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Analysis of Vitamin C Content in *Ruku-Ruku* (*Ocimum tenuiflorum* L.) Leaves

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Abstract

Ruku-ruku (*Ocimum tenuiflorum* L.) is widely recognized as a medicinal plant used in traditional medicine. The leaves are the most commonly utilized part of the plant, particularly for treating various health conditions such as fever, cough, joint and nerve pain, oral ulcers, tinea versicolor, nausea, and vomiting. *Ruku-ruku* leaves contain various essential nutrients, including calcium, iron, zinc, vitamin A, and vitamin C. This study aimed to determine the vitamin C content of *ruku-ruku* leaves. The research employed a quantitative descriptive method using the iodimetric titration technique. The samples consisted of young, mature, and old leaves collected from Tulo Village, Sigi Regency, Indonesia. Vitamin C content was measured with three replications for each sample. The results showed that the highest vitamin C content was found in young leaves, with an average value of 70.89 mg/100 g, followed by mature leaves at 54.18 mg/100 g and old leaves at 33.80 mg/100 g. Differences in vitamin C content were influenced by the level of leaf maturity, with younger leaves containing higher concentrations of vitamin C than older leaves.

Keywords: *Ruku-ruku* leaves; Vitamin C; Quantitative analysis; Iodimetric titration.

INTRODUCTION

Indonesia is one of the world's megabiodiversity countries, possessing an exceptionally high level of biological diversity and a wide variety of plant species that have long been utilized in traditional medicine. The use of these medicinal plants continues to grow due to their therapeutic properties, which have been inherited and validated through generations of traditional practice. Indonesia is estimated to have more than 30,000 species of medicinal plants with potential applications as natural remedies. According to the 2015 Research on Medicinal Plants and Traditional Herbal Medicine, a total of 1,159 medicinal plant species belonging to 156 families were documented (Klau et al., 2021). This rich biodiversity positions Indonesia as one of the world's centers of medicinal plant diversity, offering substantial potential for the development of natural bioactive compounds, including vitamins and antioxidants. One medicinal plant with various therapeutic benefits is *ruku-ruku*, which belongs to the family Lamiaceae (Rashid et al., 2018).

Ruku-ruku is widely recognized as a medicinal plant used in traditional healthcare practices. It is a shrub species that can grow up to approximately 1.5 m in height and is characterized by an erect, highly branched stem and spur-shaped leaves. The leaves are the most commonly utilized part of the plant, particularly in traditional medicine for treating various health conditions, including fever, cough, joint and nerve pain, oral ulcers, tinea versicolor, nausea, and vomiting (Andalia & Fitri, 2021). In addition, *ruku-ruku* has been reported to provide several other health benefits, such as reducing fever, stimulating appetite, alleviating diarrhea, and supporting cardiovascular health (Istiqomah, 2020). Taxonomically, *ruku-ruku* belongs to the family Lamiaceae and is closely related to sweet basil. The species is widely distributed throughout South and Southeast Asia, including Indonesia. *Ruku-ruku* contains various essential nutrients, including calcium, iron, zinc, vitamin A, and vitamin C. Furthermore, its stems contain essential oils whose major constituents include rosmarinic acid, apigenin, cirsimaritin, isotimusin, and isotimonin. The seeds also contain xylose sugars and polysaccharides (Siva et al., 2016). This plant is commonly consumed as herbal tea both fresh and dried forms. The leaves possess an aroma that differs slightly from that of sweet basil due to variations in their essential oil composition. Several studies have reported that the essential oils of *ruku-ruku* exhibit potential therapeutic properties beneficial to human health. Currently, plant-derived essential oils are increasingly being explored as natural alternatives to synthetic antioxidants, antifungal agents, and antibacterial compounds, which may be associated with adverse effects such as carcinogenicity and liver damage (Khodaei et al., 2020). In addition, the plant contains β-carotene (provitamin A), which plays an important role in maintaining visual function and enhancing antibody-mediated immune responses (Tiwari et al., 2018).

