HYDROGEN
JURNAL KEPENDIDIKAN KIMIA
https://e-journal.undikma.ac.id/index.php/hydrogen/index

December 2025. 13(6)
e-ISSN: 2656-3061
p-ISSN: 2338-6487
pp. 1086-1094

Antioxidant Capacity and Phytochemical Profile of *Crescentia cujete L.* Fruit Peel Extract from The Serang Region

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Article History
Received: 30-11-2025
Revised: 06-12-2025
Published: 31-12-2025

Keywords: *Crescentia cujete*, antioxidant, TEAC, ABTS, DPPH, phytochemical profile

Abstract
Crescentia cujete is a plant commonly found in the Serang region; however, its fruit peel remains largely underutilized, and studies regarding its bioactivity potential are minimal. This research utilized dried, pulverized *C. cujete* fruit peel, which was extracted using the Soxhlet method with 96% ethanol to yield the ethanolic extract. Qualitative phytochemical screening was conducted to identify the secondary metabolite classes . Antioxidant capacity was measured using ABTS$\bullet^+$ radicals, and DPPH values were expressed as IC_{50}. Phytochemical results showed the presence of flavonoids, phenols, alkaloids, terpenoids, steroids, and saponins. The DPPH assay yielded a high IC_{50} value of 1166.68 ppm, classifying the extract as having very weak antioxidant activity. Conversely, the TEAC method demonstrated high antioxidant capacity, yielding a value of 171.37$\pm$4.03 mM TE/g. The significant difference between the two methods suggests that antioxidant effectiveness is strongly influenced by the radical and chemical properties of the constituent compounds. The novelty of this study lies in the comparative evaluation of the antioxidant activity of *C. cujete* fruit peel using two different radical systems and in the finding that the extract exhibits selective, strong activity against the ABTS radical. *C. cujete* fruit peel is potentially a source of natural antioxidants.

How to Cite: Purnamasari, M. (2025).Antioxidant Capacity and Phytochemical Profile of Crescentia cujete L. Fruit Peel Extract from The Serang Region. Hydrogen: Jurnal Kependidikan Kimia, 13(6), 1086–1094. https://doi.org/10.33394/hjkk.v13i6.18592

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INTRODUCTION

Free radicals are reactive molecules capable of inducing oxidative damage in cells and tissues. Oxidative stress is defined as an imbalance between the production of free radicals and the body's capacity to neutralize them; this condition contributes to the development of various degenerative diseases, including cancer, diabetes mellitus, atherosclerosis, premature aging, and inflammatory disorders (Liguori et al., 2018). To counteract the damaging effects of free radicals, antioxidants play an important role by donating electrons and neutralizing them before they cause damage to biomolecules. The search for natural antioxidant sources has escalated due to concerns regarding the potential toxicity of synthetic antioxidants, such as BHA and BHT (Shahidi & Ambigaipalan, 2015). Therefore, exploring bioactive compounds from indigenous plants represents a crucial approach for developing safe, sustainable antioxidant raw materials.

Plants contain various secondary metabolites that play roles in biological defense, including flavonoids, phenolics, alkaloids, terpenoids, steroids, and saponins. These compounds are known to have antioxidant activity through free radical scavenging, oxidation inhibition, and metal chelation mechanisms (Panche et al., 2016). Phytochemical analysis of plant extracts is a prerequisite step for identifying secondary metabolites with potential pharmacological activities, including antioxidant activity (Hasanah et al., 2017). One plant with potential for study is Crescentia cujete L., also known as berenuk/beluluk fruit. Historically, various parts of the *C. cujete* plant have been utilized in traditional medicine for treating inflammation, hypertension, and infections (Gonzales, Sevilla, et al., 2023).

The leaf extract of *Crescentia cujete* exhibits moderate to high antioxidant activity as determined by both the DPPH and ABTS assays, which is attributed to its phenolic and flavonoid constituents (Gonzales, Huang, et al., 2023). In another study, the leaf and bark extracts of *C. cujete* demonstrated strong anti-inflammatory activity—particularly the bark extract—as well as notable antibacterial effects, thereby indicating substantial potential as a therapeutic agent (Parvin et al., 2015).

