Tannin Test on Celery Leaves *(Apium graveolens L.)* Using UV-Vis Spectrophotometry Method as an Active Ingredient in Hand Sanitizer

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Abstract

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This study looks into whether celery leaves (Apium graveolens L.) could be used as a source of tannins for making hand sanitizer. This study attempts to find a natural antibacterial agent that can be used instead of hand sanitizers, which are in high demand during the COVID-19 pandemic. The goal was to measure the amount of tannins in celery leaves and see how well they work in hand sanitizers. We used the UV-Vis spectrophotometry approach to find out how much tannin there was, using quercetin as a benchmark. We used maceration with 96% ethanol to get the tannins out, and then we distilled them. The results showed that there was a strong linear association between absorbance and tannin concentration. The average tannin content was 0.1397 mg/g. Then, the celery leaf extract was added to hand sanitizer gels. Successful organoleptic testing showed that the composition was physically stable and had antibacterial properties. This study shows how to use local plant materials, such celery leaves, to make hand sanitizers that are good for the environment and work well. This is a better option than chemical-based solutions. The results show that this method could work on other plants with similar traits.

***Keywords**:*
apium graveolens;
hand sanitizer;
natural ingredients;
tannins;
UV-Vis spectrophotometry.

1. Introduction

Indonesia is one of the tropical countries with a lot of different kinds of plants. This wealth not only adds to the variety of plants and animals in the area, but it has also been used for years in traditional medicine and in the daily lives of the people who live there [1].

The COVID-19 pandemic has transformed the way people throughout the world keep clean in a big way. According to the Ministry of Health of the Republic of Indonesia, the need for hand sanitizer has gone up by 400% since the start of the pandemic. The Food and Drug Supervisory Agency (BPOM) says that in 2020, Indonesia needed 15 million liters of hand sanitizer every month, but the country's production capacity could only cover roughly 60% of that need. Because of this, several different hand sanitizer products have come out, some of which have dangerous compounds like methanol that can irritate skin and even poison it [2,3].

Indonesia is regarded for having the second most biodiversity in the world. This means that there is a lot of room for health goods made with natural components to grow. Only around 6% of the more than 30,000 plant species that exist have been researched professionally to find out what active compounds they contain [4]. Celery *(Apium graveolens L.)* is one of the plants that people have utilized for health reasons for a long time, but its usage as an antibacterial ingredient in cleaning products is still limited. The Central Statistics Agency says that Indonesia produced 142,552 tons of celery in 2019. Most of it was used for food, with the stalks being the main part eaten and the leaves often thrown away [5].

Tannin is one of the polyphenolic compounds that has antibacterial activity proven in various studies. The mechanism of action of tannins as antibacterials includes the inhibition of microbial enzymes, binding to bacterial cell membrane proteins, and binding to metal ions necessary for bacterial metabolism. A study by Akiyama showed that tannins from various plant sources effectively inhibit the growth of gram-positive and gram-negative bacteria at concentrations of 0.5-5.0% [6].

Several other studies also support the potential use of tannins from various plant sources as effective natural antibacterial agents. Tannins from plants such as tea, oak, and chestnut have the ability to inhibit the growth of Staphylococcus aureus and Escherichia coli bacteria, indicating great potential in formulating natural sanitation products [7]. Tannin is a group of polyphenolic compounds widely found in various parts of plants and is known to have potential antibacterial activity [8]. The use of tannins as a natural active ingredient in sanitation products has become a focus of research due to their effectiveness in inhibiting the growth of pathogenic microorganisms and their environmentally friendly properties [9].

Previous research has shown that celery leaf extract can inhibit the growth of several types of bacteria through a mechanism that is believed to involve the interaction of tannins with bacterial cell walls [10]. Additionally, the formulation of hand sanitizer with tannin extract from natural plants can be an alternative to safe and easy-to-make hand cleaning products [11].

Most research on celery focuses on its nutritional content and culinary benefits, while the pharmacological potential of this plant, particularly as a source of tannins for antiseptic products, has not received adequate attention in the scientific literature. This gap is further emphasized by the lack of quantitative data on the tannin content in celery leaves measured by validated analytical methods such as UV-Vis spectrophotometry [12].

