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Analysis of Vitamin C (ascorbic acid) in *Cucumis mel L. var reticulatus Naudin* and *Averrhoa bilimbi* L. Fruit using UV-Vis Spectrophotometry

Hafid Syahputra[*1], Muhammad Rivai[2], Shafira Mahrani[2], and Yasmeen Nazhifah[2]
[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*
[2]*Undergraduate Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*
*Corresponding Author: hafid@usu.ac.id

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ABSTRACT
Cucumis mel L. var reticulatus Naudin and *Averrhoa bilimbi* L. are known to possess significant chemicals that offer health benefits, including the presence of vitamin C. Vitamin C, classified as a water-soluble vitamin, possesses the potential to exert a significant influence in the prevention of several diseases. The objective of this experiment was to analyze the concentrations of vitamin C present in *Cucumis mel L. var reticulatus Naudin* and *Averrhoa bilimbi* L. using the UV-Vis spectrophotometry method. The test was performed qualitatively with $FeCl_3$ reagen and quantitatively with 2,6-dichlorophenol indophenol using UV-Vis spectrophotometry. The results showed that the levels of vitamin C in *Cucumis mel* and *Averrhoa bilimbi* fruit were 44.523 mg/100g and 48.666 mg/100 g, respectively.
Keywords: *Cucumis mel*, *Averrhoa bilimbi*, Vitamin C, UV-Vis spectrophotometry

ABSTRAK
Buah timun suri (*Cucumis mel L. var reticulatus Naudin*) dan buah belimbing wuluh (*Averrhoa bilimbi* L.) mengandung senyawa-senyawa penting yang bermanfaat bagi kesehatan, salah satunya adalah vitamin C. Vitamin C merupakan vitamin yang larut dalam air yang dapat berperan penting dalam mencegah berbagai penyakit. Pengujian ini bertujuan untuk menentukan kadar vitamin C pada *Cucumis mel L. var reticulatus Naudin* dan buah *Averrhoa bilimbi* L. dengan metode spektrofotometri UV-Vis. Pengujian dilakukan secara kualitatif dengan pereaksi $FeCl_3$ dan kuantitatif dengan 2,6-diklorofenol indofenol menggunakan spektrofotometri UV-Vis. Hasil penelitian menunjukkan bahwa kadar vitamin C pada buah *Cucumis mel* dan *Averrhoa bilimbi* masing-masing adalah 44,523mg/100g dan 48,666 mg/100 g.
Kata Kunci: *Cucumis mel*, *Averrhoa bilimbi*, Vitamin C, Spektrofotometri UV-Vis.

1. Introduction

Indonesia is a nation characterized by its agricultural sector, which boasts a wealth of natural resources, particularly in the realm of agricultural products. Fruit exhibits significant promise within the context of Indonesia. The diversity of tropical fruit present in Indonesia is indicative of this phenomenon. Fruits are considered to be a valuable dietary source of essential nutrients such as vitamins A and C, dietary fiber, and various minerals. These nutrients play a crucial role in regulating many physiological processes within the human body [1], [2]. *Cucumis mel L. var reticulatus Naudin* and *Averrhoa bilimbi* L. are fruits that can produce vitamin C.

Cucumis mel and *Averrhoa bilimbi* come from Southeast Asia and can be found almost throughout Indonesia. These fruits are often found in home gardens and are often processed into common food products. *Cucumis mel* can be processed into fruit ice, cocktails, or granulated sugar, while people often use the fruit

Averrhoa bilimbi as a food flavoring to give a sour taste. The fruit *Averrhoa bilimbi* is often used as a mixture in various traditional dishes. *Averrhoa bilimbi* also treats cholesterol, gout, diabetes mellitus, acne, and ulcer [3], [4]. The ingredients contained in this fruit are very beneficial for the body, such as carbohydrates, protein, fat, fiber, water, ash, vitamin C, potassium, calcium, and phosphorus. *Cucumis mel* contains secondary metabolites in the skin, fruit, and seeds, consisting of active compounds of flavonoids, saponins, alkaloids, vitamin C, and tannins. In comparison, the *Averrhoa bilimbi* plant contains main compounds such as vitamin C, saponins, tannins, flavonoids, triterpenes, amino acids, citric acid, glucoside compounds, phenolics, potassium sugar ions, as well as vitamins and minerals, so these two fruits have health benefits [5].

Vitamin C is an essential chemical required for several metabolic activities within the human body. Lack of vitamin C in the food consumed can cause a decrease in body resistance. Vitamin C, also known as ascorbic acid, is classified as a water-soluble vitamin derived from hexose derivatives that exhibit a high susceptibility to oxidation. In addition, vitamin C has a chromophore group sensitive to light stimulation. *Cucumis mel* and *Averrhoa bilimbi* contain vitamin C [6], [7]. Various techniques have been applied for the quantification of vitamin C concentrations, such as Ultraviolet and visible spectrophotometry.