Vitamin C, also known as L-ascorbic acid, is a water-soluble antioxidant compound that plays a crucial role in protecting the body against damage caused by reactive oxygen species present in cells and blood plasma. Physically, vitamin C occurs as white crystals with the chemical formula $C_6H_8O_6$ and a molecular weight of 176.13 g mol^{-1} (Purwoko, 2017). Vitamin C performs numerous essential physiological functions, particularly as an antioxidant that protects cells from oxidative damage induced by free radicals. In addition, it is involved in collagen synthesis and contributes to the acceleration of wound healing. Vitamin C has also been reported to enhance immune function, support metabolic processes, facilitate iron absorption, and contribute to the regulation of blood pressure and uric acid levels (Safnowandi, 2022). Ascorbic acid participates in a wide range of metabolic pathways within the body, including the biosynthesis of collagen, carnitine, norepinephrine, and serotonin. Furthermore, it assists in the absorption and metabolism of iron and calcium, helps prevent infections, and may contribute to the prevention of cancer and cardiovascular diseases. These biological functions are closely associated with its antioxidant properties, which enable it to neutralize free radicals and reduce oxidative stress (Kurniawati et al., 2023). As one of the most important water-soluble vitamins, vitamin C also plays a significant role in maintaining and strengthening the immune system (Makmun et al., 2020). Vitamin C deficiency can lead to increased susceptibility to stress and infections due to impaired immune function. Severe deficiency may result in scurvy, a condition characterized by symptoms such as bleeding gums, fatigue, joint pain, and anemia (Arel et al., 2017). According to the Recommended Dietary Allowance (RDA) established by the Indonesian Ministry of Health, the daily vitamin C requirement for adults ranges from 75 to 90 mg per day (Ministry of Health of the Republic of Indonesia, 2019). This study aimed to determine the vitamin C content of *ruku-ruku* leaves (*Ocimum tenuiflorum* L.).

MATERIALS AND METHODS

Study area

This study employed a quantitative descriptive approach to accurately determine the vitamin C content of *ruku-ruku* leaves. The research was conducted on April 14, 2026. Leaf samples were collected from Tulo Village, Dolo District, Sigi Regency, Central Sulawesi, Indonesia. The samples were subsequently subjected to drying and grinding processes before being analyzed using the iodimetric titration method. The prepared extracts were titrated with a standardized 0.01 N iodine solution until a violet color endpoint was observed. The resulting extracts were then quantitatively analyzed to determine their vitamin C content. All laboratory analyses were carried out at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Tadulako University.

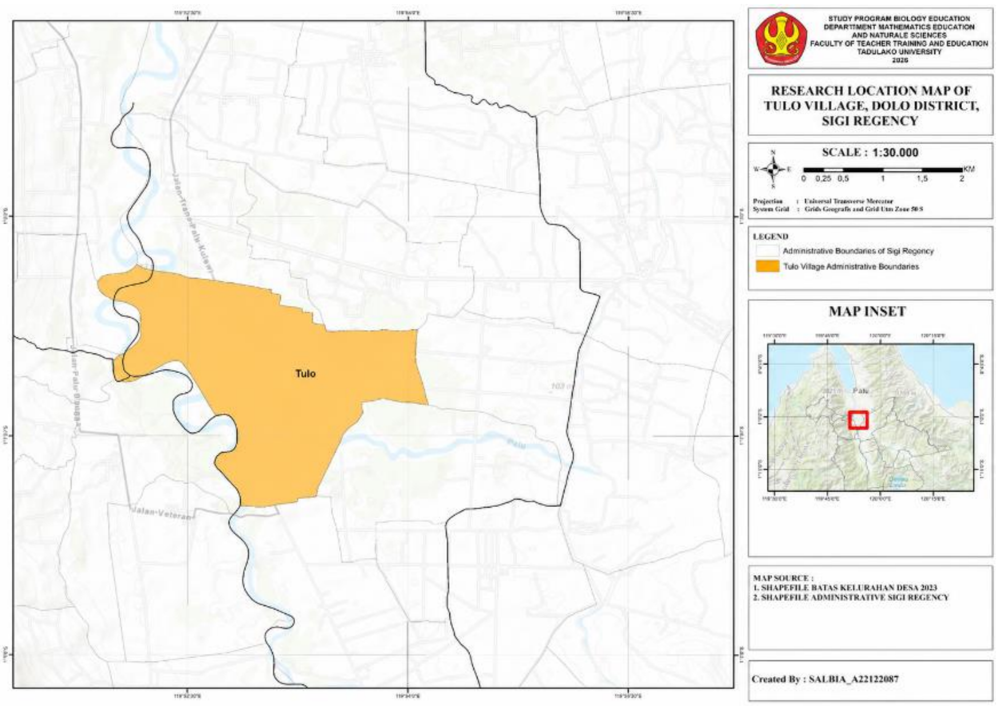

Figure 1. Map of the research location in Tulo Village, Dolo District, Sigi Regency, Central Sulawesi, Indonesia.

Procedures

Sample Preparation

During the sample preparation stage, *ruku-ruku* (*Ocimum tenuiflorum* L.) leaves (Figure 2) were thoroughly washed under running water, drained, and weighed to obtain 10 g of fresh material. The samples were then oven-dried for 24 hours and ground into a fine powder using a mortar and pestle. Subsequently, 1 g of each powdered sample was weighed and transferred into an Erlenmeyer flask, followed by the addition of distilled water to a final volume of 100 mL. The resulting solution was filtered through filter paper into a beaker. An aliquot of 25 mL of the filtrate was then transferred to an Erlenmeyer flask and mixed with 1.2 mL of 10% H_2SO_4 solution and a few drops of 1% starch indicator. The mixture was subsequently titrated with a standardized 0.01 N iodine solution until a permanent violet coloration appeared, indicating the endpoint of the titration.