Previous research by Januário et al. (2022) reported that *the Crescentia cujete* extract exhibited cytotoxic activity when evaluated using the osmotic fragility method and the *Artemia salina* lethality bioassay, resulting in a mortality rate of 45.32%. Additional studies have demonstrated that *C. cujete* possesses antimicrobial properties and can be utilized in environmentally friendly nanoparticle synthesis. Its extract has been used to produce silver nanoparticles (AgNPs) via a biosynthetic mechanism, with quercetin identified by HPLC as one of its bioactive constituents (Sánchez & Fiscal Ladino, 2024).

However, the fruit peel has not been widely utilized or scientifically investigated, despite reports indicating that plant peels often harbor high concentrations of phenolic compounds and flavonoids (Singh et al., 2020; Suleria et al., 2020; Tang et al., 2021) . In the Serang region, this plant is abundant but underutilized.

In the Serang region, Crescentia cujete is relatively easy to find. However, its use remains limited, and scientific research on its phytochemical composition and antioxidant activity, especially in the fruit peel, remains rare. Exploring the potential of local plants such as this is important to support the development of local natural resources and enrich Indonesia's biodiversity data. Antioxidant activity was evaluated using the DPPH and TEAC methods, each possessing specific advantages and limitations. Specifically, the DPPH assay assesses radical reduction, whereas the TEAC method is capable of detecting both polar and non-polar antioxidants, thus providing a more comprehensive profile (Shahidi & Samarasinghe, 2025; Wołosiak et al., 2022)

Although *Crescentia cujete* is abundant in the Serang region, scientific investigations into the antioxidant potential of its fruit peel remain limited. Moreover, no studies have directly compared the performance of the DPPH and TEAC assays in assessing the antioxidant activity of *C. cujete* fruit peel extracts from this area. This lack of information results in an insufficient scientific basis for accurately determining its antioxidant capacity and for supporting its development as a potential natural antioxidant source.

METHOD

Tools and Materials

The equipment utilized in this study comprised of a set of laboratory instruments, including extraction equipment using the Soxhlet method, stirring rods, filter paper, glass funnels, Erlenmeyer flasks, measuring cylinders, blenders, volumetric flasks, test tubes, micropipettes (BioHit 1000 µL), measuring pipettes, spatulas, and vials.

Meanwhile, the materials use included the peel of Crescentia cujete L. fruit obtained from Jawilan District, Banten, 96% ethanol, Troloxs, Quersetin, ABTS, $K_2S_2O_8$, DPPH, $AlCl_3$, Mg tape, HCl, H_2SO_4, distilled water, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, 1% $FeCl_3$ solution, glacial CH_3COOH, and NaCl.

Sample Extraction

The *C. cujete* fruit peel was initially washed, cut into small pieces, and subsequently air-dried. The dried material was then ground into a fine powder. Fifty grams of the powder was accurately weighed, placed in a thimble, and extracted using a Soxhlet apparatus with 200 mL of 96% ethanol for approximately 6 hours at 78°C. The solvent was subsequently removed using a rotary evaporator at 40–50°C to yield a concentrated viscous extract. The resulting extract was stored at 4°C (Kumar & Kumar, 2025; Okoduwa et al., 2016).

Phytochemical testing (Purnamasari et al., 2022)

Phytochemical testing of *C. cujete* L. fruit peel extract was conducted in several stages.

The phenol and tannin assay were performed by adding 2 mL of 2% $FeCl_3$ solution was added to the ethanol extract. The formation of a blue-green to black color change served as an indication for the presence of phenolic compounds and tannins.

The flavonoid test involved the addition of 2 mL of a 2% sodium hydroxide solution to the ethanol extract, followed by approximately 10 drops of dilute acid solution. The solution reverting to a colorless state indicated the presence of flavonoids.

