Comunicata Scientiae
Horticultural Journal

ORIGINAL ARTICLE
published: 30 December 2025
https://doi.org/10.14295/CS.v16.4363

Antioxidant Capacity, Phenolic and Flavonoid Content in Stalks and Leaves of Three Allium Species

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Abstract

The study compared the total phenolic content (TPC), total flavonoid content (TFC), and scavenging capacity (IC_{50}) of leaves and stalks/bulbs of lokio (*Allium chinense* G. Don), an underutilised allium species, and compared them with two commercially popular onions, i.e., spring onion (*Allium fistulosum* L.) and green onion (*Allium ascalonicum* L.). Extracts of the three alliums exhibited distinct antioxidant capacity, and significant differences existed in TPC, TFC, and IC_{50} among the three species. The allium stalk/bulb contained significantly higher TPC, TFC, and IC_{50} than its green leaf counterparts, with a strong correlation (r = 0.9064). It was observed that the TPC of the bulb/stalk of *A. chinense* (20.20 mg/g GAE FW) was higher as compared to *A. fistulosum* (19.97 mg/g GAE FW) and *A. ascalonicum* (19.62 mg/g GAE FW). Meanwhile, in terms of TFC, the bulb of *A. ascalonicum* contained the highest (5.06 mg/g QE FW), followed by *A. chinense* (3.63 mg/g QE FW) and *A. fistulosum* (1.70 mg/g QE FW). Among the three alliums, *A. chinense* had the highest antioxidant capacity, as indicated by a lower IC_{50} in the white stalks (97.40 ppm), followed by *A. ascalonicum* (98.21 ppm) and *A. fistulosum* (100.80 ppm). This study also revealed strong correlations (r > 0.90) between TPC, TFC, and the antioxidant capacity of tested allium. The results of the current assessment would be useful for future studies and applications of lokio (*Allium chinense* G. Don) as an alternative to popular spring onion and green onion in food, nutraceuticals, and cosmetic product development.

Keywords: green onion, herbs, lokio, spring onion, scavenge capacity

Introduction

A large number of aromatic plants, including the Amaryllidaceae family, contain chemical compounds with antioxidant properties. The Allium genus consists of more than 600 different species, which are cultivated globally and consumed as vegetables and seasonings. Edible alliums, including shallots, garlic, leeks, lokio, and chives, are ingredients used in foods for their distinctive pungent aroma and taste, as well as their nutritional value (Kim et al., 2023). Depending on the parts of the onion plants, from bulbs and stems to leaves, allium flavor is related to the high content of essential oils, which are dominated by compounds containing sulfur and terpenes (Kim et al., 2023; Qin et al., 2023). The Allium genus has also been used traditionally as medicine to treat vision, flu, headaches, heart problems, analgesics, and weight problems (Singh and Ramakrishna, 2017; Nazir et al., 2022).

Allium is known to be very rich in secondary metabolites, including phenolic acids, flavonoids, and flavonoid polymers, all of which have health benefits, (Dey and Khaled, 2013; Qin et al., 2023). The compound content in plants is influenced by the species (Abdel-Gawad et al., 2014), cultivar (Bernaert et al., 2012; Čeryová et al., 2023), anatomical parts (Lachowicz et al., 2018), growing environment, post-harvest handling (Biernacka et al., 2021; Kozlowska et al., 2021).

Allium fistulosum L., known as spring onion (scallion) or Welsh onion, has a wider leaf structure and smaller tubers, and the aroma of onions is softer than that of lokio (Padula et al., 2022). *Allium ascalonicum* L., known as shallot, which bulb is used more often than lokio and leeks in everyday cooking. The green onion of A. ascalonicum is an immature form of the onion, harvested earlier while the leaves are green and the bulb is very small in diameter (Fitriana and Susandarini, 2019). *Allium*

Received: 21 August 2025
Accepted: 18 September 2025

chinense G. Don is a species of onion that is popular among the Batak ethnic group in the area of Toba, North Sumatra, Indonesia, and was rarely found and consumed by other ethnic groups in Indonesia (Anggriani and Anggarani, 2022). It was just a few studies on *A. chinense* that have been found. Syahrajabian et al. (2020) reported from their review that A. chinense has high nutritional value and can be used as a food ingredient, antioxidant, and source of dietary flavonoids in a number of nations.