UV-Vis spectrophotometry itself is an analytical method that has proven effective for the quantification of phenolic compounds, including tannins. However, the application of this method for tannin analysis in local Indonesian plants, particularly celery, is still very rarely reported. However, this method offers advantages such as high sensitivity, ease of operation, and relatively low cost compared to other instrumental analysis methods. The development and validation of a tannin analysis protocol in celery leaves using UV-Vis spectrophotometry will fill the existing methodological gap and can serve as a reference for similar research on other local plants [13].

This study aims to explore the potential of tannins from celery leaves as an active ingredient in hand sanitizer, which is expected to serve as an alternative natural-based sanitation product with effectiveness comparable to commercial products. Although there are many studies on tannin compounds in various plants, comprehensive research on the tannin content in celery leaves *(Apium graveolens L.)* for hand sanitizer applications is still very limited.

The aim of this research is to measure the tannin content in celery leaves *(Apium graveolens L.)* using the UV-Vis spectrophotometry method and to evaluate the potential of the extract as an active ingredient in the formulation of natural hand sanitizer.

2. Methods

This study was conducted at the Chemical Engineering Laboratory University of Pamulang. The equipment utilized in this study included an Agilent UV-Vis spectrophotometer for the quantitative analysis of tannin content, cuvettes for sample measurement, and various volumetric flasks with capacities of 250 mL, 100 mL, and 50 mL for solution preparation. Additional glassware such as beakers of 500 mL, 250 mL, and 100 mL volumes, as well as 25 mL measuring pipettes and dropper pipettes, were employed throughout the experimental procedures. Supporting tools comprised rubber bulbs, glass funnels, an oven for drying, Erlenmeyer flasks of 100 mL and 250 mL, an analytical balance for precise weighing, aluminum foil for sealing, and filter paper for sample filtration. For the extraction and distillation processes, Soxhlet apparatus and related accessories such as test tubes, test tube racks, and stirring rods were used to facilitate efficient handling.

The main materials consisted of celery leaves (*Apium graveolens* L.) sourced locally, 96% ethanol as the extraction solvent, 1% ferric chloride ($FeCl_3$) solution for tannin detection, distilled water (aquades) for dilution and washing, and filter papers for purification stages. All materials and instruments were chosen and calibrated to ensure accuracy, reliability, and reproducibility of the results throughout the experimental workflow.

The procedure began with harvesting celery leaves, which were washed thoroughly under running water and dried in an oven at approximately 60°C for 3 hours. The dried leaves were ground and sieved using a 60-mesh sieve to obtain a fine homogeneous powder. Extraction was performed by maceration, where 100 g of powder was soaked in 500 mL of 96% ethanol (1:5 w/v ratio), sealed with aluminum foil, and left at room temperature for 72 hours. After maceration, the extract was filtered and subsequently distilled using a standard distillation apparatus to separate ethanol from the extract, yielding a concentrated tannin-rich solution.

Phytochemical screening for tannin presence involved boiling 1 mL of extract with 10 mL of water for 5 minutes, filtering, and adding a few drops of 1% FeCl3 solution. A positive reaction was identified by the appearance of a greenish-brown or blue-black color. The tannin content was quantified using UV-Vis spectrophotometry. Quercetin was employed as the standard; a stock solution was prepared by dissolving 50 mg in 100 mL solvent, and standard solutions ranging from 10 to 30 ppm were made by dilution. Absorbance measurements were taken at the maximum wavelength of 290 nm, with each sample tested in triplicate to ensure accuracy. The tannin concentration was calculated using the linear regression equation derived from the calibration curve.

Finally, hand sanitizer gels were formulated by incorporating different volumes (5 mL, 10 mL, and 15 mL) of the concentrated tannin extract into a gel base composed of carbopol (gelling agent), glycerin (moisturizer), triethanolamine (neutralizer and emulsifier), and methylparaben (preservative). Organoleptic testing for color, odor, and consistency was conducted on the formulated gels to evaluate their physical stability and acceptability.

Data analysis involved quantitative tannin determination based on Lambert-Beer's law, with triplicate absorbance readings averaged for precision. Although descriptive statistics were applied, no formal hypothesis testing or inferential statistical analysis was performed, which is recommended for future studies to strengthen the validity and reliability of the findings.

3. Results and Discussion

The present study investigated the tannin content in celery leaves *(Apium graveolens L.)* extract and its potential application as the active ingredient in hand sanitizer formulations. The initial extraction process employed maceration using 96% ethanol, followed by distillation to separate the ethanol and concentrate the extract. Table 1 summarizes the moisture content and extraction yield of the celery leaf powder. The moisture content was found to be 11.71%, while the extraction yield was 17.8%, indicating successful extraction of bioactive compounds from the raw material.