UV-Vis spectrophotometry is one of the most useful qualitative and quantitative analysis methods. UV-Vis spectrophotometry exhibits notable attributes like a comparatively elevated sensitivity, heightened selectivity, and a commendable level of accuracy. In addition, testing with UV-Vis spectrophotometry is cheap and easy. The UV-Vis spectrophotometry method can provide results for measuring vitamin C levels, almost like the nutritional values contained in the extract [8]. Based on the description above, the authors were interested in conducting qualitative and quantitative analysis of ascorbic acid from *Cucumis mel* and *Averrhoa bilimbi* L. Fruit by UV-Vis Spectrophotometry.

2. Methods

2.1 Materials and Sample Preparation

The glassware used in the test is standardized glassware with the brands iwaki and Pyrex. Meanwhile, the materials used in this test were 2,6-dichlorophenol indophenol (Merck), distilled water, 1 N ammonium hydroxide (Merck), ascorbic acid (Merck), 0.4% oxalic acid (Merck), 3% ferric chloride b /v (Merck), and sodium bicarbonate 0.84% w/v (Merck).

Cucumis mel and *Averrhoa bilimbi* wealth is obtained from the market in Medan, Sumatera Utara. After cleaning the fruit thoroughly, the total weight of each fruit was calculated. Then, each fruit was cut into small pieces and blended until smooth. A sample weighing approximately 100 g was precisely measured and thereafter transferred into a 100 mL measuring flask. The flask was then filled with a 0.4% oxalic acid solution until reaching the mark line. The resulting mixture was thoroughly mixed and subsequently filtered. All chemical reagents used in this study were purchased from Merck.

2.2 Qualitative Analysis of Vitamin C with the Color Reaction Method

2 ml of the filtrate sample was transferred into a test tube, neutralized to a pH range of 6-8 by the addition of NH_4OH, and finally supplemented with a small quantity of $FeCl_3$. The ascorbic acid is evidenced by the formation of a purple color in the sample [9].

2.3 Quantitative Analysis with UV-Vis Spectrophotometry Method

The procedure involved extracting 2.5 mL of the sample solution for each fruit . The extracted solution was then transferred into a 25 mL volumetric flask. Subsequently, a 0.4% oxalic acid solution was added to the flask until it reached the mark. Finally, 1 mL of the resulting solution was pipetted and transferred into a 10 mL flask. After that, 2,6 - dichlorophenol indophenol was added to the mark limit, and then shaken until homogeneous, Subsequently, the measurement of absorption was conducted at a specific wavelength of 513 nm using a UV-Vis spectrophotometer [8].

Vitamin C levels were determined by utilizing the linear regression equation derived from the standard curve of vitamin C. The determination of vitamin C levels in the given sample can be accomplished through the utilization of a regression equation $Y = aX+b$.

The formula for calculating vitamin C levels:

$$K = \frac{X \times V \times FP}{\text{Weight of sample (g)}}$$

Note:
K = level of vitamin C in the sample (µg/g)
X = level of vitamin C in the sample solution after dilution (µg/mL)
V = volume of sample solution before dilution (mL)
FP = Dilution factor sample weight

3. Results and Discussion

3.1 Qualitative Analysis of Vitamin C

A preliminary qualitative analysis was conducted to ascertain the presence of vitamin C in the sample solution. Figure 1 displays the outcomes of a qualitative examination conducted on the presence of vitamin C in *Cucumis mel* and *Averrhoa bilimbi*.

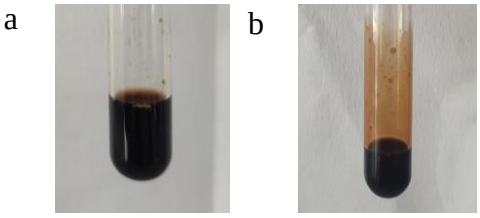

Figure 1. Results of Qualitative Analysis of Vitamin C (a) *Cucumis mel*; (b) *Averrhoa bilimbi*

Figure 1 shows that the fruit samples of *Cucumis mel* and *Averrhoa bilimbi* using $FeCl_3$ gave positive results. This showed that the sample contained vitamin C. A qualitative test of vitamin C with $FeCl_3$ reagent will form a purple solution, which indicates an inflammation of the vitamin C content [9], [10].

3.2 Quantitative Analysis of Vitamin C

Vitamin C is an antioxidant, and antioxidants are substances that can counteract free radicals. Vitamin C is abundant in fruits and vegetables. Vitamin C deficiency can cause mild symptoms such as fatigue, anorexia, muscle aches, easier stress, and infection, while severe vitamin C deficiency can cause scurvy. Scurvy is characterized by bleeding gums, weakness, joint pain, and anemia [4]. Vitamin C is classified as a hydrophilic vitamin, meaning it is soluble in water. Hence, in this research endeavor, a sterile aqueous solvent was employed as a precautionary measure to mitigate the potential presence of contaminants and ensure the absence of pyrogens. A solution of ascorbic acid was prepared at a concentration of 1000 µg/mL and subsequently diluted to a concentration of 200 µg/mL to determine the wavelength at which maximum absorption occurs. The resulting measurement, obtained using a UV-visible spectrophotometer, yielded a wavelength of 513 nm (figure 2). A set of concentrations ranging from 4-10 µg/mL was prepared using the 100 µg/mL stock solution . The absorbance of these concentrations was then measured at a wavelength of 513 nm (figure 3). Subsequently, a calibration curve was constructed using the concentration and absorbance data. The equation for the calibration curve is expressed as $y = 0.0747x + 0.0161$, where y represents the dependent variable and x represents the independent variable. The correlation coefficient (r) associated with this equation is 0.998, indicating a strong linear relationship between the variables [8], [11].