This research aimed to investigate the antioxidant activity and related compounds, such as phenolics and flavonoids, of underutilized *A. chinense* G. Don and the commercially important onion species *Allium fistulosum* L. and *Allium ascalonicum* L. Further, it aimed to discover promising natural antioxidants for future usage in various fields such as food, medicine, and cosmetics.

Materials and Methods

Materials

Allium species (*A. chinense* G. Don; *A. fistulosum* L.; and *A. ascalonicum L*) were collected in April 2024 from a local market in Medan, Indonesia, and transported directly to the laboratory of Politeknik Teknologi Kimia Industri – Medan. After stripping the root and wilted leaves, each sample species was thoroughly cleaned. Each was divided into green leaves and a white stalk portion (**Figure 1**). They all went through extraction procedures before measurement.

Determination of the Free Radical Scavenging Activity

The free radical scavenging activity, which was expressed as IC_{50}, was determined by the 1,1-Di[phenyl-2-picrylhydrazyl] (DPPH)-Assay, as described by Muflihah et al. (2021).

Preparation of ascorbic acid standard solutions. By dissolving 25 mg of the standard ascorbic acid in 25 mL of ethanol, a standard stock solution of 1000 ppm ascorbic acid was created. A series of dilutions were conducted from this stock solution to obtain ascorbic acid concentrations of 50, 75, 100, 125, and 150 ppm. A UV-Vis spectrophotometer was used to detect the absorbance at 516 nm. The calibration curve, which plotted absorbance against concentration, was then established.

Measurement of IC_{50}. Prior to measurement, 0.025 g of the allium sample were dissolved in 25 mL of ethanol to create a stock extract solution. To obtain a series of 50, 75, 100, 125, and 150 ppm extract samples by adding ethanol, 1 mL of DPPH solution was added to each extract. Ascorbic acid and the DPPH solution were each employed as the standard and control. After giving each combination solution a good shake, it was allowed to stand at room temperature for half an hour. A UV-visible spectrophotometer was used to determine the extract sample's absorbance at 516 nm in comparison to a blank. Using Eq. 1, the scavenging rate was determined as the percentage of scavenging activity:

$$\%\text{radical scavenging activity}=[(Abs_{Blank}-Abs._{sample})/Abs._{Blank}]x100\%$$

The concentration versus percentage of each sample's free radical scavenging activity was then plotted as a standard graph. The sample's IC_{50} was determined using an equation derived from a standard graph. All determinations were carried out in triplicate.

Determination of Total Phenolic Content

The Folin-Ciocalteu colorimetric method, modified by Chandra et al. (2014) was used to determine the total phenolic content (TPC) of a substance. To establish a standard calibration curve, gallic acid was used.

Preparation of the standard calibration curve. 50 mg of gallic acid were dissolved in 100 mL of methanol to create a stock gallic acid solution (500 ppm). After completely diluting 0.5 mL of gallic acid solution (at concentrations of 20, 30, 40, 50, and 60 µg/mL) with 0.5 mL of 10% Folin-Ciocalteu's reagent, the mixture was stirred for approximately 60 seconds. One milliliter of a 10% Na_2CO_3 solution was added after five minutes, and the mixture was left to stand at room temperature for 35 minutes. A UV-Vis spectrophotometer was used to detect the absorbance at 742 nm. The calibration curve, which plotted absorbance against concentration, was then established.

