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Phytochemical Profile and Antioxidant Activity of Green Onion (Allium fistulosum L.) Extract

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Abstract: Oxidative stress plays a pivotal role in the progression of various degenerative diseases due to excessive production of reactive oxygen species (ROS) that overwhelms the body's antioxidant defense system. Green onion (Allium fistulosum L.) is an herbal plant known to contain diverse bioactive compounds with potential as natural antioxidants. This study aimed to analyze the phytochemical profile and antioxidant activity of green onion extract in vitro. Extraction was performed using the percolation method with methanol. Qualitative phytochemical analysis was conducted using specific reagents. Antioxidant capacity was subsequently evaluated using DPPH, ABTS, and FRAP assays, each referenced against Trolox as the standard compound. Results showed that the extract had a yield of 20.36% and contained alkaloids, betacyanins, cardiac glycosides, glycosides, coumarins, flavonoids, phenols, quinones, saponins, steroids, terpenoids, and tannins. Antioxidant activity yielded IC_{50} values of 185.86 µg/mL (DPPH), 91.60 µg/mL (ABTS), and 15.39 µg/mL (FRAP). These findings indicate that green onion extract possesses considerable potential as a natural antioxidant source for further development.

Keywords: Allium Fistulosum, Antioxidant, Phytochemical, DPPH, ABTS, FRAP.

INTRODUCTION

Noncommunicable diseases (NCDs), including cardiovascular disease, diabetes mellitus, cancer, and degenerative diseases, are the leading cause of death worldwide, accounting for more than 70% of total global deaths each year (WHO, 2023). The rising prevalence of NCDs is closely linked to lifestyle changes, such as unhealthy diets, lack of physical activity, and increased exposure to environmental pollutants (Muniri et al., 2023).

One of the primary mechanisms underlying the development of NCDs is oxidative stress, defined as a condition in which the production of reactive oxygen species (ROS) exceeds the capacity of the body's antioxidant system. The accumulation of ROS can cause biomolecular damage, trigger chronic inflammation, and contribute to the development of various degenerative diseases, including atherosclerosis, type 2 diabetes mellitus, cancer, and neurodegenerative diseases (Liguori et al., 2018; Pizzino et al., 2017; Butterfield & Halliwell, 2019). Therefore, antioxidants play a crucial role in neutralizing free radicals, thereby potentially reducing the risk of oxidative damage and the development of NCDs (Chaudhary et al., 2023; Gorrepati et al., 2024).

The genus *Allium* comprises a group of plants known for their various biological activities, such as antioxidant, anti-inflammatory, antimicrobial, anticancer, and cardioprotective effects. These activities are primarily attributed to the presence of organosulfur compounds, flavonoids, and phenolic compounds (Iwar et al., 2024; Shang et al., 2019). One species widely consumed in Asia is green onion (Allium fistulosum L.), which contains various bioactive compounds, including organosulfur compounds, flavonoids (particularly quercetin and isorhamnetin), phenolic compounds, vitamin C, vitamin B6, and selenium (Kim et al., 2023). Several studies have reported that *Allium fistulosum* has high levels of phenolic and flavonoid compounds and exhibits promising antioxidant, antidiabetic, anticancer, and cardioprotective activities (Jimenez-Lopez et al., 2022; Sagar et al., 2021; Reboredo-Rodríguez et al., 2017).

The characteristics of plant extracts are influenced by the extraction method and the type of solvent used. Methanol is an effective solvent for extracting polar compounds such as phenolics and flavonoids (Azwanida, 2015). Furthermore, the use of several antioxidant assay methods, such as DPPH, ABTS, and FRAP, allows for a more comprehensive evaluation of antioxidant capacity because each method employs a distinct measurement mechanism (Pisoschi et al., 2016).

Although the pharmacological potential of *Allium fistulosum* has been widely reported, studies integrating the phytochemical profile and antioxidant capacity of methanol extracts from Indonesian green onion remain limited. Therefore, this study aims to explore the phytochemical composition and antioxidant activity of methanol extracts from Indonesian green onion (Allium fistulosum L.) as a scientific basis for developing this plant as a source of raw materials for medicinal and nutraceutical products.

