

Identification of Flavonoid Content in *Cucumis Sativus L.* Peels with Dissimilar Polarity Solvents

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Abstract

The study aimed to quantify flavonoid content in two types of *Cucumis Sativus L.* peels (regular and Japanese variants) using solvents with different polarities. The solvents utilized were water, 50% ethanol and 50% acetone which the yield was compared, and the flavonoid was extracted. The Shinoda test was employed to qualitatively determine the presence of flavonoids, indicated by changes to pale pink colour and colour disappearance upon the addition of HCl and magnesium ribbon. Quantitative analysis was performed using the aluminum chloride colorimetric method. Japanese cucumber peel extracted with 50% ethanol had the highest total flavonoid content (TFC) of 2.20 mg QE/g, while regular cucumber peel extracted with 50% ethanol had a total flavonoid content (TFC) of 1.20 mg QE/g. The study indicated that solvent polarity significantly influences flavonoid extraction, with ethanol being more effective for Japanese cucumber variants.

1. Introduction

Cucumis sativus L., also known as cucumber, is a highly esteemed fruit vegetable that holds great importance in the global economy because of its diverse applications. It is globally cultivated and is one of the most ancient, cultivated crops, thought to be indigenous to Asia [1]. The presence of ample amounts of water, nutrients, and phytochemicals in cucumbers enhances their health-promoting properties and highlights their economic significance as a versatile fruit vegetable that finds applications in various healthcare and food industries.

Regular and Japanese cucumbers are among the several varieties of cucumbers that exist because of variances in farming methods, regional tastes, and particular applications. Farmers have planted cucumbers that fit many climates, soil conditions, and cooking requirements over time [2]. Usually grown for their size and robustness, regular cucumbers fit a range of dishes and have a longer shelf life [3]. Conversely, Japanese cucumbers, also known as *Kyuri*, which are favoured for their thin form, soft skin, and mild taste which is desired in salads and sushi. Different kinds of cucumbers reflect the agricultural creativity and cultural tastes influencing food systems.

Cucumbers are not only refreshing and hydrating, but they also provide notable health advantages because of the presence of flavonoids. These compounds are secondary metabolites that possess antioxidant properties and have been extensively investigated for their potential therapeutic benefits [4,5]. The health-promoting properties of cucumbers are enhanced by the presence of flavonoids, such as quercetin and kaempferol, which help to eliminate harmful free radicals and decrease oxidative stress. The antioxidant properties of this action can aid in the prevention of chronic illnesses and have been linked to a reduced likelihood of developing cardiovascular diseases, specific forms of cancer, and neurodegenerative disorders [5].

The solubility of a compound in a solvent is largely determined by the principle of "like dissolves like", as flavonoids also differ in their polarity. Polar solvents are more efficient in extracting polar compounds, such as flavonoid glycosides, whereas less polar solvents are more suitable for non-polar compounds, such as flavonoid aglycones. As a result, different polarities of solvents can be utilized to selectively extract flavonoids. Palaiogiannis et al. (2023) claim, for example, that a wider range of polyphenolic compounds can be selectively dissolved using a series of solvents with increasing polarity, improving the general extraction yield [6].

The extraction of flavonoids from cucumber peel is a critical process that requires careful consideration of the solvents used to ensure maximum yield. Based on the previous study of flavonoid content from different parts of cucumber, according to Yunusa et al. (2018), ethanolic peel extract has the highest value of flavonoid content compared to aqueous extract by using the maceration method [7]. In another study with a different method, according to Ji et al. (2011), no flavonoids were detected in cucumber peels with aqueous extract [8]. The choice of solvents like water, ethanol, and acetone are also relatively safe and cost-effective. With being effective in dissolving phenolic compounds, their use minimizes health risks and environmental impact compared to more hazardous solvents. Such a ratio that was used is essential for plant extraction since a higher solid-to-solvent ratio can increase the concentration gradient, enhancing the diffusion rate and allowing more efficient solids extraction by the solvent [9].

The Shinoda test is a qualitative method used to detect the presence of flavonoids in plant extracts. The test specifically targets flavonoids, making it a reliable method for their identification. When flavonoids are present, the reaction produces a distinct color change, typically pink or red, which is easy to observe [10]. While the aluminum chloride colorimetric assay is widely used for the quantitative detection of flavonoids. The method is highly sensitive, capable of detecting even small amounts of flavonoids. This makes it suitable for analyzing samples with low flavonoid concentrations [11]. Thus, proper extraction techniques are vital to maximizing the flavonoid yield. This includes optimizing the solvent concentration, temperature, extraction time, and the ratio of plant material to solvent. Hence, the objective of this study was to quantify the flavonoid content in regular and Japanese cucumber by utilizing different solvents with varying polarity (water, 50% ethanol and 50% acetone).

2. Materials and Methods

2.1 Chemicals

95% Quercetin was purchased from Sigma Aldrich (M) Sdn. Bhd. (Malaysia). 99.8% ethanol, 99.5% acetone, 37% hydrochloric acid, and 97% aluminum chloride (III) were analytical grades from QRec (Malaysia).

