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Research Article

Quantitative Determination of Flavanone Content in Teki Grass Tuber (*Cyperus rotundus* L) using Ultraviolet-Visible Spectrophotometry

Laeli Fitriyati[1]*, Laela Hayu Nurani[2], Dwi Utami[2], and Wahyu Widyaningsih[2]

[1]Faculty of Health Sciences, Universitas Muhammadiyah Gombong, Kebumen, Indonesia
[2]Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
*Corresponding author: Laeli Fitriyati | Email: laelifitriyati@unimugo.ac.id

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Abstract: Teki grass (*Cyperus rotundus* L.) tubers are medicinal plants that have been widely utilized for their antioxidant properties, along with anti-inflammatory, antibacterial, and anticancer activities. The antioxidant activity is primarily attributed to the presence of polyphenolic compounds, particularly flavanones, which play a crucial role in scavenging free radicals and protecting against oxidative stress. This study aims to determine the flavanone content in teki grass tubers using UV-Vis spectrophotometry to support their antioxidant potential. The research was conducted as a laboratory-based experimental study, involving several steps: sample preparation, herbal drug processing, extraction, and quantitative analysis of flavanone content by comparison with a flavanone standard. The UV-Vis spectrophotometric analysis revealed the presence of flavanones in the extract of teki grass tubers. Quantitative results showed that the flavanone concentration in the extract was 128.29 µg/mL, indicating significant antioxidant compound availability. These findings confirm that teki grass tubers are a potential natural source of flavanones, with promising antioxidant capacity, supporting their traditional use in herbal medicine for conditions related to oxidative stress and cellular protection.

Keywords: flavanones, medicinal plants, phytochemicals, quantitative analysis, teki grass, UV-Vis spectrophotometry

1. INTRODUCTION

Teki grass (*Cyperus rotundus*) is a plant that grows extensively in Indonesia. The tuber is the most beneficial portion of the teki grass. However, it is rarely used as a treatment [1]. Teki grass tubers are regarded as a promising medicinal plant for development as an antioxidant source due to their ease of availability and flavonoids content [2]. Previous studies have reported the total flavonoid concentration of teki grass extract using UV-Vis spectrophotometry at 442 nm, yielding 108.37 mg/g QE (quercetin equivalent) [1]. Flavonoids are an essential secondary metabolite in plants. Flavonoids are classified broadly as flavones, flavonols, flavanols, flavanones, anthocyanidins, and chalcones [3]. Flavanones are colourless substances that cannot be detected by chromatography unless utilising a chromogen sprayer. Flavanones are also known as isomeric flavonoids, and one type can easily convert to another. Flavanone is typically easier to generate in acidic settings, whereas chalcone is easier to obtain in alkaline conditions [4]. Although flavanones

(e.g. naringenin) can be found in citrus foods, flavones (e.g apigenin) in green leafy spices, isoflavones in soybean foods, and flavonols in practically all foods, flavanone molecules can also be found in teki grass tubers. Researchers conducted tests to determine the levels of flavanones in extracts of teki grass tubers using the UV-Vis spectrophotometric method, using aluminium chloride and pure flavanone as a comparison, where aluminium chloride forms a stable complex with the flavone compound, causing the complex compound to absorb electromagnetic radiation in the UV-Vis region via a transition event, specifically excitation [4].

2. MATERIALS AND METHODS

This study's equipment includes a cutting knife, rotary evaporator, measuring cup, Beaker glass, Erlenmeyer flask, dropper pipette, test tube, chemicals glass, sample glass bottle, and UV-Vis spectrophotometer. The ingredients employed in this study included ethanol, $AlCl_3$, acetic acid, and teki grass tuber.

2.1. Sample Preparation

Preparation of teki grass tubers (*Cyperus rotundus* L) collected from Argosari Village, Ayah District, Kebumen Regency, Central Java. The teki grass tubers were collected, properly washed under running water, and dried. The teki grass tubers were classified according to their size, freshness, and pest-free status. Plant determination was carried out at the Plant Systematics Laboratory of Ahmad Dahlan University (UAD) with the number of determination certificate 287/Lab.Bio/B/VI/2023.

The tubers were subsequently extracted using the maceration process, and the total flavanone concentration was measured at Ahmad Dahlan University's Plant Systematics Laboratory. The maceration process is used to create a crude extract of teki grass tubers, which involves dissolving the dry powdered simplicia of teki grass tubers in 96% ethanol at a 1:10 ratio. The maceration procedure lasted 72 hours, with three 20-minute stirring sessions per day. The extract is thickened using a rotary evaporator [5].

