to the stem extract. Quantitative analysis further confirmed that the stem contained a marginally higher total phenolic content (7.43 mg GAE/100 g), whereas the leaf had a significantly greater flavonoid level (1.62 mg QE/g). By offering a clear comparison of the phytochemical profiles of the stem and leaf, this work fills an important gap in the existing

literature on *K. ceratophylla*. The findings highlight the plant's potential as a natural source of bioactive compounds, supporting its relevance in the development of plant-based therapeutic and nutraceutical agents. Future investigations are needed to build on these preliminary results, particularly through systematic evaluation of biological activities, isolation of key active constituents, and elucidation of their mechanisms of action. Further studies focusing on extract standardization, safety assessment, and synergy with conventional drugs would also strengthen the foundation for considering *K. ceratophylla* as a promising natural resource for biomedical applications.

5. Acknowledgements

We would like to express our gratitude to the National Research and Innovation Agency (BRIN) and the Postgraduate School of Chemistry, University of North Sumatra for their support in conducting this research.

6. Conflict of Interest

Authors declare no conflicts of interest

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