Extraction and measurement of phenolic. A stock extract solution of the allium sample was obtained by dissolving 25 mg of freshly ground allium in 50% water-ethanol (v/v) in a 25-mL volumetric flask. Then, the same procedure as for the standard curve was carried out with 0.5 mL of an ethanol extract of allium instead of gallic acid. The total phenolic content in the sample was calculated from the equation of the standard calibration curve and expressed as mg gallic acid equivalents (GAE) per g fresh weight (FW). All determinations were carried out in triplicate.

Determination of Total Flavonoids Content

With a slight modification, the aluminum chloride colorimetric method described by Chandra et al. (2014) was used to assess the total flavonoid content (TFC).

Figure 1. *Allium chinense* G. Don, *Allium ascalonicum* L., and *Allium fistulosum* L. (from left to right)

Quercetin was utilized to create a standard calibration curve.

Preparation of the standard calibration curve. By dissolving 10 mg of quercetin in 100 mL of methanol, a stock quercetin solution (100 ppm) was created. After thoroughly diluting 0.5 mL of stock quercetin (at concentrations of 10, 20, 30, 40, and 50 µg/mL) with 0.1 mL of 2% $AlCl_3$ and 0.1 mL of CH_3COONa, the mixture was stirred for approximately one minute. Following a 5-minute incubation period, 2.8 milliliters of distilled water were added, well mixed, and allowed to stand at room temperature for 25 minutes. A UV-Vis spectrophotometer was used to measure the absorbance at 431 nm. The calibration curve, which plotted absorbance against concentration, was then established.

Extraction and measurement of flavonoids. A stock extract solution of the allium sample was obtained by dissolving 1g of freshly ground allium in 50% water-methanol (v/v) in a 100-mL volumetric flask. Then, the same procedure as for the standard curve was carried out with 1 mL of methanol extract of allium instead of gallic acid. The total flavonoid content in the sample was calculated from the equation of the standard calibration curve and expressed as mg quercetin equivalents (QE) per g fresh weight (FW). All determinations were carried out in triplicate.

Statistical analysis

Quantitative data are presented as mean values with the respective standard deviation in triplicate. For comparisons, a two-way analysis of variance (ANOVA) and Duncan-test were employed. A difference that was denoted as statistically significant was indicated by a p-value < 0.05. The strength and direction of the relationship between the bioactive phytochemicals (TCP, TFC, and IC_{50}) and between various parts of the allium sample ($p < 0.05$) were expressed through analyses of the Pearson's correlation coefficient. All analyses were performed using statistical software SPSS.

Results and Discussion

The green leaves and white stalk portions of *Allium chinense* G. Don (lokio), *Allium fistulosum* L. (spring onion), and *Allium ascalonicum* L. (green onion*)* were rich in phenolics and flavonoids and had potential scavenging activity. The total phenolic content *(*TPC), total flavonoid content *(*TFC), and scavenging activity (IC_{50}) values differ significantly within species of allium, as well as within the plant portions.

Total phenolic content (TPC)

Phenolic compounds, also known as polyphenols, are some of the most prevalent substances produced

during secondary metabolism in plants and are crucial for their interaction with the surrounding environment (Zagoskina et al., 2023). The phenolic content (TPC) in three allium samples was determined spectrophotometrically, calculated from the gallic acid calibration curve (y = 0.0071x + 0.0332; R^2 = 0.9837), and expressed as mg of gallic acid equivalent (GAE) in the fresh weight of the allium sample. Ethanol extracts of three *Allium* species evaluated, both from green leaves and white stalks/bulbs, demonstrated the high content of phenolic substances. The average TPC value of *A. chinense* did not significantly differ compared to the TPC of *A. fistulosum* but was significantly higher than that of *A. ascalonicum*. The TPC (mg GAE/g FW) of *Allium spp.* ranged from 19.25 to 20.20 for *A. chinense*, while it ranged from 19.14 to 19.97 for *A. fistulosum* and 19.05 to 19.62 for *A. ascalonicum* (**Table 1**).