2.2. Determination of Flavanone Content

The preparation of the flavanone standard solution began with weighing 10 mg of flavanone standard, which was then dissolved in ethanol to a final volume of 10 mL, yielding a stock solution with a concentration of 1000 ppm. From this stock, aliquots of 20, 40, 60, 80, and 100 μL were each diluted with ethanol p.a to obtain working standard solutions with concentrations of 2, 4, 6, 8, and 10 ppm, respectively.

To prepare the calibration curve, each standard solution was treated with 3 mL of ethanol p.a, 0.2 mL of 10% aluminum chloride ($AlCl_3$), and 0.2 mL of 1 M potassium acetate. The mixture was incubated for 30 minutes at room temperature. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 440 nm. Each measurement was conducted in triplicate to ensure accuracy and reproducibility.

For the determination of flavanone content in the teki grass (*Cyperus rotundus* L.) tuber extract, 10 mg of the extract was accurately weighed and diluted with ethanol to a final volume of 10 mL, resulting in a 1000 ppm solution. From this, 1 mL was taken and further diluted with ethanol to a total volume of 10 mL to obtain a 100 ppm working solution. A 5 mL aliquot of this solution was

then mixed with 3 mL of ethanol, 0.2 mL of 10% $AlCl_3$, and 0.2 mL of 1 M potassium acetate. The mixture was incubated at room temperature for 30 minutes before the absorbance was measured at 440 nm using a UV-Vis spectrophotometer. This measurement was also performed in triplicate.

2.3. Data Analysis

The yield of the extract was calculated using the following formula:

$$Extract\ Yield\ (\%) = \frac{Weight\ of\ extract\ obtained\ (grams)}{Weight\ of\ herbal\ drug\ powder\ (grams)} \times 100\%$$

This calculation was used to determine the efficiency of the extraction process by comparing the mass of the extract obtained to the initial mass of the powdered herbal drug. The result was expressed as a percentage to indicate the proportion of extract recovered from the raw plant material.

The concentration and corresponding absorbance values of flavanone standards were plotted and analyzed using Microsoft Excel to generate a linear regression equation in the form y = bx + a, where y represents absorbance and x denotes flavanone concentration (expressed as flavanone equivalence/FE). This calibration curve equation was then used to determine the flavanone concentration in the extract sample.

The total flavanone content (TFC) was calculated using the following formula:

$$Total\ Flavanone\ Content = \frac{(C \times V \times df)}{\mathrm{w}}$$

Where C is flavanone concentration obtained from the regression equation (mg/mL), V is the volume of extract used (mL), df is the dilution factor, and w is the weight of the extract sample used (grams) [6].

3. RESULTS AND DISCUSSION

3.1. Production of Herbal drug

The teki grass tubers used to make simplicia were collected from the city of Kebumen, specifically from Argosari village in the Ayah Subdistrict. Sampling was done during the rainy season, when the plants were productive. The roots were extracted, the tubers were gathered, wet sorted, washed, diced, and dried.

3.2. Teki Grass Tuber Extract

Dried teki grass tubers were extracted using the maceration process, with 96% ethanol as a solvent [7]. Ethanol 96% is employed as a solvent because it is a semi-polar solvent that may attract polar and non-polar chemicals found in teki grass tubers better than other solvents [8]. This solvent is also widely used in herbal extraction due to its safety, cost-effectiveness, and ability to preserve the stability of bioactive compounds. Moreover, ethanol facilitates the efficient extraction of flavonoids, which are among the key phytochemicals targeted in this study.

Table 1. Extraction results of *Cyperus rotundus*

Amount of sample powder (g)	Volume of solvent (L)	Amount of crude extract (g)	Extraction efficiency (%)	Criteria by IHP	Justification
250	2.5	35.18	17.59	≥7.2%	Meets the criteria [9]

Table 1 presents the results of the extraction process of teki grass (*Cyperus rotundus* L.) tubers, including the amount of sample powder used, volume of solvent, yield of crude extract, and extraction efficiency. The extract yield was 17.59%. The efficiency is then evaluated based on the criteria established by the Indonesian Herbal Pharmacopoeia (IHP), which states that a minimum yield of 7.2% is required. The results indicate that the extraction meets the required standard [9].