For **the steroid test**, the ethanol extract is mixed with 2 mL of chloroform to form two phases. Subsequently, concentrated H_2SO_4 solution was slowly introduced down the inner wall of the test tube. The formation of a red color in the chloroform layer (upper phase) was used to indicate the presence of steroid compounds.

For **the terpenoid test**, the concentrated ethanol extract is dissolved in 2 mL of chloroform and evaporated to dryness. Subsequently, 2 mL of concentrated H_2SO_4 was added, and the mixture was heated in a water bath for approximately 2 minutes A grayish color change indicates the presence of terpenoids.

The alkaloid test was performed by aliquoting the ethanol extract into two separate test tubes, followed by treating each with 2 mL of 1% HCl and slow heating in a water bath. The first tube is then added with Mayer's reagent, and the second tube with Wagner's reagent. The formation of a precipitate in both tubes indicates the presence of alkaloid compounds.

In **the saponin test,** the ethanol extract is diluted with 5 mL of distilled water, then shaken vigorously. The formation of stable foam following vigorous shaking was used to indicate the presence of saponin compounds.

In vitro Antioxidant Activity

Trolox equivalent antioxidant capacity assay (TEAC)

The TEAC assay was conducted as follows: a freshly prepared $ABTS^{\cdot+}$ working solution was prepared by dissolving 38.4 mg of ABTS in 10 mL of 2.5 mM $K_2S_2O_8$, thoroughly mixed, and incubated in the dark at ambient temperature for 12–16 hours. The resulting solution was subsequently diluted with methanol to achieve an absorbance of 0.70 ± 0.02. A standard calibration curve was generated using Trolox solutions prepared within the concentration range of 0–45 mM. For sample preparation, 10 mg of the *C. cujete* L. fruit peel extract was dissolved in 1 mL of methanol, sonicated for 15 minutes, and subsequently diluted to achieve a final concentration of 100 μg/mL. The antioxidant capacity, expressed as Trolox Equivalent Antioxidant Capacity (TEAC), was determined in triplicate. All measurements were reported as the mean ± standard deviation, with data processing conducted using Microsoft Excel (Purnamasari, 2024; Widodo et al., 2020).

DPPH radical scavenging activity test

The DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was prepared by dissolving it in methanol to a concentration of 0.4 µM. A volume of 825 µL of the *C. cujete* L. fruit peel extract (prepared in a final concentration series of 0–150 µg /mL) was mixed with 175 µL of DPPH solution (70 µM) and homogenized. Controls were made using the same sample volume and concentration, but without the addition of DPPH solution. The sample and control mixtures were subsequently incubated in the dark at 37°C for 30 minutes. After incubation, the samples were measured for absorbance at 517 nm using a spectrophotometer. Quercetin was used as a reference compound. The IC_{50} value was calculated using the formula:

$$\left(\frac{A_0 - A_1}{A_0}\right) x\ 100$$

Where A0 is the absorbance of the control, and A1 is the absorbance of the sample or standard (Widodo et al., 2019)

RESULTS AND DISCUSSION

Phytochemistry

Phytochemical screening of the *C. cujete* L. fruit peel extract revealed the presence of several secondary metabolites, namely flavonoids, phenolics, alkaloids, terpenoids, steroids, and saponins.

Table 1. Results of Phytochemical screening of the *C. cujete* L. fruit peel extract

Secondary Metabolites	Results
Flavonoids	+
Phenolic	+
Alkaloids	+
Terpenoids	+
Steroid	+
Saponin	+

The presence of flavonoids and phenolics generally suggests robust antioxidant activity. Flavonoids and phenolics are known to act as radical scavengers through electron donation (single electron transfer) and hydrogen atom transfer mechanisms, thereby stabilizing radicals through the formation of resonance structures (Panche et al., 2016; Shahidi & Ambigaipalan, 2015).

