

A Comparative Study on the Total Antioxidant and Antimicrobial Potentials of Ethanolic Extracts from Various Organ Tissues of *Allium* spp.

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Received March 12th, 2013; revised April 12th, 2013; accepted April 19th, 2013

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ABSTRACT

The extracts from different tissues of *Allium fistulosum* L. and *Allium sativum* L. were investigated to evaluate their antioxidant and antimicrobial capacity. The highest yields of the *Allium* extracts were prepared from the extraction of 30% ethanol solution. The DPPH scavenging activity was the highest in *A. fistulosum* L. leaves, which IC₅₀ is 14.61 µg·mL⁻¹. The highest antioxidant activity using TEAC assay and total phenolic content were observed in *A. sativum* L. stems and *A. fistulosum* L. stems, where they are determined to be 15.51 mM and 191.04 mg GAE·g⁻¹, and 14.59 mM and 182.60 mg GAE·g⁻¹, respectively. Statistic analysis revealed that the DPPH IC₅₀ value was significantly correlated with total phenolic contents and antioxidant activity using TEAC assay. The extracts of *A. sativum* L. bulbs were found to exhibit antimicrobial activity against *Staphylococcus aureus* with the MIC and MBC of 0.2 mg·mL⁻¹ and 0.4 mg·mL⁻¹, respectively. In addition, the extract of *A. fistulosum* L. stems was more active against *Bacillus subtilis*, with an MIC and MBC of 0.2 and 0.4 mg·mL⁻¹, respectively. The inhibitory activity of various *Allium* extracts against the test bacteria was greater than that of 10 µg·mL⁻¹ allicin. The results indicated that *Allium* spp. extracts could be used as a potential source of natural antioxidants and antimicrobial agents.

Keywords: *Allium* spp.; Antioxidants; Antimicrobial Activity; DPPH IC₅₀; TEAC; Total Phenolic Content

1. Introduction

The economically important *Allium* spp. (onion and garlic) are used worldwide as spices, vegetables, and medicinal plants. Traditionally, they also play a very essential role in the daily diet in many Asia countries, and many used to flavor foods. The green onions (*Allium fistulosum* L.), also referred to as the Welsh onion, and garlic (*Allium sativum* L.) belong to the family *Alliaceae*, which are very economically important vegetables in most East Asian countries [1-3]. The *Allium* spp. materials were acquired from Sunshin Area, Yi-Lan, Taiwan. This area, mostly run by local small-scale farmers, is famous for its top-quality, fragrance-rich green onions. Green onions and garlic are commonly consumed vegetables that possess markedly bioactive potentials, such as antioxidant capacity [4], antimicrobial activity [5,6], antiplatelet ac-

tivities [7] and anticancer activity [8]. With successful demonstration of the functioning properties of the Sunshin green onions, this study would help the local farmers to market their produce with value adding strategies and benefit economically from their production.

Polyphenols and quercetin in plants have been reported to be the major compounds exhibiting antioxidant activity [4,9]. In addition, the antioxidant activity of quercetin and its functional effects have been fully demonstrated in a few previous studies [10,11]. On the other hand, *Allium* spp. contains other antioxidant components, such as flavonoids and volatile sulfur compounds [10,12]. Statistical analysis revealed that the total phenolic contents and the DPPH free radical-scavenging activity were positively correlated [13]. Besides, it had been showed a positive correlation between the dynamics of DPPH free radical-scavenging activity and the antioxidant capacity

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to scavenge the ABTS⁺ radicals [14]. Studies of the antioxidant activity of vegetables in South America also pointed out that the DPPH free radical-scavenging ability and total phenolic contents showed a good linear relationship and that both methods could be used to measure the antioxidant activity in vegetables [15].

Antioxidant and antimicrobial effects of *Allium* plants have been reported for times [1,4,5,10]; however, a study on the top-quality green onions and garlic produced in Taiwan is still scarce. In this study, different types of tissue of Taiwan Sun-Shin green onion and garlic were extracted from roots, stems and leaves using ethanol. Methods of determining the antioxidant activities of ethanol extracts from various *Allium* tissues include the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and trolox equivalent antioxidant capacity (TEAC) assay, and total phenolics were determined using the Folin-Ciocalteu method. In order to evaluate the potential use of different tissues of *Allium* spp. in Taiwan, their antioxidant capacity and antimicrobial activity were measured. The objectives of this study were to compare the antioxidant activity of the different parts of *Allium* spp. from Taiwan, to compare the antimicrobial activities of various *Allium* extracts, and to correlate the antioxidant load with the antioxidant activity of the extracts.