TPC in evaluated allium was found to be a lower value than the reported TPC (93.34 mg GAE/g) of *A. porrum* in Serbia (Radovanovic et al., 2015) and the TPC (102.45 mg GAE/g) of *A. porrum* in Egypt (Abdel-Gawad et al., 2014). Plant polyphenol production and accumulation are influenced by a variety of factors, including physiological-biochemical, molecular-genetic, and environmental factors (Zagoskina et al., 2023).

The polyphenol distribution across the parts of the plants also varied. This study also revealed that bulb extract of *Allium spp.* was found to be significantly richer ($p < 0.05$) than the leaf part extract in terms of total phenolic contents. The average value of TPC (mg GAE/g) in white stalks/bulbs, which ranged from 19.62 to 20.20, was statistically higher ($p<0.05$) compared to the TPC in green leaves, which ranged from 19.05 to 19.25.

The TPC in the bulb was higher as compared to the TPC in the leaves of *A. chinense* and *A. ascalonicum*, but not of *A. fistulosum*. Our results agree with those reported that higher TPC in bulbs than leaves of *A. chinense* (Rhetso et al., 2020) and of *A. sphaerocephalon* (Emir & Emir, 2020[b]). However, other groups reported contrary findings. Inferior TPC in bulb/stalk than in leaves/shaft has been reported in *A. ursinum* (Lachowicz et al., 2018; Kumar and Kumar, 2023), in *A. scorodoprasum* (Mollica et al., 2018), and in *A. porrum* (Biernacka et al., 2021). Meanwhile, similar to our finding on *A. fistulosum*, Bernaert et al. (2012) reported a quite comparable TPC (mg GAE/g) in green leaves (from 5.47 to 15.14) and in white stalks (from 6.98 to 13.96). Likewise, the TPC in green leaves of *A. fistulosum* L. is 25.07±2.02 mg GAE/100 g (Yuasa et al., 2022), and in white stem *A. fistulosum* L. is 182.80 mg GAE/g (Chang et al., 2013). These results on TPC can vary due to differences among cultivars, environmental conditions (Bibi et al., 2022), and agricultural practices. The variations in applied total phenolic determination assays could also contribute to the variation.

According to this study, some polyphenols tend to be more concentrated in bulbs than in the leaves. It may be caused by a number of things, such as the bulb's role as the plant's defence and storage mechanism. Though the leaves may produce more polyphenol when the plant is subjected to more environmental stressors (Zagoskina et al., 2023), the bulb typically contains a greater variety of polyphenols than the leaf portions because polyphenols are synthesized or accumulated in the bulb as part of its metabolic processes, contributing to the overall polyphenol content. Furthermore, polyphenols contributed to the protection of the bulbs, which were particularly vulnerable as subterranean sections, from pathogens and certain pests.

Total flavonoid content (TFC)

Flavonoids are the most isolated, identified, and diverse group of polyphenolic substances in plants. In this study, the flavonoid content of the allium samples was determined based on the quercetin calibration curve (y = 0.0111x + 0.0519, R^2 = 0.9809) and reported as mg of quercetin equivalent (QE) in the fresh weight of the allium sample. Ethanol extracts of three *Allium* species tested, both from green leaves and white stalks, demonstrated the high content of flavonoid substance. The TFC (mg QE/g FW) of *Allium spp.* ranged from 2.03 to 3.63 for *A. chinense*, while it ranged from 1.53 to 1.70 for *A. fistulosum* and 2.34 to 5.06 for *A. ascalonicum*. There was also a significant ($p < 0.05$) difference in TFC among the three

Table 1. Total phenolics (TPC), total flavonoids (TFC) content, and $IC_{50\ of}$ white stalks and green leaves extracts of *Allium* species[a].

Species	Green leaves	TPC[b] (mg GAE/g FW)	TFC[b] (mg QE/g FW)
Allium chinense G. Don	White stalks	20.20±0.08[b]	3.63±0.11[e]
	Green leaves	19.25±0.25[a]	2.03±0.06[c]
Allium fistulosum L.	White stalks	19.97±0.20[ab]	1.70±0.04[b]
	Green leaves	19.14±0.08[a]	1.53±0.04[a]
Allium ascalonicum L.	White stalks	19.62±0.70[b]	5.06±0.03[f]
	Green leaves	19.05±0.22[a]	2.34±0.05[d]

[a]Values are expressed as means of triplicate determination ± standard deviation.