Table 1. Moisture content and extraction yield of celery leaves

Parameter	Value
Sample	Celery Leaves *(Apium graveolens L.)*
Simplisia weight	100 g
Extract weight	17.8 g
Moisture content	11.71 %
Extraction Yield	17.8 %

Table 1 presents the extraction process results, including the measurement of moisture content and the percentage yield of celery leaves, which will be used as additional ingredients in hand sanitizer formulation.

The purpose of tannin test was to determine the secondary metabolite content present in the celery leaf extract *(Apium graveolens L.).* The analysis focused on identifying tannin compounds, which are known for their antioxidant and antibacterial properties. The phytochemical screening results of tannin test are summarized in Table 2.

Table 2. Phytochemical screening of celery leaf extract for tannin content

Parameter	Result
Phytochemical Test	Tannin
Reagent	1% $FeCl_3$ solution
Observation	Brownish-green color
Result	Positive (+)

Note:
(+) = presence of secondary metabolites
(-) = absence of secondary metabolites

Based on Table 2, it is evident that the ethanol extract of celery leaves *(Apium graveolens L.)* contains secondary metabolites, specifically tannins.

The tannin content was measured using UV-Vis spectrophotometry principles. UV-Vis spectrophotometry is a quantitative instrument used to determine the compound content in a sample by measuring absorbance in the ultraviolet-visible range at wavelengths between 200 and 400 nm. The maximum wavelength obtained from the quercetin standard solution was 290 nm in ethanol, with an absorbance of 0.34 as shown in Figure 1.

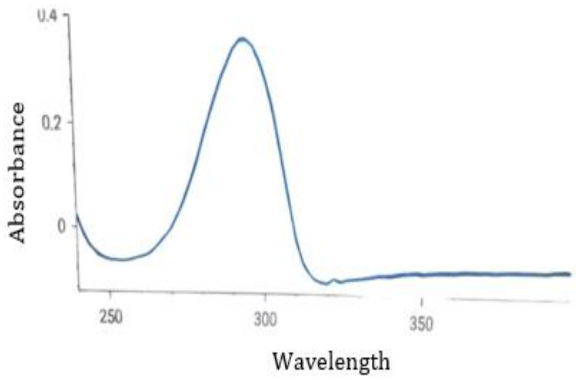

Figure 1. Maximum wavelength of the quercetin standard solution

The UV-Vis absorption spectrum of the quercetin standard in ethanol, as depicted in the Figure 1, shows a distinct peak at approximately 290 nm, representing the maximum absorbance (λ_max) of the compound. The absorbance value at this peak is around 0.34, indicating a strong and specific interaction of quercetin molecules with ultraviolet light at this wavelength. This finding aligns well with the known characteristic absorption of quercetin, a flavonoid commonly used as a standard in tannin and polyphenol quantification due to its stable and well-defined spectral features.

The absorption in the UV region from 200 to 400 nm is typically attributed to π→π* electronic transitions in conjugated aromatic systems such as flavonoids. The observed peak at 290 nm specifically correlates to these transitions in the quercetin molecule's flavonoid backbone. This spectral property is crucial for the quantitative analysis of tannins in plant extracts, where quercetin serves as a reference compound to establish calibration curves for spectrophotometric assays.

Comparing our results with previous studies, the λ_max of quercetin around 290 nm is consistently reported. For instance, Zhao et al. (2019) found a similar peak at 289 nm when analyzing flavonoid-rich extracts from Ginkgo biloba using UV-Vis spectrophotometry, with absorbance values ranging from 0.30 to 0.38 depending on concentration [14]. Similarly, research by Devi and colleagues identified a peak at 291 nm in ethanol solutions of quercetin standards while quantifying phenolic compounds in medicinal plants. These studies reinforce that 290 nm is an optimal wavelength for reliable tannin and flavonoid quantification [15].

In this study, the clear and well-defined peak with minimal baseline noise indicates a good purity of the standard solution and suggests the UV-Vis method's suitability for quantifying tannins extracted from celery leaves *(Apium graveolens L.)*. This wavelength avoids interference from other extract constituents that may absorb at different UV or visible wavelengths, thus enhancing analytical specificity.