2. Materials and Methods

2.1. Plant Materials

Plant materials including *Allium fistulosum* L. (the Welsh onion) and *A. sativum* L. (Garlic) were collected and purchased from the Farmer's Association in the Yi-Lan Sunshin Area in Taiwan. The plant tissues, which included bulbs, roots, stems and leaves of *Allium* spp., were dried in an oven at 50°C for 7 d. The lengths of the root, stem and leaf from *A. fistulosum* L. were 8.2 ± 4.6 cm, 16.9 ± 1.9 cm and 46.1 ± 6.3 cm, respectively, and those from *A. sativum* L. were 9.7 ± 4.1 cm, 21.9 ± 5.4 cm and 50.4 ± 12.2 cm, respectively. The dried plant tissues were then extracted using various concentrations of ethanol and 100°C boiling water. Adequate amounts (10 g) of the dried tissues were extracted with 30%, 60%, 95% ethanol for 7 d at ambient temperature and boiling water for 30 min, respectively. Fractions of the extracted material were stored at 4°C prior to analysis. The abbreviations AFR, AFS and AFL represent respectively represent the roots, stems, and leaves of *A. fistulosum*, and ASB, ASR, ASS, and ASL respectively represent the bulbs, roots, stems, and leaves of *A. sativum*.

2.2. Chemicals

2,2'-Diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS),

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu reagent quercetin and (+)-catechin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Potassium persulfate was purchased from Merck. All other solvents and reagents were purchased from Sigma Chemical Co.

2.3. Antioxidant Activity Assays

The DPPH radical-scavenging capacity of *Allium* spp. was determined according to the method described by Gyamfi *et al.* [16]. One milliliter of ethanolic extract and 5 mL of freshly prepared 0.1 mM DPPH ethanolic solution were mixed thoroughly and kept in the dark. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm by a Jasco V-550 UV-visible spectrophotometer (Tokyo, Japan). The blank was prepared by replacing the extract with various concentrations of ethanol or water. The percentage of free radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \left[1 - \frac{A_{517} \text{ nm of sample}}{A_{517} \text{ nm of blank}} \right] \times 100$$

The decrease in absorbance, which was induced by the tested sample and illustrated by a change of color from deep-violet to light-yellow, was compared to that of the positive control using (+)-catechin as a standard. The IC₅₀ value, representing the concentration of extract that required for 50% inhibition of DPPH radicals, was determined. The assay was carried out in triplicate and results were averaged.

In order to determine the free radical scavenging capacity, the TEAC assay, using the ABTS^{•+} radical cation, was carried out according to the method of Re *et al.* [17], with the following slight modification. The relative antioxidant capacity of the test tissues was measured in comparison to that of Trolox (as the control) by scavenging the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS^{•+}) radical. The ABTS^{•+} radical was generated by mixing a solution of 7 mM ABTS^{•+} with 2.45 mM K₂S₂O₈. The ABTS^{•+} solution was diluted with water prior to use and an absorbance of 0.70 ± 0.02 at 734 nm was obtained. Upon adding 1485 µL of the diluted ABTS^{•+} solution to 15 µL of tested sample or Trolox, the absorbance at 734 nm was read by a Jasco V-550 UV-visible spectrophotometer after incubating the 1.5 mL mixed solution for 6 min. Decreases in absorbance were recorded and the concentrations of the standard and samples were calculated with respect to the calibration plot. The final TEAC value of the tested samples was calculated by comparing ABTS^{•+} decolorisation with that of Trolox. The Trolox solution

was used as a reference standard and the results were expressed as mmol equivalent.

2.4. Determination of Total Phenolics Contents

The amount of total phenolics was measured by the Folin-Ciocalteu method described by Kujala *et al.* [18], using gallic acid as the standard. A calibration curve was obtained using concentrations of standard solutions in the range of 0.005 - 0.08 mg·mL⁻¹. A 0.4 mL aliquot of diluted extract (all fractions were diluted with methanol to adjust the absorbance within the calibration limits), 0.4 mL of 1 M Folin-Ciocalteu reagent, and 0.8 mL of Na₂CO₃ (20%, w/v) were mixed. After 8 min of incubation, the mixture was centrifuged at 15,000 × g for 10 min. Then the absorbance of the supernatant was measured at 730 nm against a blank (using distilled water) with a Jasco V-550 UV-visible spectrophotometer. The concentration of phenolics thus obtained was multiplied by the dilution factor, and the results were expressed as the equivalent of milligrams of gallic acid per gram of extract (mg GAE·g⁻¹).