3.3. Determination Results of Flavanone Content

The findings of the flavanone screening test in the tubers of teki grass (*Cyperus rotundus* L) were positive for containing flavanones. Flavanone chemicals are member of the flavonoids [10]. Figure 1 shows the chemical structure of flavanone, a subclass of flavonoids characterized by a 15-carbon skeleton consisting of two aromatic rings (A and B) and a heterocyclic ring (C). This structure is a key feature in many bioactive compounds found in plants and contributes to their antioxidant and pharmacological activities.

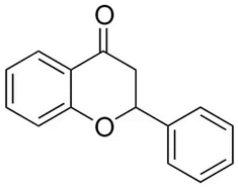

Figure 1. Structure of Flavanone

Flavonoids are phenolic compounds that occur naturally in a variety of plants. Their fundamental structure consists of a phenol ring bonded to a pyran ring by three carbon atoms. Flavanones, on the other hand, are flavonoid derivatives with a pyran ring rather than a phenolic ring and are connected with various health benefits due to their capacity to ward off free radicals [11].

3.4. Determination of Flavanone Concentration

The flavanone level was determined at a wavelength of 350-450 nm [10], with a maximum wavelength of 319.5 nm after scanning the standard. The selection of 319.5 nm as the measurement wavelength is justified based on the UV-Vis scanning results of the flavanone standard, which showed maximum absorbance at this specific wavelength. This represents the λmax (lambda maximum) where flavanones exhibit their highest light absorption, ensuring maximum sensitivity and accuracy in quantitative analysis. The absorbance of 0.9 was achieved at a concentration of 100 ppm. On a measurement of flavonoid levels, $AlCl_3$ was added to induce a complex formation with flavanones, resulting in a yellow solution [10].

The $AlCl_3$ reagent forms stable chelate complexes with flavonoids, including flavanones, through coordination with the carbonyl group at the C-4 position and hydroxyl groups at either the C-3 or C-5 positions of the flavonoid skeleton. This complexation alters the electronic configuration of the flavonoid molecule, resulting in characteristic absorption changes. Upon derivatization with $AlCl_3$, most flavonoids exhibit a distinct bathochromic shift, with their absorption maxima (λmax) shifting from native values around 280–290 nm to approximately 380–400 nm, depending on the specific subclass of flavonoids. This red shift is indicative of the formation of flavonoid–Al(III) chelates and is consistently observed across different flavonoid types, including flavonols, flavan-3-ols, flavanones, and flavones. In this study, the observed λmax at 319.5 nm represents an intermediate shift, likely influenced by the specific structural features of the flavanones present. These findings are in agreement with previous reports highlighting the utility of $AlCl_3$ derivatization for flavonoid identification through UV–Vis absorbance shifts [12].

Table 2. Results of absorbance measurements of flavanone solutions

Concentration (µg/ml)	Absorbance			Average±SD
	1	2	3	
2	0.3056	0.3057	0.306	0.3058 ± 0.0002
4	0.3362	0.3364	0.3365	0.3364 ± 0.0002
6	0.3775	0.3776	0.3778	0.3776 ± 0.0002
8	0.3987	0.3992	0.399	0.3990 ± 0.0003
10	0.4472	0.4477	0.448	0.4476 ± 0.0004

Table 2 displays the absorbance values obtained from standard flavanone solutions at varying concentrations (2–10 µg/mL). Each measurement was conducted in triplicate to ensure accuracy and precision. The average absorbance values were then used to construct the calibration curve, which served as the basis for determining the flavanone content in the plant extract samples.

In this research, the standard curve was determined at concentrations of 6, 8, 10, 12, and 14 µg/mL. The results reveal that the higher the concentration value, the higher the absorbance, as shown in Figure 2. When calculating the standard curve, the equation y = 0.0115x + 0.0158 was obtained with an R^2 value of 0.9674, indicating that 96.74% of the variation in absorbance can be explained by the concentration. This equation was then used to determine the amount of flavanone in teki grass (Cyperus rotundus) tubers.