Figure 2. *Ruku-ruku* (*Ocimum tenuiflorum* L.) leaves.

Preparation of Reagents

Preparation of 10% H_2SO_4 Solution

An amount of 10% sulfuric acid (H_2SO_4) solution was prepared by carefully diluting 10 mL of concentrated H_2SO_4 with distilled water in a 100 mL volumetric flask and adjusting the volume to the calibration mark.

Preparation of 0.1 N Iodine Solution

An amount of 0.1 N iodine solution was prepared by dissolving 1.27 g of iodine powder (I_2) and 2.3 g of potassium iodide (KI) in a small volume of distilled water with continuous stirring until completely dissolved. The solution was then transferred to a 100 mL volumetric flask and diluted to the mark with distilled water.

Preparation of 0.01 N Iodine Solution

An amount of 0.01 N iodine solution was prepared by pipetting 10 mL of the 0.1 N iodine stock solution into a 100 mL volumetric flask, followed by dilution with distilled water to the calibration mark.

Preparation of 1% Starch Indicator Solution

An amount of 1% starch solution was prepared by dissolving 1 g of starch in 100 mL of hot distilled water in a beaker. The solution was used as an indicator during the iodimetric titration process.

Data analysis

The vitamin C content was calculated using the following equation (Nisa, 2020):

$$\text{Vitamin C levels mg/gram} = \frac{\text{VI2 x NI2 x Mr C6H8O6}}{\textit{Sample weight (g)}}\ 100\%$$

Where:

V I_2	= volume of iodine solution used in the titration (mL)
N I_2	= normality of the iodine solution (N)
Mr vitamin C	= sample weight

RESULT AND DISCUSSION

Results

This study was conducted to determine the vitamin C content of young, mature, and old *ruku-ruku* (*Ocimum tenuiflorum* L.) leaves. The analysis was performed using the iodimetric titration method with three replicates for each sample. Iodimetric titration is a direct titration technique that employs iodine solution as the standard reagent and is typically carried out under neutral or slightly acidic conditions (Jumi et al., 2023). This method was selected because it is highly selective for vitamin C, a strong reducing agent capable of reducing iodine (I_2) to iodide ions (I^-). The reaction proceeds stoichiometrically, allowing the vitamin C concentration to be accurately determined based on the volume of iodine consumed during the titration process (Sari et al., 2021). The titration was conducted in triplicate, and the resulting data were used to calculate the vitamin C content of each sample. The results are presented in Table 1.

Table 1. Vitamin C Content in *Ruku-Ruku* Leaves.

Samples	Sample Weight (g)	I_2 Titration Volume (mL)	Vitamin C Content (mg/100 g)
Young leaves	1.05	0.84	70.45
	1.02	0.82	70.79
	1.06	0.86	71.44
Average	–	–	70.89
Mature leaves	1.03	0.62	53.01
	1.06	0.66	54.83
	1.03	0.64	54.72
Average	–	–	54.18
Old leaves	1.09	0.42	33.93
	1.10	0.42	33.62
	1.02	0.40	34.64
Average	–	–	33.80

Discussion

Based on the differences in vitamin C content observed among the samples, leaf maturity appears to have a significant influence on the concentration of vitamin C in *ruku-ruku* leaves. Young leaves exhibited substantially higher vitamin C levels than mature and old leaves. This pattern is likely associated with the intense physiological activity and cellular metabolism that occur during the early stages of leaf development. During this phase, plant tissues actively synthesize primary and secondary metabolites required for growth, including ascorbic acid (vitamin C). As leaves age, vitamin C content tends to decline due to oxidative processes and alterations in metabolic activity within leaf tissues. According to Fadhli and Susanti (2024), fresh young leaves generally contain higher concentrations of vitamin C because photosynthetic and metabolic activities are more intensive, thereby promoting the synthesis of bioactive compounds. However, as leaf maturity increases, vitamin C concentrations gradually decrease as a consequence of bioactive compound degradation and the transition of metabolic processes from active growth to tissue maintenance. Similar findings were reported by Sari et al. (2021) in tomatoes, where vitamin C levels declined with increasing fruit maturity. This reduction was attributed to elevated activity of the enzyme ascorbate oxidase, which catalyzes the oxidation of ascorbic acid into dehydroascorbic acid, a compound with reduced antioxidant activity. These findings suggest that leaf developmental stage is an important factor influencing the nutritional quality of *ruku-ruku* leaves, particularly with respect to their antioxidant properties.