2.5. Assay of Antibacterial Activity

The tested bacteria were Gram-negative *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 9027, and Gram-positive *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6051. The strains were purchased from the Bioresource Collection and Research Center (BCRC) of the Food Industry Research Institute in Hsinchu, Taiwan. These bacteria were cultured on a nutrient agar plate for 24 h at 37°C, and the colonies that formed were picked up twice with a platinum loop and inoculated with a sterilized cotton stick onto the surface of a new nutrient agar plate. Then 50 µL of 1 mg·mL⁻¹ *Allium* extract were applied to an ethanol-sterilized paper disc (8 mm in diameter), and were placed on the plate. After incubation at 37°C for 24 h, the inhibition zone around the disc was measured [6]. Penicillin and tetracycline were used as reference controls in the antimicrobial assay. The experiments were run in triplicate, and the developing inhibition zones were compared with those of the reference discs. In addition, the minimal inhibitory concentration (MIC) of the samples was determined by the broth dilution method using the serially diluted *Allium* extracts as described [6,9]. Then, bacterial cultures were prepared in a nutrient broth and incubated at 37°C for 24 h. The bacterial cultures were adjusted with sterilized saline to bring the concentration to 10⁷ mL⁻¹. The media containing various *Allium* extracts were diluted with distilled water to give concentrations ranged from 2 to 0.05 mg·mL⁻¹. One hundred microlitres of the diluted bacterial culture, 0.6 mL of nutrient broth, and 0.6 mL of the

Allium extract were mixed well in a test tube. The mixture was then incubated at 37°C for 24 h to determine the minimal concentration at which growth of bacterial cells was fully inhibited. The MIC and MBC, the lowest concentration of *Allium* extracts that inhibits the growth of the test microorganism, was determined.

2.6. Statistical Analysis

The triplicate data were subjected to an analysis of variance for a completely random design, using SAS statistics software. The data were presented as mean ± standard deviation of three determinations. Comparison of means was analyzed by Scheffe's test of the SAS system, and differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. The Characteristics of Plant Material

Both the water contents and extraction yields with ethanol of different parts of plant tissues were determined to comprehend the general characteristics of the *A. fistulosum* L. and *A. sativum* L. The water contents of different plant tissues, including root, stem, and leave, on a drying basis, obtained from *A. fistulosum* and *A. sativum* were analyzed and found to be in the range of 83.47% to 94.76%, whereas the bulb of *Allium sativum* L. had the lowest water content of 69.80% (data not shown).

Next, the extraction yields from whole *Allium* spp. plants prepared with various concentrations of ethanol (30%, 60%, and 95%) were examined. These extraction yields were then compared with those of the same *Allium* spp. extracted with 100°C hot water. The results show that the highest extraction yields, which were 42.87% ± 8.74% and 42.43% ± 4.63% for *A. fistulosum* and *A. sativum*, respectively, were prepared from 100°C hot water. The second highest extraction yields, which were 39.06% ± 6.89% and 41.39% ± 3.63% for *A. fistulosum* and *A. sativum*, were extracted from 30% ethanol solution. Nevertheless, these extraction yields of the tissues extracted from hot water and 30% ethanol solution were not significantly different at 95% confidence interval. However, it was found in our experiments that the hot water extracts contain many visible remnants of plants, and that their antioxidant and antimicrobial activities were less than those of the ethanolic extracts (results not shown). Ethanol has been proved to be a better solvent than water for the extraction of allicin in plant tissues [6]. The subsequent extraction of *Allium* spp. in the study was prepared with a solution that contained 30% of ethanol to generate the high extraction yields. The extraction yields of various parts of the plant tissues extracted from *A.*

fistulosum and *A. sativum* with 30% (w/w) ethanol, on a drying weight basis, were also investigated. The extraction yields of ethanol ranged from 15.47% to 43.56% for *A. fistulosum*, while it ranged from 25.31% to 39.05% for *A. sativum* (data not shown). The results also show that the extract of stem had the highest yields, followed by that of the leaf and root.