[b]different letters within the same column indicate a significant difference at p<0,05Apereisu ntempec tatium seniquo ceroridicem Romandetis firi, vocre, nonsus spios at deraes? Quam se consi publina, quostif

species, as presented in Table 1.

The average value of TFC (mg QE/g) in white stalks (ranged from 1.70 to 5.06) was statistically higher ($p < 0.05$) compared to the TFC in green leaves (ranged from 1.53 to 2.34). There was a significant ($p < 0.05$) difference in TFC among the three species as well as between parts of the plants. The extract of allium contained a smaller portion of flavonoids than phenolic compounds, as could be expected. These results agree with a previous study on the TFC of *A. chinense* by Rhetso et al. (2020), where they reported that the TFC in the bulb or stalk (9.27±0.39) was higher than in the leaves (6.86±0.59). Conversely, Bibi et al. (2022) found that TFC in leaves was higher than that in bulbs.

The highest TFC was found in stalks of *A. fistulosum* at 5.06 mg QE/g, and the lowest was in leaves of *A. ascalonicum* at 1.53 mg QE/g. The TFC in the stalks and leaves decreased in the same order as follows: *A. fistulosum* > *A. chinense* > *A. ascalonicum*. Similar to TPC, the TFC could also differ to a large extent with varying species and cultivars, agricultural practices, and environmental conditions (Bibi et al., 2022). The variations in extraction applied to total flavonoid determination assays could also contribute to the variation. Differences in the compound content in plants, including phenolic and flavonoid content, are influenced by the species, cultivar, and anatomical parts, besides the growing environment and post-harvest handling (Bernaert et al., 2012; Abdel-Gawad et al., 2014; Lachowicz et al., 2018; Biernacka et al., 2021; Kozlowska et al., 2021; Čeryová et al., 2023).

DPPH scavenging capacity (IC_{50})

DPPH free radicals have been found to be scavenged by allium extracts, as confirmed by the IC_{50} of ethanol extracts of three *Allium* species tested, both from green leaves and white stalks. The scavenging capacity of the allium extracts is shown in **Figure 2**, which was expressed by the IC_{50}. The DPPH free radical-scavenging activity of *Allium spp.* ranged from 97.40 to 114.89 ppm for *A. chinense*, while it ranged from 106.79 to 121.68 ppm for *A. fistulosum* and 98.21 to 117.22 ppm for *A. ascalonicum*. The smaller the IC_{50} value of a sample, the stronger its ability to scavenge free radicals and terminate the radical chain reaction, which suggests their antioxidant properties (Gulcin and Alwasel, 2023). The result value indicates that the antioxidant activity of the *A. chinense* extract is significantly higher than its two sister species.

The result also showed that the IC_{50} value of the fresh white stalks (range from 97.40 to 106.79 ppm) was significantly ($p<0.05$) lower than that of the fresh green leaves of allium (range from 114.89 to 121.68 ppm), and it was confirmed in three Allium species tested. Among the extracts of various parts of the *Allium spp.,* the strongest potential scavenging activity was in the white stalks or bulb of *A. chinense* (IC_{50} = 97.40 ppm), which did not differ significantly from the white bulb of *A. ascalonicum* (IC_{50} = 96.20 ppm), and the weakest was in the green leaves of *A. fistulosum* (IC_{50} = 121.68 ppm).

These results also indicate that white stalks or bulbs exhibited a significantly stronger potential antioxidant activity than green leaves. The free radical antioxidant activity of allium ethanol extracts obtained from fresh white stalks or bulbs decreased in order as follows: *A. chinense* > *A. fistulosum* > *A. ascalonicum*, a similar order as in green leaves.