The vitamin C content recorded in young *ruku-ruku* leaves (70.89 mg/100 g) can be considered relatively high when compared with several other medicinal and culinary plants. For example, vitamin C levels in basil, a species belonging to the same genus as *ruku-ruku*, have been reported to range from 18 to 61 mg/100 g, whereas shallots contain approximately 7.4 mg/100 g of vitamin C (Jumi et al., 2023). These comparisons indicate that young *ruku-ruku* leaves possess considerable potential as a natural source of vitamin C and may provide greater nutritional benefits than several commonly consumed plant-based foods. It should also be noted that the vitamin C values obtained in the present study were determined from dried samples. The use of a dry-weight basis may result in relatively higher concentrations than those measured in fresh materials because the drying process removes water from plant tissues, thereby increasing the concentration of nutrients per unit weight. Consequently, caution should be exercised when directly comparing these values with data obtained from fresh samples (Andalia & Fitri, 2021).

A comparison of the vitamin C content of young *ruku-ruku* leaves (70.89 mg/100 g) with that of several locally consumed leafy vegetables further demonstrates their nutritional value. According to the Indonesian Food Composition Table (TKPI) and previous studies, fresh cassava leaves contain approximately 275 mg/100 g of vitamin C, while fresh papaya leaves contain around 140 mg/100 g (Ministry of Health of the Republic of Indonesia, 2019). In contrast, commonly consumed vegetables such as water spinach contain approximately 35 mg/100 g, spinach contains around 28 mg/100 g, and *katuk* leaves contain approximately 50–60 mg/100 g of vitamin C (Indriyati et al., 2023). Although the vitamin C content of young ruku-ruku leaves is lower than that of cassava and papaya leaves, it remains higher than that of water spinach and spinach, which are among the most widely consumed vegetables in Indonesia. This comparison highlights the potential of ruku-ruku leaves as a valuable dietary source of vitamin C and supports their incorporation into local food systems and nutrition-based health promotion programs (Jumi et al., 2023).

The determination of vitamin C content in this study was carried out using the iodimetric titration method. This analytical technique is widely used because vitamin C readily reacts with iodine, allowing its concentration to be quantified based on the volume of iodine solution consumed during titration. In general, a greater volume of iodine required to reach the endpoint indicates a higher concentration of vitamin C in the sample (Sari et al., 2021). During the reaction, ascorbic acid reduces iodine (I_2) to iodide ions (I^-), resulting in the disappearance of the characteristic iodine color. This occurs because iodine molecules are bound and reduced by ascorbic acid molecules present in the sample. Therefore, samples with higher vitamin C concentrations are capable of reacting with and reducing larger amounts of iodine than samples with lower vitamin C concentrations (Setiawan et al., 2021). The endpoint of the titration is indicated by the appearance of a blue coloration, which results from the formation of a starch–iodine complex after all available vitamin C has reacted completely with iodine. The blue color develops because the glucose units in starch form helical structures capable of trapping iodine molecules, thereby producing a stable blue-colored complex (Fitriana et al., 2020). Iodimetric titration offers several advantages, including procedural simplicity, relatively low operational costs, and the absence of a requirement for sophisticated instruments such as spectrophotometers. However, the method is susceptible to interference from impurities and other reducing substances that may affect analytical accuracy. For this reason, appropriate sample preparation and purification procedures are essential prior to analysis to minimize potential interference and ensure reliable results (Erwanto et al., 2018).

The findings of this study demonstrate that ruku-ruku leaves have considerable potential as a natural source of vitamin C with possible applications in nutrition and public health. The relatively high vitamin C content observed, particularly in young leaves, suggests that this plant may contribute significantly to dietary antioxidant intake. Vitamin C is an essential nutrient involved in immune function, collagen synthesis, wound healing, and protection against oxidative stress. Therefore, the consumption of *ruku-ruku* leaves may provide additional health benefits beyond their traditional medicinal uses. Furthermore, when converted from dry-weight values to fresh-weight equivalents, the consumption of 100 g of fresh young ruku-ruku leaves could contribute a substantial proportion of the recommended daily vitamin C intake for adults. These results support the utilization of ruku-ruku as a locally available functional food and highlight its potential role in promoting nutritional security and community health through the use of indigenous plant resources (Octaviani et al., 2019).

CONCLUSIONS

Based on the results of this study, it can be concluded that *ruku-ruku* (*Ocimum tenuiflorum* L.) leaves contain vitamin C at varying concentrations depending on the stage of leaf maturity. Young leaves exhibited the highest vitamin C content, with an average value of 70.89 mg/100 g, followed by mature leaves at 54.18 mg/100 g and old leaves at 33.80 mg/100 g. These findings indicate that leaf maturity significantly influences vitamin C concentration, with younger leaves generally containing higher levels of vitamin C than older leaves. Therefore, young *ruku-ruku* leaves may serve as a more valuable natural source of vitamin C and have greater potential for nutritional and health-related applications.

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

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