METHOD

This study employed a laboratory experimental design with an *in vitro* approach to evaluate the phytochemical composition and antioxidant activity of green onion (Allium fistulosum L.) extract. The study was conducted from January to May 2026 at the Biochemistry and Molecular Biology Laboratory, Faculty of Medicine, Tarumanagara University.

Samples of fresh green onions were obtained from Bogor, West Java. Inclusion criteria included fresh green onions showing no physical damage or signs of decay. Samples were washed thoroughly with running water, air-dried at room temperature for seven days away from direct sunlight to preserve thermolabile compounds, and ground into a dry powder (simplicia).

The extraction process was carried out using the percolation technique using 96% technical-grade methanol. The simplicia were initially macerated for 24 hours, followed by percolation until the filtrate became clear. The liquid extract was concentrated using a rotary evaporator at 40°C to obtain a thick extract. Yield was calculated as the ratio of thick extract mass to the initial simplicia mass.

Qualitative phytochemical screening was conducted to identify alkaloids (Wagner), flavonoids (NaOH), phenols (Folin-Ciocalteu), tannins ($FeCl_3$), saponins (foam test with distilled water), steroids and terpenoids (Liebermann-Burchard), glycosides (Borntrager), quinones (H_2SO_4), coumarins (NaOH), anthocyanins (NaOH), betacyanins (NaOH), and cardiac glycosides (Keller-Killiani).

Evaluation of antioxidant capacity was conducted through three distinct yet complementary assays, namely DPPH, ABTS, and FRAP, with Trolox as a reference standard. All tests were conducted in triplicate to improve the reliability and reproducibility of the results. The extract concentrations for each method were determined based on preliminary tests to ensure they covered a concentration range that produced an inhibitory response or sufficient reducing capacity for the construction of concentration–response curves and the accurate determination of IC_{50} values. All absorbance measurements were performed using a Genesys 30 visible spectrophotometer at the appropriate wavelength for each method. Antioxidant activity was expressed as IC_{50} values derived from linear regression analysis using GraphPad Prism software. Data were presented in tables and figures and analyzed using quantitative and qualitative descriptive approaches.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical analysis of the green onion extract revealed the presence of diverse secondary metabolites including alkaloids, betacyanins, cardiac glycosides, glycosides, coumarin derivatives, flavonoids, phenolics, quinones, saponins, steroidal compounds, terpenoids, and tannins. Notably, anthocyanins were absent from the extract. The complete screening results are summarized in Table 1.

Table 1. Phytochemical Screening Results of Green Onion Extract

Phytochemical	Result (Green Onion Extract)	Reagent
Alkaloids	+	Wagner
Anthocyanins	−	NaOH
Betacyanins	+	NaOH
Cardiac glycosides	+	Keller-Killiani
Glycosides	+	Borntrager
Coumarins	+	NaOH
Flavonoids	+	NaOH
Phenols	+	Folin-Ciocalteu
Quinones	+	H_2SO_4
Saponins	+	Distilled water
Steroids	+	Liebermann-Burchard
Terpenoids	+	Liebermann-Burchard
Tannins	+	$FeCl_3$

The yield of the green onion extract obtained in this study was 20.36%, within the range of 15.47–43.56% reported by Chang et al. (2013). Yield variation may be influenced by solvent type, extraction method, temperature, and duration of extraction. The use of methanol facilitated optimal extraction of polar compounds such as flavonoids and phenolics due to its high polarity (Syamsul et al., 2020). Variations in yield can also be attributed to differences in geographical and agroclimatic conditions, which influence the accumulation of secondary metabolites (Pant et al., 2021).

The phytochemical findings are consistent with those of Maulana et al. (2026), who reported the presence of flavonoids, phenols, steroids, glycosides, and saponins in *Allium fistulosum* Maulana et al. (2026), and Țigu et al. (2021) who provided a comparative phytochemical analysis between *Allium fistulosum* and *Allium sativum*. Kim et al. (2023) also described a comprehensive phytochemical composition of *Allium fistulosum*, including quercetin, isorhamnetin, and kaempferol as dominant phenolics.