The detection of terpenoids and steroids also contributes to antioxidant activity. These two metabolite classes exhibit greater compatibility with $ABTS^{\bullet+}$ radicals due to their semi-polar and lipophilic properties, which typically leads to higher observed reactivity in the TEAC method compared to the DPPH method (Floegel et al., 2011; Wołosiak et al., 2022). This explains the high TEAC values obtained in this study.

Saponins and alkaloids were detected, and although their contribution to antioxidant activity is not as high as that of flavonoids, several studies show that alkaloids can act as antioxidants through radical complexation and lipid oxidation inhibition mechanisms (Rubio et al., 2016).

In vitro Antioxidant Activity

The antioxidant activity of *C. cujete* fruit peel extract was analyzed using two approaches, namely the DPPH method and the Trolox Equivalent Antioxidant Capacity (TEAC) method. These two methods have different principles, providing complementary insights into the extract's ability to reduce free radicals. The fundamental principle of this method relies on measuring the capacity of antioxidant compounds to reduce the $ABTS^{\bullet+}$ cation (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), which are stable radicals that have an intense blue-green color. The $ABTS^{\bullet+}$ radical is generated via a reaction between the ABTS solution and an oxidizing agent, such as potassium persulfate ($K_2S_2O_8$), under specific conditions (typically involving incubation for 12–16 hours at ambient temperature in the dark), producing a blue-green solution that has an absorption peak at a wavelength of 734–743 nm, depending on the solvent and solution conditions. When antioxidant compounds from plant extracts are added to the $ABTS^{\bullet+}$ solution, these compounds donate electrons or hydrogen atoms to reduce $ABTS^{\bullet+}$ to the non-radical form ABTS, causing a decrease in color intensity. This reduction in absorbance is quantified using a UV-Vis spectrophotometer, typically at approximately 743 nm. The higher the antioxidant activity in the sample, the greater the decrease in absorbance (Karaulova et al., 2021; Rubio et al., 2016).

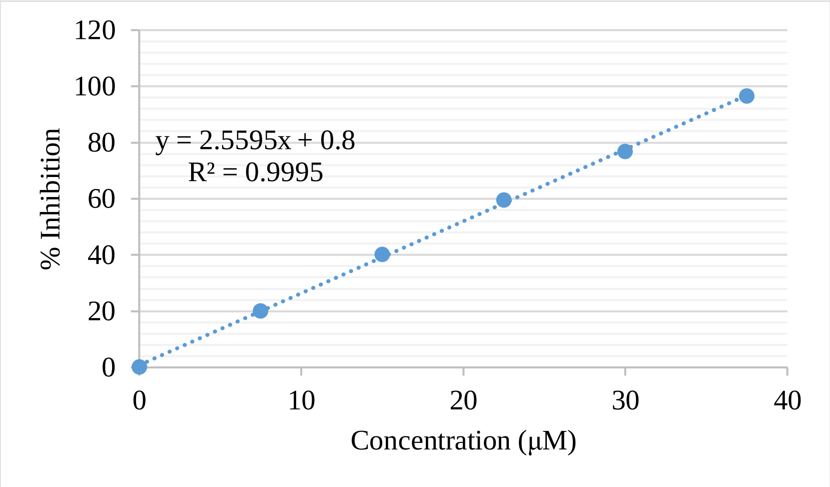

Figure 1. Trolox Standard Curve

The *C. cujete* L. fruit peel extract demonstrated significant antioxidant activity based on the TEAC assay results, yielding a value of 171.37 ± 4.03 mM TE/g extract. This value indicates that the extract has a reasonably high ability to counteract or reduce free radicals, especially $ABTS^{\bullet+}$ radicals used in this method.