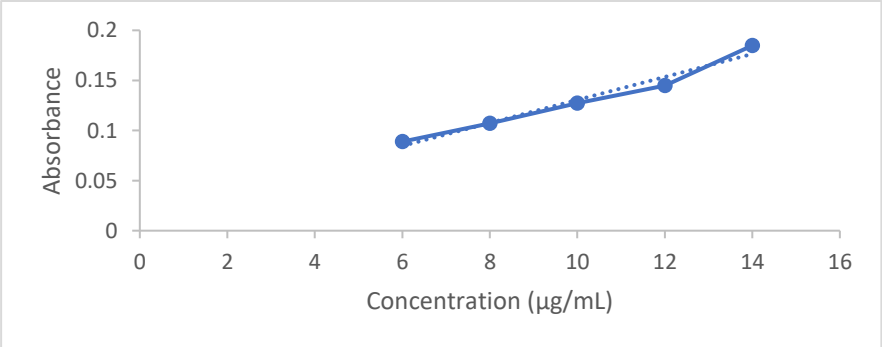

Figure 2. Calibration curve of flavanone

The flavanone concentration of teki grass tuber extract was determined using UV-Vis spectrophotometry at a wavelength of 319.5 nm, as shown in Table 3.

Table 3. Flavanone content of teki grass tuber extract

Sample	Concentration (µg/mL)	Absorbance			Average±SD	Rate X (µg/ml)
		1	2	3		
Extract	100	0.1633	0.1633	0.1633	0.1633 ± 0.00	128.29

Based on Table 3, the absorbance of the teki grass tuber extract at a concentration of 100 µg/mL was measured in triplicate and showed consistent values of 0.1633 in all three trials, resulting in an average absorbance of 0.1633. This high consistency indicates good repeatability and precision in the measurement process. By substituting the average absorbance value into the linear regression equation $y = 0.0115x + 0.0158$, the flavanone content (X) in the sample was calculated. This result indicates that the extract contains 128.29 µg/mL flavanone equivalence (FE), which reflects a relatively high concentration of flavanones in the teki grass tuber extract.

3.5. Methodological Limitations and Comparative Analysis with Related Cyperus Species

3.5.1. Methodological Limitations (Spectrophotometric Specificity)

This study successfully quantified the flavanone content in *Cyperus rotundus* tuber extract using UV-Vis spectrophotometry, revealing a concentration of 128.29 µg/mL. This finding supports the traditional use of *Cyperus rotundus* as a potential natural antioxidant source due to the known free radical scavenging ability of flavanones. Flavanones are well-documented for their capacity to neutralize reactive oxygen species, contributing to cellular protection and the management of oxidative stress-related disorders. However, the spectrophotometric method employed in this study presents inherent limitations, particularly in terms of specificity. The use of aluminium chloride ($AlCl_3$) as a derivatizing agent allows for the formation of chelate complexes with flavonoid hydroxyl groups, but it is not entirely selective for flavanones. Other polyphenolic or hydroxylated compounds present in the extract may also react with $AlCl_3$, potentially leading to an overestimation of flavanone content. Future studies employing more selective and validated analytical techniques, such as high-performance liquid chromatography (HPLC) coupled with mass spectrometry, are recommended to achieve more accurate quantification [12].

3.5.2. Comparative Analysis with Related *Cyperus* species

The flavanone content of 128.29 µg/mL obtained in this study demonstrates significant bioactive potential when compared to previous research on *Cyperus rotundus* and related plant species. Zhou and Fu (2013) isolated a new flavanone compound, 7,8-dihydroxy-5,6-methylenedioxyflavone, from *Cyperus rotundus* rhizomes along with other known flavonoids including quercetin, kaempferol, and luteolin, confirming the presence of diverse flavonoid compounds in this species [13]. Recent comprehensive reviews indicate that over 192 natural products have been isolated from *Cyperus rotundus*, with terpenoids and flavonoids being the major bioactive constituents [14]. The quantitative flavanone content found in this study (128.29

μg/mL) suggests that tuber extracts may contain higher concentrations of these compounds compared to other plant parts previously studied, though most literature focuses on rhizomes rather than tubers. When compared to related *Cyperus* species, *C. esculentus* (tiger nut) has been reported to contain flavonoids in stems and leaves reaching 13.2 mg/g dry weight, though specific flavanone quantification was not performed. The flavonoid content in *C. esculentus* tubers shows crude protein (10-15%), starch (20-30%), and sugar (10-20%), indicating substantial secondary metabolite production within this genus [15].