The absence of anthocyanins is likely attributable to the variety used; Xu et al. (2022) reported that anthocyanins are more predominant in red-stemmed varieties of *Allium fistulosum* compared to white-stemmed varieties. The identification of cardiac glycosides, coumarins, quinones, terpenoids, and betacyanins adds to the limited information available on the complete phytochemical profile of green onion. Coumarins in *Allium* have been reported to possess anticoagulant and anti-inflammatory activities, while terpenoids contribute antimicrobial and cytotoxic effects (Jimenez-Lopez et al., 2022). Saponins detected contribute to hemolytic, immunomodulatory, and antitumor activities reported in *Allium* species (Iwar et al., 2024), while tannins act as antioxidants through metal chelation and free radical scavenging mechanisms (Smeriglio et al., 2017).

Total Antioxidant Capacity Assessment

DPPH Method (2,2-diphenyl-1-picrylhydrazyl)

The Trolox standard assay using the DPPH method yielded the linear regression equation $Y = 1.690X + 1.845$ ($R^2 = 0.9943$; Figure 1), with an IC_{50} of 28.49 µg/mL (Table 3).

Table 2. Mean Absorbance, Percent Inhibition, and IC_{50} of Trolox (DPPH Method)

Concentration (µg/mL)	Mean Absorbance	Inhibition (%)	IC_{50} (µg/mL)
10	0.531	16.90%	
20	0.409	35.99%	
30	0.286	55.24%	28.49
40	0.189	70.42%	
50	0.101	84.19%	

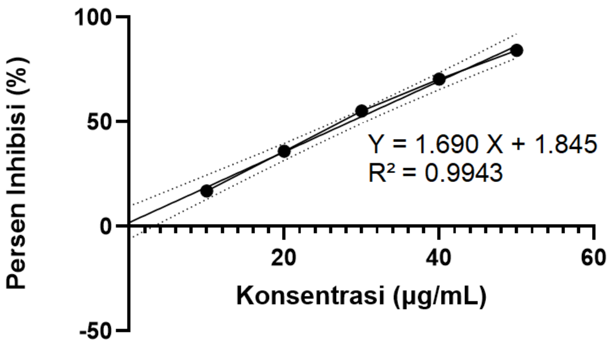

Figure 1. Trolox DPPH Calibration Curve

The green onion extract tested with the DPPH method at 515 nm demonstrated a dose-dependent increase in free radical inhibition. Linear regression yielded $Y = 0.07823x + 35.46$ ($R^2 = 0.9867$; Figure 2), with an IC_{50} of 185.86 µg/mL (Table 4).

Table 3. Mean Absorbance, Percent Inhibition, and IC_{50} of Green Onion Extract (DPPH Method)

Concentration (µg/mL)	Mean Absorbance	Inhibition (%)	IC_{50} (µg/mL)
100	0.299	46.82%	
200	0.249	52.42%	
300	0.217	58.72%	185.86
400	0.185	64.63%	
500	0.126	75.95%	

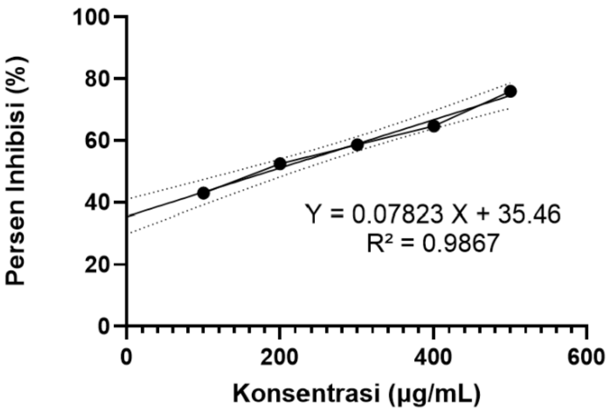

Figure 2. Green Onion Extract DPPH Curve

Based on the results of the DPPH assay, Trolox exhibited an IC_{50} value of 28.49 µg/mL, while the methanol extract of green onion (Allium fistulosum L.) had an IC_{50} value of 185.86 µg/mL. According to the Blois classification, these values fall into the category of weak antioxidant activity (IC_{50} > 150 µg/mL) (Pisoschi et al., 2016). The higher IC_{50} value of the extract compared to Trolox indicates lower radical-scavenging capacity, as Trolox is a pure antioxidant, whereas the green onion extract is a complex mixture of secondary metabolites. Consequently, the concentration of active compounds is lower, and there is a possibility of interactions among components that affect antioxidant activity.