3.2. The DPPH Free Radical-Scavenging Activity of Different *Allium* Extracts

The DPPH free radical-scavenging activities of different *Allium* extracts are presented in **Table 1**. The DPPH free radical-scavenging activity of *Allium* spp. ranged from 67.34% to 90.03% for *A. fistulosum*, while it ranged from 63.63% to 88.33% for *A. sativum*. AFL extracts (90.0%) and ASL (88.3%) had the highest DPPH free radical-scavenging activity among the extracts of various parts of the *Allium* spp. Furthermore, the leaf extracts had higher DPPH free radical-scavenging activity than that of the stem, bulb and root. The corresponding IC₅₀ values of the various *Allium* spp. required to scavenge 50% of free radicals are also shown in **Table 1**. Compared to the IC₅₀ values of various *Allium* spp., the free radical-scavenging potency of the AFL and ASL was 14.61 and 14.89 µg·mL⁻¹, respectively, which were higher than that of (+)-Catechin (2.19 µg·mL⁻¹). In addition, the extracts of leaf had higher DPPH free radical-scavenging activity than that of the other parts of *Allium* spp.

The DPPH free radical-scavenging activity of *A. fistulosum* extracts in this study was higher than the results described by Huang *et al.* [15]. In their study, the green onion extract showed a lower antioxidant capacity, at a

Table 1. Effects of ethanolic extracts on the *in vitro* DPPH radical-scavenging activity and DPPH free radical IC₅₀ value of various *Allium* spp.

Extracts	DPPH radical-scavenging activity (%)	DPPH IC ₅₀ (µg·mL ⁻¹)
AFR	67.34 ± 1.73 ^{bc}	19.54 ± 0.34 ^b
AFS	85.95 ± 7.05 ^{ab}	15.21 ± 0.24 ^c
AFL	90.03 ± 5.38 ^a	14.61 ± 0.11 ^c
ASB	85.79 ± 3.50 ^{ab}	15.24 ± 0.16 ^c
ASR	63.63 ± 7.69 ^c	20.68 ± 0.14 ^a
ASS	86.49 ± 5.72 ^a	15.13 ± 0.10 ^c
ASL	88.33 ± 6.48 ^a	14.89 ± 0.19 ^c
(+)-Catechin*	88.26 ± 0.83 ^a	2.19 ± 0.02 ^d

Data are expressed as the average of three determinations ± SD. Data with different superscript lower-case letters in the individual column are significantly different at p < 0.05, according to the Scheffe's test. * (+)-Catechin (5 µg·mL⁻¹) was used as a positive reference.

concentration of 10.0 mg dried vegetable equivalent mL⁻¹, about only 60% of the DPPH free radical-scavenging activity obtained from our study. The discrepancy among the DPPH radical-scavenging activities in different studies may be partially due to different antioxidant potentials of different compounds, the antioxidant activity of the plant extracts strongly depends on the extraction solvent. For example, the DPPH radical-scavenging activity of *Cynara cardunculus* EtOAc extracts showed the strongest activity (IC₅₀ = 21.50 µg·mL⁻¹), when compared to those of other extraction solvents [19].

3.3. TEAC Assay of Different *Allium* Extracts

The antioxidant activity obtained from the TEAC assay is presented in **Figure 1**. The TEAC values of the various *Allium* extracts ranged from 6.74 to 14.59 mM for *A. fistulosum*, while it ranged from 6.79 to 15.51 mM for *A. sativum*. ASS had the highest antioxidant activity, followed by AFS, ASL, AFL, ASB, while ASR and AFR appeared to have the lowest ability to scavenge the ABTS⁺. The most interesting result is that the TEAC values of the leaf and stem were significantly higher than those of the root (**Figure 1**). Another significant finding is that the extracts from the stem had higher antioxidant activities than those taken from other tissues of *Allium* spp. This finding adheres to the finding of Aoyama and Yamamoto [10], showing that the flavonoid-rich *Allium* vegetables demonstrated a high antioxidant activity using