The result on antioxidant activity differs from some observations that (1) the green part of the leek has higher DPPH scavenging and reducing ability than its white stalk counterpart (Biernacka et al., 2021), (2) leaves have higher antioxidant potential than bulb extracts of *Allium ursinum* L. (Lachowicz et al., 2018), and (3) aerial parts of several *Allium* species have higher antioxidant activity compared to bulbs. A noteworthy observation through this study is the comparable higher antioxidant capacity of lokio (*A. chinense*), an underutilized allium species, aligning its performance with the two sister species.

Correlation analysis

This study analyzed the strength and direction of the linear relationship and correlation between bioactive phytochemicals (TPC and TFC) and the IC_{50} value in green leaves and white stalks of allium by adopting the Pearson correlation coefficient (PCC), also referred to as Pearson's r, as presented in **Table 2**.

The coefficient correlation (r) between TPC and TFC reveals a strong positive ($r > 0.8$; $p < 0.05$) relationship, as we expected. Our results showed that r-values of 0.9582, 0.9409, and 0.9043 (p-value < 0.05) between TPC and TFC in *A. chinense*, *A. ascalonicum*, and *A. fistulosum*,

Table 2. Coefficients of Pearson correlations (r) between total phenolic content (TPC), total flavonoid content (TFC) and IC_{50}

Species		TPC	TFC
Allium chinense G. Don	TPC		
	TFC	0.9582	
	IC_{50}-DPPH	-0.9615	-0.9976
Allium fistulosum L.	TPC		
	TFC	0.9410	
	IC_{50}-DPPH	-0.9590	-0.9487
Allium ascalonicum L.	TPC		
	TFC	0.9043	
	IC_{50}-DPPH	-0.9050	-0.9991

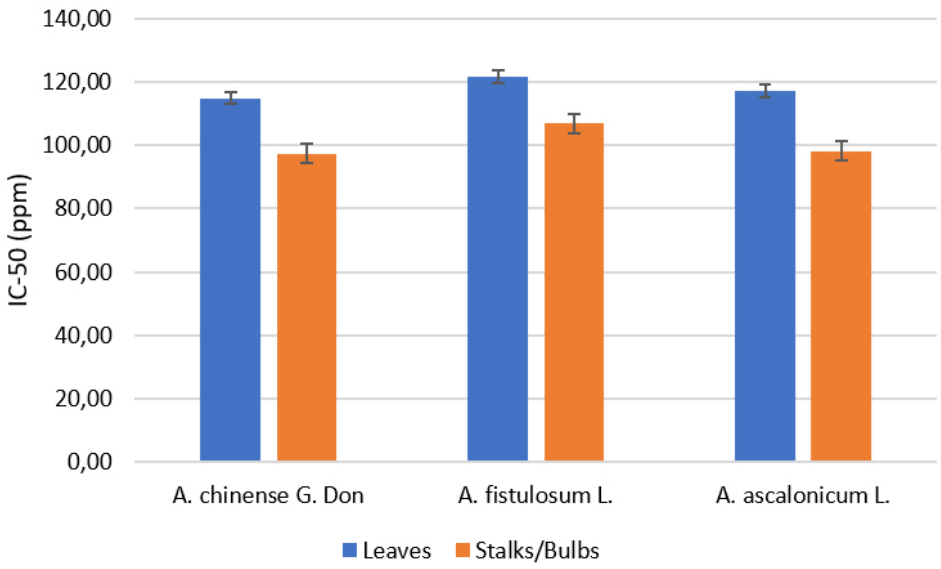

Figure 2. IC_{50} value (ppm) of selected *Allium* species

respectively, confirmed that TFC was contributed mainly by TPC. The TPC of allium was approximately 10-fold higher than the total flavonoid content. Our findings concur with those of Bibi et al. (2022) who observed that TPC < TFC in most cases. These findings showed that the high phenolic content is not primarily due to total flavonoids and their derivatives. In general, *A. ascalonicum* contained the highest average value of TPC, and the TFC decreased in order as follows: *A. ascalonicum* > *A. chinense* > *A. fistulosum*.