Phytochemical screening results indicate that the green onion extract contains flavonoids, phenolic compounds, tannins, and other secondary metabolites known to act as antioxidants. These compounds function as hydrogen or electron donors via hydroxyl groups to stabilize free radicals and halt oxidative chain reactions. However, antioxidant activity is determined not only by the presence of these compounds but also by their concentration, chemical structure, and the potential for synergistic or antagonistic interactions among metabolites. This explains why, despite containing antioxidant compounds, the DPPH radical scavenging capacity of the extract remains relatively low.

Compared to other studies, the IC_{50} value in this study was higher than that reported by Maulana and Amananti (2026), who used a 70% ethanol extract prepared via the Soxhlet method and obtained an IC_{50} of 90 µg/mL. However, the IC_{50} value obtained in the present study was lower than that reported for *Allium tuberosum*, which had an IC_{50} of 312.55 µg/mL (Aziz et al., 2019). These differences may be influenced by the type of solvent, the extraction method, and the origin of the plant material. Furthermore, the DPPH method primarily reflects the hydrogen atom transfer (HAT) mechanism, although electron transfer (ET) also contributes (Apak et al., 2016). The relatively high IC_{50} value indicates that the antioxidant activity of the extract is more likely dominated by the electron transfer mechanism rather than hydrogen atom transfer, which is also consistent with the likely results from other methods such as ABTS and FRAP, which are more sensitive to ET.

ABTS Method (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))

The Trolox standard using the ABTS method yielded $Y = 14.44X + 1.067$ ($R^2 = 0.9901$; Figure 3), with an IC_{50} of 3.39 µg/mL (Table 5).

Table 4. Mean Absorbance, Percent Inhibition, and IC_{50} of Trolox (ABTS Method)

Concentration (µg/mL)	Mean Absorbance	Inhibition (%)	IC_{50} (µg/mL)
1.25	0.267	21.01%	
2.50	0.210	37.87%	
3.75	0.165	51.18%	3.39
5.00	0.096	71.60%	
6.25	0.019	94.38%	

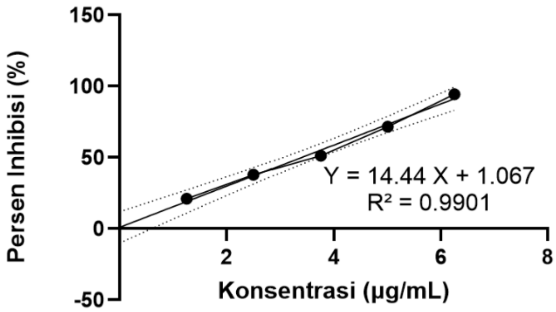

Figure 3. Trolox ABTS Calibration Curve

The green onion extract demonstrated consistent dose-dependent inhibition of the $ABTS^{•+}$ radical cation, yielding $Y = 0.9428X - 36.36$ ($R^2 = 0.9757$; Figure 4), with an IC_{50} of 91.60 µg/mL (Table 6).

Table 5. Mean Absorbance, Percent Inhibition, and IC_{50} of Green Onion Extract (ABTS Method)

Concentration (µg/mL)	Mean Absorbance	Inhibition (%)	IC_{50} (µg/mL)
60	0.252	16.00%	
70	0.201	33.00%	
80	0.178	40.67%	91.60
90	0.151	49.67%	
100	0.125	58.33%	
110	0.105	65.00%	