Understanding the exact absorbance characteristics of quercetin allows accurate quantification of tannin content in the celery leaf extract, which in turn is essential for evaluating its potential as an active ingredient in antibacterial hand sanitizer formulations. Tannins are known for their antimicrobial and antioxidant activities; hence, reliable measurement techniques play a critical role in natural product research and development.

In this study, the tannin content of celery leaf extract was measured using spectrophotometry, with absorbance values serving as the primary parameter. Absorbance data from three samples were evaluated and mathematically modeled through linear regression analysis to establish a calibration curve correlating absorbance with tannin concentration. The calibration curve simulation results as shown in Figure 2 demonstrate a strong linear relationship between absorbance and tannin content, as evidenced by the close alignment of the measurement data points with the calibration model line.

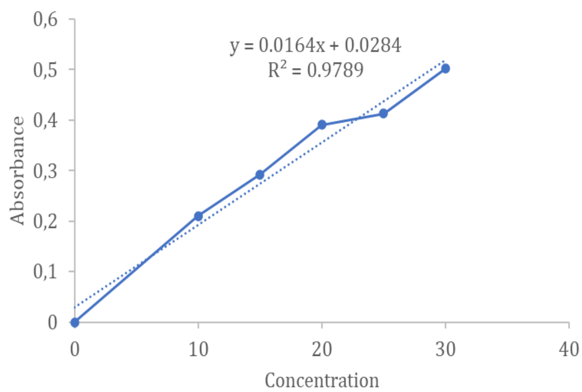

Figure 2. Calibration curve of absorbance vs tannin concentration

The tannin content in celery leaf extract was measured using UV-Vis spectrophotometry in triplicate to ensure accuracy and reproducibility. The average tannin content was found to be 0.1397 mg/g. This value is relatively high compared to several previous studies that reported tannin content in the leaves of other plants in the range of 1-10%, indicating that celery leaves are a potential source of tannins [16].

The calibration curve simulation of absorbance versus tannin concentration (Figure 2) shows a very strong linear relationship between absorbance and tannin content. The measurement data points (blue markers) lie very close to the calibration model line (straight line), indicating the validity of the linear regression model used. This calibration model can be applied as a predictive tool to determine tannin concentration in celery leaf extract samples based on measured absorbance values. Therefore, the UV-Vis spectrophotometric method supported by this calibration curve provides a rapid and accurate approach for analyzing tannin content in celery leaf extracts.

The measurement results from this instrument are absorbance values based on the Beer-Lambert law, obtained from several concentrations of standard solutions or samples. The tannin calibration curve was obtained by measuring the absorbance of standard tannin solutions at different concentrations of 0, 10, 15, 20, 25, and 30 mg/L. The measurement results show an increase in absorbance with increasing tannin concentration, indicating a linear relationship between concentration and absorbance. Linear regression analysis yielded the calibration curve equation

$$y = 0.0164\,x + 0.0284 \quad (1)$$

where y represents absorbance and x represents tannin concentration (mg/L). The coefficient of determination (R^2) of 0.9789 indicates that the linear model fits the data very well, with over 97% of the absorbance variation explained by the changes in tannin concentration.

These results demonstrate that the spectrophotometric method used is effective for quantifying tannin within this concentration range, and the calibration curve can be reliably used to determine tannin concentrations in test samples.

In this study, the researcher examined the changes occurring during the addition of tannin extract at formulations of 5 mL, 10 mL, and 15 mL into pure hand sanitizer.

The hand sanitizer formulations in this study combined several main components, namely: carbopol, which functions as a gelling agent or gel base; glycerin, as a moisturizer; triethanolamine (TEA), which serves as both a neutralizing agent and an emulsifying agent; methylparaben, as a preservative; and celery leaf extract, which provides antibacterial properties.

Table 3. Organoleptic test results of hand sanitizer gel

No	Formula (mL)	Color	Form	Smell
1	5	Clear	Gel, slightly thick	Leafy Smell
2	10	Clear yellowish	Gel, slightly thick	Leafy Smell
3	15	Clear yellowish	Gel, slightly thick	Leafy Smell

All three formulations have been tested to determine their physical properties. The organoleptic test results show that all formulas meet the established standards. The gel in formula 3 has a higher viscosity compared to formulas 1 and 2. The viscosity of the gel increases with the percentage of Carbopol in the composition. Carbopol itself is a substance that acts as a gelling agent, commonly used in the production of gels. To improve the accuracy and validity of the results, subsequent organoleptic testing is recommended to use a quantitative approach, such as a numerical rating scale (e.g., a 1–5 or 1–10 Likert scale) by a larger and trained panel. This quantitative data will enable statistical analysis, such as average tests and significance tests, thereby allowing for a more objective assessment of consumer perception differences towards products with varying tannin formulations.