The relationship between TPC and IC_{50} is indicated by the r-values of -0.9615, -0.9590, and -0.9050 in *A. chinense*, *A. fistulosum*, and *A. ascalonicum*, respectively, revealing a strong positive relationship. Those are similar to the IC_{50}-TFC relationship, with r-values of -0.9976, -0.9487, and -0.9991 in lokio, spring onion, and green onion, respectively. Negative relationships indicate that an increase in TPC and TFC will decrease the ID_{50} value. The smaller the IC_{50} value of a sample, the stronger its potential to inhibit free radicals. Therefore, higher TPC and TFC are associated with the higher potential antioxidant activity of the samples. Phenolic compounds, including flavonoids, are the most widely distributed phenolic compounds in plants, and other phenolic family members such as terpenoids and tannins contribute highly to the antioxidant activity of plant material owing to their capability to scavenge reactive oxygen species (Platzer et al., 2022).

This study also analyzed the relationship between each parameter in green leaves and white stalks. Regarding TPC, the coefficient between leaves and stalks was 0.3821, indicating a weak positive relationship. This suggested that higher TPC in leaves did not always lead to higher deposits in stalks. However, in terms of TFC, it was found to be strongly positive (r = 0.9940) between leaves and stalks/bulbs. This suggested that higher TFC in leaves coincided with higher TFC in stalks/bulbs. Further, a strong relationship (r = 0.9604) exists between leaves and stalks regarding the IC50 value, indicating stronger scavenging activity in leaves, followed by stronger activity in stalks/bulbs. The latter confirmed the contribution of flavonoids to antioxidant activity. As TPC also has a significant positive contribution to antioxidant activity, the role of other phenolic compounds cannot be excluded.

Polyphenols have the ability to scavenge hydroxyl radicals (OH) and superoxide anion radicals (O_2) while also neutralizing active oxygen species such as hydrogen peroxide (H_2O_2) or singlet oxygen (O_2). With this action, polyphenol can act as an antioxidant by preventing radical reactions instigated by reactive oxygen species (ROS) and characterizing their antioxidant properties and their sensitivity to oxidation.

The finding of this study suggested that higher phenolics, coinciding with the higher potential antioxidant activity in both leaves and stalks of allium, and TFC have been considered significant contributors to antioxidant activity. This result is in agreement with the finding in *A. pallens* L. (Emir and Emir, 2020[a,b]) and in spring onion (*A. fistulosum* L.) (Biernacka et al., 2021). A noteworthy observation through this study is the comparable higher antioxidant capacity of *A. chinense*, an underutilized allium species, aligning its performance with the two sister species.

Conclusions

In this study, *Allium chinense* G. Don (lokio), *Allium fistulosum* L. (spring onion), and *Allium ascalonicum* L. (green onion) exhibited high free radical-scavenging activities, indicating their antioxidant properties. The properties were contributed significantly by the total phenolics and total flavonoids, as revealed by strong correlations between bioactive compounds and scavenging capacity ($r > 0.90$). The white stalks/bulbs of allium exhibited significantly higher levels of TPC, TFC, and antioxidant activity compared to those of the green leaves. In terms of scavenging capacity measured by the DPPH method, the best was the ethanol extract bulb of the *Allium chinense* G. Don (IC_{50} = 87.40 ppm), which has no significant difference from the bulb of *Allium ascalonicum* L. (IC_{50} = 98.21 ppm). This information would be useful to increase the utilization of underutilized lokio (*Allium chinense* G. Don) as an alternative to spring onion and green onion in food preparation as well as in cosmetic and medicine product development.

Acknowledgements

This study was partly financially supported by a grant from Universitas HKBP Nommensen. The analysis was performed in laboratory of Politeknik Teknologi Kimia Industri – Medan, Indonesia.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.