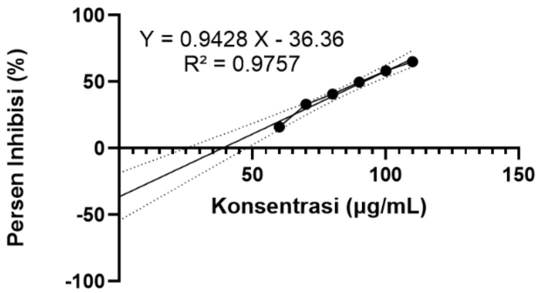

Figure 4. Green Onion Extract ABTS Curve

Based on the results of the ABTS assay, the methanol extract of green onion (Allium fistulosum L.) had an IC_{50} value of 91.60 µg/mL, which falls into the category of strong antioxidant activity (IC_{50} 50–100 µg/mL), although it is still lower than that of Trolox (IC_{50} 3.39 µg/mL) (Pisoschi et al., 2016). This difference reflects the complexity of the extract, which consists of various secondary metabolites with varying concentrations and antioxidant potencies, unlike Trolox, which is a pure compound.

The higher ABTS activity compared to DPPH indicates that the bioactive compounds in the green onion extract are more effective against ABTS•$^{+}$ radicals, which can be neutralized through either electron transfer (ET) or hydrogen atom transfer (HAT) mechanisms, and are capable of reacting with both hydrophilic and lipophilic antioxidants (Pisoschi et al., 2016). These findings are consistent with the results of phytochemical screening, which revealed the presence of flavonoids, phenolic compounds, tannins, and organosulfur compounds. The hydroxyl groups in flavonoids and phenolic compounds act as effective electron donors in stabilizing the ABTS•$^{+}$ radical, while organosulfur compounds contribute to maintaining redox balance by enhancing the endogenous antioxidant system. Thus, the antioxidant activity of the green onion extract is likely the result of the synergistic action of various classes of secondary metabolites, rather than originating from a single compound.

The results of this study are consistent with the report by Elkhawas et al., which showed that *Allium fistulosum* exhibits higher ABTS radical-scavenging activity than several other *Allium* species, a finding attributed to its content of phenolic compounds, vitamin B6, and vitamin C, which act synergistically as electron and hydrogen atom donors (Elkhawas et al., 2026; Kim et al., 2023). Quercetin and isorhamnetin, the dominant flavonoids in *Allium fistulosum*, have been demonstrated to possess potent ABTS radical scavenging capacity (Kim et al., 2023; Jimenez-Lopez et al., 2022). By comparison, *Allium tuberosum* from Vietnam was reported to have an ABTS IC_{50} value of 83.13 µg/mL, which is slightly lower than the results of this study (Nhut et al., 2020).

FRAP Method (Ferric Reducing Antioxidant Power)

The Trolox standard using the FRAP method yielded $Y = 1.644X + 38.85$ ($R^2 = 0.9953$; Figure 5), with an IC_{50} of 6.78 µg/mL (Table 7).

Table 6. Mean Absorbance, Percent Inhibition, and IC_{50} of Trolox (FRAP Method)

Concentration (µg/mL)	Mean Absorbance	Inhibition (%)	IC_{50} (µg/mL)
5	0.078	46.15%	
10	0.096	56.25%	
15	0.115	63.48%	6.78
20	0.153	72.55%	
25	0.201	79.10%	

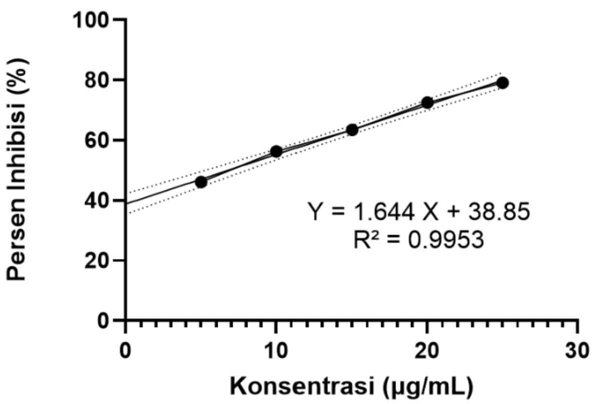

Figure 5. Trolox FRAP Calibration Curve

The green onion extract demonstrated consistent dose-dependent FRAP activity ($R^2 = 0.9941$) with the equation $Y = 1.608X + 25.26$, yielding an IC_{50} of 15.39 µg/mL (Table 8). This low IC_{50} indicates strong capacity for Fe^{3+} to Fe^{2+} reduction, categorized as very strong antioxidant activity under classifications emphasizing electron transfer mechanisms.