To provide a broader perspective, the flavanone content of *Cyperus rotundus* was compared with that of citrus species, which are widely recognized as some of the richest natural sources of flavonoids. Recent studies on 27 local citrus cultivars revealed that total flavanone content in citrus ranges from 29.84 to 2554.70 mg/100 g dry weight (DW) in peels, 52.94 to 916.58 mg/100 g DW in pulps, and 28.30 to 392.48 μg/mL fresh weight (FW) in juices. Among the identified flavanones, hesperidin was the most abundant, with levels reaching up to 2298.99 mg/100 g DW in citrus peels, followed by naringin (up to 1676.31 mg/100 g DW) and eriocitrin (up to 268.69 mg/100 g DW) depending on the citrus species and plant parts. Consistently, citrus peels exhibited higher flavonoid concentrations than pulps and juices, with cultivars such as *C. reticulata*, *C. sinensis*, and *C. grandis* identified as the richest sources of hesperidin, naringin, and eriocitrin, respectively. Although the flavanone concentration in *Cyperus rotundus* tuber extract is considerably lower than the levels reported in citrus peels, it remains within a pharmacologically relevant range, particularly when viewed in the context of traditional medicinal applications. The antioxidant potential of *Cyperus rotundus* may also arise from synergistic effects between flavanones and other secondary metabolites present in the extract [16].

The strong correlation coefficient (R^2 = 0.9674) supports the reliability of the standard curve used for quantification. The relatively high flavanone concentration (128.29 μg/mL) obtained from *Cyperus rotundus* tubers appears comparable to or exceeding flavanone levels reported in other medicinal plants, positioning this species as a potentially valuable source of bioactive flavanones within the *Cyperaceae* family.

3.6. Pharmacological Significance and Bioactivity Thresholds

The findings of this study demonstrate that the flavanone concentration of 128.29 μg/mL (equivalent to 572.1 μM) found in *Cyperus rotundus* tuber extract exhibits substantial pharmacological relevance when evaluated against established bioactivity thresholds for flavanones [17]. This concentration significantly exceeds the minimum effective concentrations reported for various biological activities of flavanone compounds. For instance, Zhou and Fu (2013) reported that a new flavanone isolated from *Cyperus rotundus* rhizomes exhibited superoxide radical scavenging activity with an IC_{50} value of 3.1 μM, indicating potent antioxidant potential at relatively low concentrations [13]. Furthermore, previous studies on flavanols, particularly (−)-epicatechin, have demonstrated that meaningful biological effects, such as nitric oxide-dependent vasodilation, can occur at plasma concentrations as low as 30–100 nM, with typical post-consumption peak plasma levels remaining below 1 μM. Notably, the antioxidant

capacity in plasma only increases at supraphysiological concentrations of flavanones (⩾10 μM), emphasizing that biological effects are not solely dependent on antioxidant mechanisms. When compared to these established thresholds, the concentration of flavanones in *Cyperus rotundus* extract (572.1 μM) is substantially higher, suggesting a strong potential for both antioxidant and other pharmacological activities. These findings not only support the traditional use of *Cyperus rotundus* in herbal medicine but also highlight its potential application in the development of therapeutic agents targeting oxidative stress and vascular health[18].

In light of the quantified flavanone concentration and the well-established structure activity relationships of flavonoid compounds, the *Cyperus rotundus* tuber extract is anticipated to possess multiple bioactive properties. Firstly, the exceptionally high flavanone content measured at a higher concentration than the reported IC_{50} for antioxidant activity, strongly indicates potent free radical scavenging capacity. Secondly, the flavanone concentration falls within or exceeds the typical bioactive ranges associated with anti-inflammatory effects, suggesting potential efficacy in modulating inflammatory pathways. Additionally, the concentration achieved in this extract surpasses the minimum inhibitory concentrations commonly reported for the antimicrobial activity of flavanones, pointing to a possible role in combating microbial infections. Furthermore, the hepatoprotective potential of the extract is supported not only by the bioactivity profile of flavanones but also by the traditional use of *Cyperus rotundus* in liver-related ailments [19]. Taken together, these findings highlight that the flavanone-rich *Cyperus rotundus* tuber extract represents a promising natural source for the development of therapeutic agents, particularly for conditions related to oxidative stress, inflammation, and microbial infections.

4. CONCLUSION

The presence of flavanones in teki grass tubers (*Cyperus rotundus* L.) was confirmed through quantitative analysis. Quantitative evaluation using UV-Vis spectrophotometry revealed that the flavanone content was 128.29 μg/mg extract. These findings suggest that *Cyperus rotundus* tubers possess a considerable flavanone content, supporting their potential as a natural source of bioactive compounds with prospective applications in health and pharmaceutical fields.

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