2.2 Plant Materials

The cucumber fruits, both regular and Japanese variants, were obtained from a supermarket in Pagoh, Malaysia. The entire cucumber was rinsed with tap water and properly stripped of its peel. The peel sections were washed with distilled water, sliced into small fragments and dried under shade conditions [7].

2.3 Extract Preparation

Essentially, the ratio of 1:10 was used where 10 g of fresh regular cucumber peels were weighed and placed in a beaker with 100 mL of distilled water. The mixture was heated at 60 °C for 30 minutes, with occasional stirring. It was allowed to cool to room temperature [8]. The mixture was then filtered through Whatman filter paper. The steps were repeated using various solvents, including 50% ethanol and 50% acetone, as well as Japanese cucumber [12]. The extracts were kept in the fridge for qualitative and quantitative analysis.

2.4 Plant Yield Determination

The percentage yield of all extracts was calculated using the following formula:

$$\% \text{ yield} = \frac{W_2 - W_1}{W_0} \times 100 \quad (\text{equation 1})$$

where W_1 is the initial weight of filter paper, W_2 is the weight of dried sample with filter paper after extraction, and W_0 is the initial weight of dried sample.

2.5 Qualitative Determination (Screening)

2.5.1 Flavonoid Test (Shinoda)

Four drops of concentrated HCl were added to the extract solutions, followed by the addition of two pieces of 9 mm of Magnesium ribbon. The presence of flavonoids was indicated by colour changes where the appearance of a pale pink colour and the disappearance of colour in certain solutions were observed [13].

2.6 Quantitative Determination

2.6.1 Total Flavonoid Content (TFC)

The concentration of flavonoids in plant extracts was measured using the aluminum chloride colorimetric method with minor adjustments [14,15]. The standard calibration curve was constructed using Quercetin. A solution of quercetin was made by dissolving 1.5 mg of quercetin in 15 mL of ethanol. The standard solutions of quercetin were then prepared by diluting the stock solution with ethanol in a range of 20-500 µg/mL. 885 µL of standard or sample solution was added, followed by 935 µL of ethanol. Subsequently, 865 µL of AlCl₃ (prepared in distilled water at a concentration of 10% w/v) and 1000 µL of distilled water were added to the mixture. Following the mixing process, the solution was left to incubate for 30 minutes at room temperature. The absorbance of the reaction mixtures was measured at a wavelength of 417 nm using a Hitachi UV-VIS spectrophotometer U-3900/3900H, against a blank (without AlCl₃) that was prepared simultaneously [15]. The total flavonoid content in the test samples was determined by using the calibration curve plot in Figure 1 ($y = 0.0022x + 0.0156$, $R^2 = 0.995$) and expressed as mg of quercetin equivalent (QE) per g of sample. All measurements were taken in triplicate.

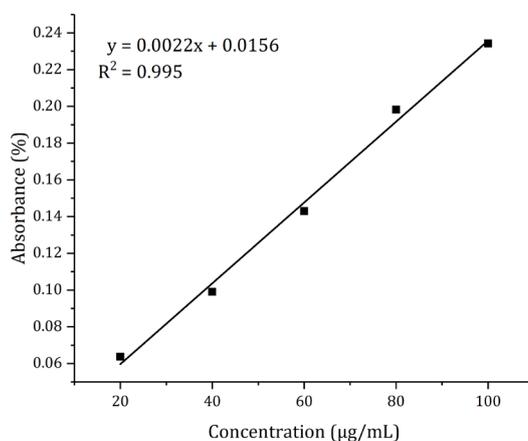


Fig. 1 Calibration curve for quercetin solution as standard

3. Results and Discussion

The percentage yield of each sample following the extraction process was determined using (equation 1) and the findings are presented in Table 1 below.

Table 1 Percentage yield of cucumber extraction (%) in different solvents

Cucumber variant	Yield (%)		
	Distilled water	50% Ethanol	50% Acetone
Regular	41.41	26.41	21.04
Japanese	38.33	25.36	29.57

The results demonstrated that the utilization of different solvents led to varying extract yields, which can be attributed to the composition and quantity of secondary metabolites extracted. In summary, the data presented in Table 1 indicates that distilled water resulted in a greater yield when extracting regular cucumber and Japanese cucumber. The high solubility of water as a solvent can enhance the dissolution of certain flavonoids, especially those with greater polarity, resulting in a greater yield. Flavonoids can also establish hydrogen bonds with water

because they contain a significant number of hydroxyl groups. According to Van Ngo et al. (2017), these interactions can increase the ability of flavonoids to dissolve and be extracted into the water phase. This leads to a greater number of flavonoids being obtained compared to using less polar solvents such as ethanol and acetone [16]. The efficacy of water in extracting flavonoids can be ascribed to its capacity to penetrate plant tissues and dissolve water-soluble compounds efficiently. This can result in an increased extraction yield. Although ethanol and acetone mixtures with water can extract a diverse array of compounds, they may not be as efficient as pure water in selectively extracting specific polar flavonoids. The use of ethanol or acetone may also result in the precipitation of certain flavonoids, thereby decreasing the overall yield based on Ziani et al. (2022) findings [17]. Simply put, when used as a solvent, distilled water produced a greater amount of extract compared to a mixture of 50% ethanol and 50% acetone in both types of cucumbers.