Table 7. Mean Absorbance, Percent Inhibition, and IC_{50} of Green Onion Extract (FRAP Method)

Concentration (µg/mL)	Mean Absorbance	Inhibition (%)	IC_{50} (µg/mL)
10	0.103	41.75%	
15	0.118	49.15%	
20	0.137	56.20%	15.39
25	0.181	66.85%	
30	0.223	73.09%	

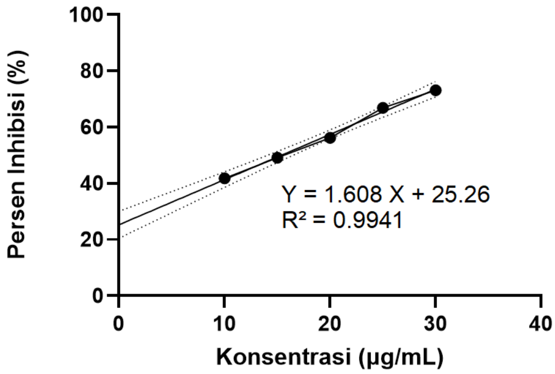

Figure 6. Green Onion Extract FRAP Curve

The results of antioxidant activity testing using the FRAP method showed that Trolox had an IC_{50} value of 6.78 µg/mL, while the green onion extract had an IC_{50} value of 15.39 µg/mL. The higher IC_{50} value of the extract indicates that its ability to reduce Fe^{3+} ions to Fe^{2+} is still lower than that of Trolox, which is a pure antioxidant with high reducing capacity. Nevertheless, the FRAP activity of the green onion extract is considered good and is supported by phytochemical screening results showing the presence of flavonoids, phenolic compounds, terpenoids, and saponins. Flavonoids and phenolic compounds act as electron donors through their hydroxyl groups, thereby reducing Fe^{3+} to Fe^{2+}, while terpenoids and saponins are thought to contribute synergistically to the extract's reducing capacity.

These findings indicate that the antioxidant mechanism of green onion extract is dominated by its electron-transfer capacity, which is also consistent with the ABTS assay results. Biologically, this ability to reduce oxidants and maintain redox balance has the potential to help protect cells from oxidative damage, which plays a role in the development of degenerative diseases. These results are consistent with studies by Elkhawas et al. and Balkrishna et al., which reported good iron-reducing capacity in *Allium fistulosum* (Elkhawas et al., 2026; Balkrishna et al., 2023). Variations in FRAP IC_{50} values across studies are likely influenced by differences in extraction methods, solvent types, sample preparation, and the geographical origin of the plants (Pant et al., 2021; Rokayya et al., 2025).

Overall, these findings support the potential of green onion as a source of natural antioxidants that could be developed as a raw material for functional foods or nutraceuticals to help reduce oxidative stress, which plays a role in the development of degenerative diseases (Chaudhary et al., 2023; Gorrepati et al., 2024).

CONCLUSION

Phytochemical screening of the methanol extract derived from green onion (Allium fistulosum L.) revealed the presence of numerous secondary metabolite classes, namely alkaloids, betacyanins, cardiac glycosides, glycosides, coumarin derivatives, flavonoids, phenolics, quinones, saponins, steroidal compounds, terpenoids, and tannins, whereas anthocyanins were not detected.

Evaluation of antioxidant activity through three distinct assays yielded differing results. The DPPH assay produced an IC_{50} value of 185.86 µg/mL, placing it in the weak activity category, while the ABTS assay returned an IC_{50} of 91.60 µg/mL, corresponding to strong activity. The lowest IC_{50} was recorded via the FRAP assay at 15.39 µg/mL, representing very strong activity. This pattern suggests that electron transfer and metal ion reduction are the predominant mechanisms underlying the antioxidant response. Taken together, these results support the prospective application of green onion extract as a naturally derived antioxidant agent worthy of further scientific investigation.

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