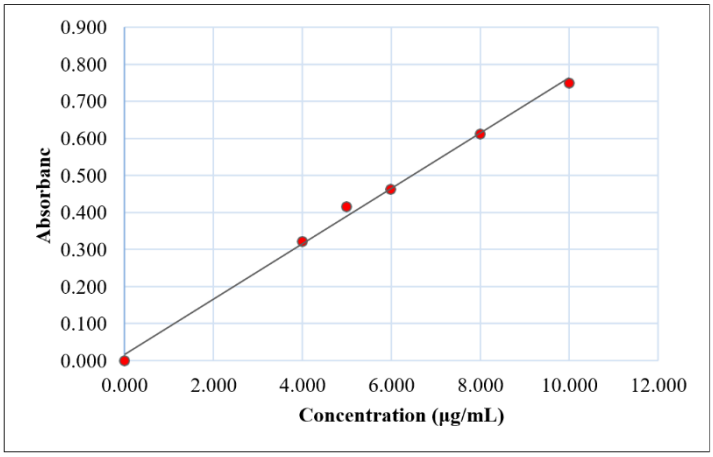

Figure 2. Maximum wavelength spectrum

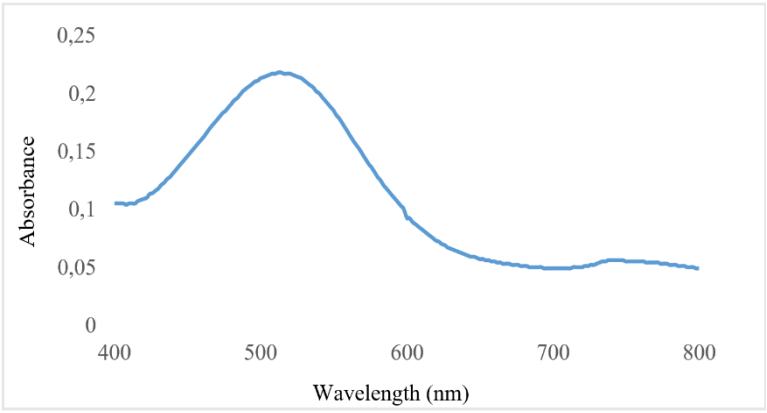

Figure 3. Vitamin C standard calibration curve

Table 1: Levels of Vitamin C in *Cucumis mel*

Cucumis mel	Absorbance	Concentration (µg/mL)	Content (mg/100 g)	Average content (mg/100 g)
S1	0.305	3.874	38.598	44.523
S2	0.420	5.400	53.967	
S3	0.323	4.111	41.004	

Table 2: Levels of Vitamin C in *Averrhoa bilimbi*

Averrhoa bilimbi	Absorbance	Concentration (µg/mL)	Content (mg/100 g)	Average content (mg/100 g)
S1	0.362	4.634	46.216	48.666
S2	0.399	5.121	51.160	
S3	0.380	4.875	48.622	

Differences in the results of vitamin C levels can be influenced by storage time, temperature, exposure, and sunlight, as well as being in line with the storage process. This is related to fruit respiration, where vitamin C is easily degraded by temperature, sugar concentration, pH, oxygen, metal catalysis, and enzymes during storage. The storage time of the fruit can affect the work of the ascorbic acid oxidase enzyme, where the reshuffling process will continue continuously along with the length of storage so that a decrease in vitamin C levels can occur. Decreasing levels of vitamin C in fruit can be overcome by storing the fruit properly. Storage

at low temperatures can inhibit enzyme activity and chemical reactions, thereby preventing a decrease in vitamin C levels. Preventing fruit exposure to air and sunshine can serve as a means of averting the oxidation process of vitamin C within fruits [12].

The vitamin C levels in *Cucumis mel* and *Averrhoa bilimbi* were determined using the UV-Vis spectrophotometry method, and three repetitions of measurements were carried out to improve accuracy during testing [13]. Previously, the sample was blended and dissolved using 0.4% oxalic acid, then added with 2,6-dichlorophenol indophenol. Oxalic acid is useful as a solvent to prevent the oxidation of vitamin C in processing so that the determination of vitamin C levels can be carried out optimally. The 2,6-dichlorophenol indophenol reagent was used to measure the amount of 2,6-dichlorophenol indophenol solution removed by vitamin C. The color intensity of 2,6-dichlorophenol indophenol was very time-dependent because the reaction results of 2,6-dichlorophenol indophenol with Vitamin C are getting lost over time. This can affect the absorbance obtained and directly affect the determination of vitamin C levels [14].

3. Conclusion

In conclusion, this study showed the potential of *Cucumis mel* and *Averrhoa bilimbi* as natural sources of vitamin C. Further studies about the potential content of other antioxidants in these fruits is advisable.

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