Conversely, the DPPH assay yielded a high IC_{50} value obtained was 1166.68 ppm, which indicates that the extract possesses very weak antioxidant activity. A high IC_{50} value indicates that the concentration of extract required to inhibit 50% of DPPH free radicals is relatively large. This diminished activity may be attributed to a low content of polar compounds participating in electron or hydrogen-atom donation, which constitutes the primary reaction mechanism in the DPPH assay (Gulcin & Alwasel, 2023; Widyasanti et al., 2025). Although phytochemical testing showed that the extract contained flavonoids, phenols, alkaloids, terpenoids, steroids, and saponins, not all of these compounds have strong potential to neutralize DPPH radicals, especially when their concentration is low, or their chemical structures are less reactive toward stable nitrogen radicals such as DPPH.

Unlike DPPH, the TEAC method showed higher antioxidant capacity. In this method, the extract had a value of 171.37 ± 4.03 mM TE/g, indicating that it was relatively more effective at neutralizing $ABTS^{\bullet+}$ radicals. Several studies have shown that the TEAC method is better at measuring antioxidants of various polarities (hydrophilic and lipophilic) and often yields higher activity values than DPPH, making ABTS/TEAC more 'broad' for diverse samples (Floegel et al., 2011; Wołosiak et al., 2022)

The remarkable divergence between the DPPH and TEAC results can be rationalized by the differential sensitivity of each method to the functional groups of the constituent compounds in the extract. DPPH is more selective for polar phenolic and flavonoid compounds. At the same time, TEAC can detect the combined activity of polar and semi-polar compounds, including terpenoids and steroids detected in phytochemical tests. Thus, the higher antioxidant capacity in the TEAC assay indicates that these compounds contribute more strongly to the electron-donating mechanism in ABTS radicals than in DPPH (Abramovič et al., 2018; Gulcin & Alwasel, 2023; Munteanu & Apetrei, 2021).

Overall, the combination of the two methods shows that although the antioxidant activity of the extract against DPPH is relatively weak, it still has a good antioxidant capacity as measured by the TEAC method. These observed differences underscore the critical importance of utilizing multiple analytical methods for evaluating antioxidant activity, particularly in samples containing diverse groups of secondary metabolites. These results also indicate that Crescentia cujete fruit peel extract has the potential for further development, either through fractionation, optimization of extraction methods, or exploration of other bioactive activities (Gonzales, Sevilla, et al., 2023; Hasanah et al., 2017; Januário et al., 2022; Krisna et al., 2022; Parvin et al., 2015; Pereira et al., 2017; Rinaldi et al., 2025; Sánchez & Fiscal Ladino, 2024; Suhita et al., 2023; Suzana & Handayanti, 2022).

CONCLUSION

The ethanol extract of *C. cujete* fruit peel was found to contain diverse secondary metabolites—namely flavonoids, phenolics, alkaloids, terpenoids, steroids, and saponins—all of which contribute to its overall biological activity. Antioxidant activity assessment revealed a significant disparity between the results obtained from the DPPH and TEAC methods. The DPPH assay yielded an IC_{50} value of 1166.68 ppm, classifying the extract as having very weak antioxidant activity due to its limited ability to scavenge DPPH free radicals. In contrast, the TEAC method showed an antioxidant capacity of 171.37 ± 4.03 mM TE/g, indicating a higher ability of the extract to reduce $ABTS^{\bullet+}$ radicals. This divergence highlights the importance of employing multiple analytical approaches to obtain a more comprehensive characterization of antioxidant activity, particularly in samples containing diverse secondary metabolites. Overall, the findings of this study indicate that the fruit peel of *Crescentia cujete* holds considerable potential for further development through fractionation, optimization of extraction techniques, or exploration of additional biological activities. Moreover, these results provide a scientific foundation for its prospective application in the phytopharmaceutical, functional food, and cosmetic industries. This observed difference demonstrates that the antioxidant activity of the *C. cujete* fruit peel extract is method-dependent and highly influenced by the chemical characteristics of its constituent compounds. The novelty of this study lies in providing the first comparative evaluation of DPPH and TEAC assays specifically for *C. cujete* fruit peel from the Serang region, thereby revealing a distinct antioxidant response profile that has not been previously documented. Overall, *C. cujete* fruit peel extract has potential as a natural antioxidant source, particularly against ABTS radicals.

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