The presence of flavonoids in both cucumber variants is determined using the Shinoda test. The outcomes of this test are depicted in Figure 2, where (a) refers to the sample conditions before the screening test and (b) after the sample has carried out the test. The samples of regular (R) and Japanese (J) cucumber were placed in test tubes with different solvents as shown in (a). The addition of concentrated HCl and Mg ribbons resulted in the observation of all color changes as shown in (b) where a reduction reaction occurs. The vibrant color of each sample extract changed to a pale pink color of R in 50% ethanol and for both cucumber variants in 50% acetone. However, the color completely disappeared when the samples were placed in distilled water for both samples. When J was placed in a solution of 50% ethanol, it transformed and became a highly concentrated yellow hue. These findings demonstrate the presence of flavonoids in every sample of cucumber peel.

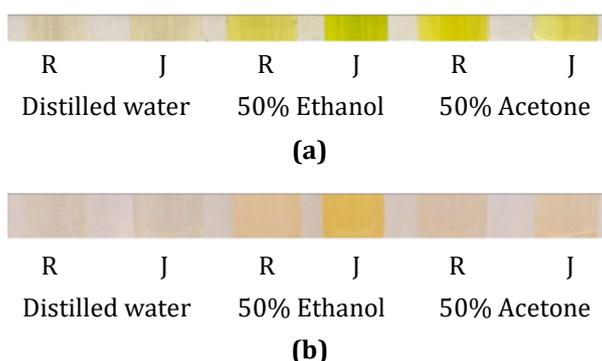


Fig. 2 Test tubes showing the color changes from (a) to (b) of the cucumber peel extracts in different solvents

The total flavonoid contents (TFC) of extracts obtained by using different solvents were measured and tabulated in Table 2 below. In this study, the total flavonoid contents (TFC) of the extracts obtained were measured using different solvents, as shown in Table 2. The Japanese cucumber variant extracted with 50% ethanol has the highest total flavonoid content (TFC) of 2.20 mg QE/g. For both cucumber variants, the 50% acetone extracts showed higher TFC values compared to those obtained with distilled water. The regular cucumber variant had a higher TFC when extracted with 50% ethanol (1.20 mg QE/g) compared to distilled water (0.66 mg QE/g) and 50% acetone (0.96 mg QE/g). So, the choice of ethanol and acetone, being polar organic solvents, are particularly effective in dissolving phenolic compounds, leading to higher extraction efficiency and better bioactivity results. Based on El-Chaghaby et al. (2011) findings, these solvents are known to be more efficient than water in extracting phenolics, which are crucial for antioxidant and antibacterial activities [18]. This finding suggests that 50% ethanol is a more effective solvent for extracting flavonoids from cucumbers, especially the Japanese variant, compared to distilled water and 50% acetone [19].

Table 2 Total flavonoid contents (TFC) of 6 extracts from two cucumber variants

Cucumber variant	TFC (mg of quercetin equivalent/g of sample), mg Qe/g		
	Distilled water	50% Ethanol	50% Acetone
Regular	0.66	1.20	0.96
Japanese	0.60	2.20	1.07

4. Conclusion

The study demonstrated that different solvents affect the yield of flavonoid extraction from cucumber peels. Distilled water yielded a higher extraction rate for both regular and Japanese cucumber variants. However, the

Japanese cucumber variant extracted with 50% ethanol showed the highest total flavonoid content (2.20 mg QE/g), suggesting that ethanol is a more effective solvent for extracting flavonoids for both cucumbers, particularly in the Japanese variant. The Shinoda test results also showed color changes for all samples indicating the presence of flavonoids, supporting that solvent polarity influences extraction efficiency with polar solvents like water being efficient in yield extraction, while ethanol is more efficient for polar flavonoids.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

*The authors confirm their contribution to the paper as follows: **study conception and design:** Muhammad Azib Mu'azzam Shah, Lim Zhe Yi, Muhammad Amin Fathihin Zarizan; **data collection:** Muhammad Azib Mu'azzam Shah, Lim Zhe Yi, Muhammad Amin Fathihin Zarizan; **analysis and interpretation of results:** Muhammad Azib Mu'azzam Shah, Lim Zhe Yi, Muhammad Amin Fathihin Zarizan; **draft manuscript preparation:** Muhammad Azib Mu'azzam Shah, Lim Zhe Yi, Muhammad Amin Fathihin Zarizan, Saliza Asman. All authors reviewed the results and approved the final version of the manuscript. The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.*

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