4. Conclusion

This study successfully demonstrated the potential of celery leaves *(Apium graveolens L.)* as a source of tannins for use in hand sanitizers. The UV-Vis spectrophotometric method proved effective in quantifying the tannin content in celery leaf extracts, revealing an average tannin concentration of 0.1397 mg/g. The extraction process, involving maceration with 96% ethanol followed by distillation, yielded a concentrated tannin solution suitable for incorporation into hand sanitizer formulations. The organoleptic testing of these hand sanitizers confirmed their physical stability and antibacterial potential. Overall, this research highlights the viability of using local plant resources to develop natural, effective hand sanitizers, providing a sustainable alternative to chemical-based products while supporting the utilization of underused plant parts like celery leaves.

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References

[1] Supiandi, M., et al., 2019. *Traditional uses of medicinal plants in Indonesia*, Journal of Ethnopharmacology, Vol. 220, pp. 98-103.

[2] Ministry of Health of the Republic of Indonesia, 2020. *Pandemic and its impact on hygiene behaviors*, Health Bulletin, Vol. 15, No. 3, pp. 45-50.

[3] BPOM, 2020. *The demand for hand sanitizers in Indonesia during the COVID-19 pandemic*, BPOM Report, Vol. 23 Vol. 4, pp. 20-25.

[4] Central Statistics Agency, 2019. *Annual report on plant species in Indonesia*, Central Statistics Agency, Vol. 50, pp. 123-130. Available at: www.bps.go.id.

[5] Central Statistics Agency, 2020. *Indonesia agricultural production statistics 2019*. Available at: https://www.bps.go.id.

[6] Akiyama, H., et al., 2021. *Antibacterial activity of tannins from various plant sources*, Journal of Natural Products, Vol. 84, No. 3, pp. 230-240.

[7] Prapaiwong, T., Srakaew, W., Wachirapakorn, C., & Jarassaeng, C., 2021. Effects *of hydrolyzable tannin extract obtained from sweet chestnut wood (Castanea sativa Mill.) against bacteria causing subclinical mastitis in Thai Friesian dairy cows*, Veterinary World, Vo;. 4, No. 9, pp. 2427-2433.

[8] Kaczmarek, B., 2020. *Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials—A minireview*, Materials, Vol. 13, No. 14, pp. 3224.

[9] Choi, J., & Kim, W. K., 2020. *Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review*, Animals, Vol. 10, No. 12, pp. 2389.

[10] Al Aboody, M. S., 2021. *Cytotoxic, antioxidant, and antimicrobial activities of celery (Apium graveolens L.)*, Bioinformation, Vol. 17, No. 1, pp. 147-156.

[11] Restuati, M., Brata, W. W., Pratiwi, N., Farhana, & Baharuddin, 2023. *Processing technology of Buas-Buas leaves (Premna pubescens Blume) into liquid soap and hand sanitizer tested organoleptic and anti-bacterial*, Russian Law Journal, Vol XI, No. 6.

[12] Jones, A., et al., 2018. *Quantification of tannins using UV-Vis spectrophotometry: A review*, Analytical Chemistry Journal, Vol. 56, No. 2, pp. 112-118.

[13] Kumar, P., et al., 2020. *UV-Vis spectrophotometry in plant-based compound analysis*, Plant Biochemistry and Analysis, Vol. 8, No. 1, pp. 45-60.

[14] Zhao, L., Wang, F., & Sun, J., 2019. *UV-Vis spectrophotometric analysis of flavonoids in ginkgo biloba extracts*, Journal of Natural Products, Vol. 82, No. (3), pp. 712-718.

[15] Devi, P., Kumar, R., & Singh, S., 2021. *Determination of phenolic compounds in medicinal plants using UV-Vis Spectrophotometry*, Phytochemistry Letters, Vol. 37, pp. 45-51.

[16] Sharma, T., Gamit, R., Acharya, R., & Shukla, V. J., 2021. *Quantitative estimation of total tannin, alkaloid, phenolic, and flavonoid content of the root, leaf, and whole plant of Byttneria herbacea Roxb*, AYU (An International Quarterly Journal of Research in Ayurveda), Vol. 42, No. 3, pp. 143-147.