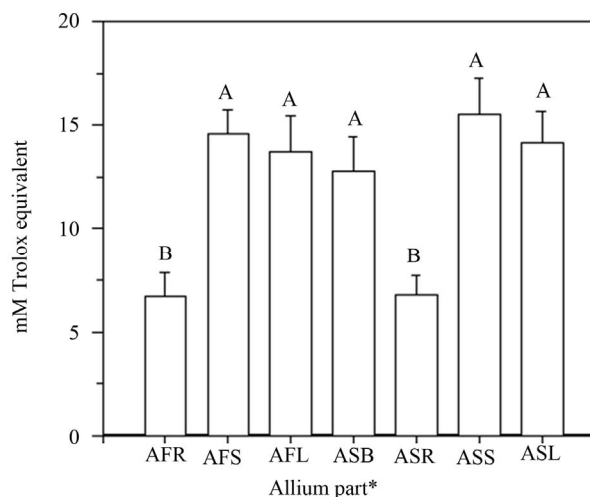


Figure 1. Antioxidant activity of various *Allium* extracts using a TEAC assay. Data are expressed as the average of three determinations ± SD. Data that do not share the same capital case letter on the top of vertical bar are significantly different at p < 0.05, according to the Scheffe's test. The symbols AFR, AFS, AFL represent the roots, stems and leaves of *Allium fistulosum*, respectively; ASB, ASR, ASS, ASL represent the bulbs, roots, stems and leaves of *Allium sativum*, respectively.

the TEAC assay. The finding of the current study is also consistent with that of Jang *et al.* [4], who found that the antioxidant activity of the Welsh onion extract, with a concentration of $50 \mu\text{g}\cdot\text{mL}^{-1}$, was higher than that of other *Allium* spp. Moreover, Vågen & Slimestad [20] analyzed the antioxidant capacities of *Allium* spp. using a TEAC assay and also found that TEAC values of the methanol extracts from 15 different onion cultivars were in the range of 0.7 to $5.1 \mu\text{mol}\cdot\text{g}^{-1}$ fresh sample weight. The discussion above shows that the antioxidant capacity of green onion can differ from cultivar to cultivar. Further, the antioxidant capacity also varies depending on the location of the onion. In general, the onion extracts were proven to act as excellent antioxidants, especially the stem part of the onion.

3.4. Total Phenolic Contents of Different *Allium* Extracts

The total phenolic content of various *Allium* extracts is presented in **Figure 2**. Total phenolic contents from different *Allium* extracts ranged from 75.27 to $191.04 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$ for *A. fistulosum*, while they ranged from 82.86 to $182.60 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$ for *A. sativum*. Our results showed the total phenolic contents in the various tissue extracts of *Allium* spp. as: AFS ($191.04 \pm 8.02 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$) > ASS ($182.60 \pm 1.73 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$) > ASB ($179.68 \pm 8.48 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$) > ASL ($176.94 \pm 7.67 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$)

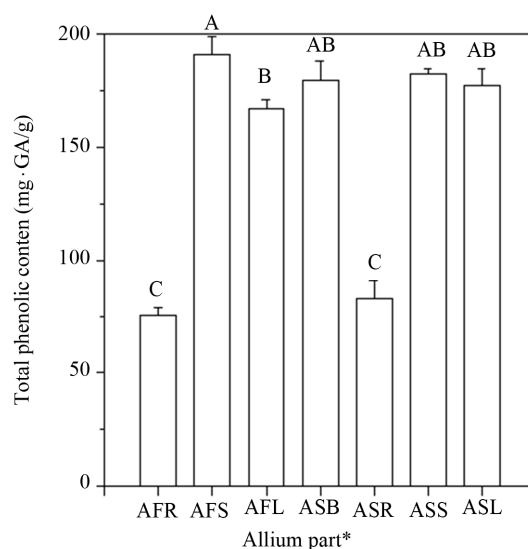


Figure 2. Total phenolic contents of various *Allium* extracts. Data are expressed as the average of three determinations \pm SD. Data that do not share the same capital case letter on the top of vertical bar are significantly different at $p < 0.05$, according to the Scheffe's test. The symbols AFR, AFS, AFL represent the roots, stems and leaves of *Allium fistulosum*, respectively; ASB, ASR, ASS, ASL represent the bulbs, roots, stems and leaves of *Allium sativum*, respectively.

> AFL ($167.16 \pm 3.78 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$) > ASR ($82.86 \pm 7.82 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$) > AFR ($75.27 \pm 3.56 \text{ mg}\cdot\text{GAE}\cdot\text{g}^{-1}$). The highest total phenolic content was observed in the stem extracts and the lowest in the root extracts of *Allium* spp. with a 30% ethanol extract solution. The results also showed that various tissue extracts of *Allium* spp. contain significantly high concentrations of phenolic compounds, which could act as the potential antioxidants. In recent studies, it was proven that free radical-scavenging activity and antioxidant activity are greatly influenced by the phenolic composition of the tested sample [13,21]. However, phenolic compounds can act as antioxidants and can also easily be oxidized. In addition, according to the study of Aoyama & Yamamoto [10], which was about the determination of the phenolic compound contents, suggested that the antioxidant activity of the green Welsh onion was higher than that of the yellow onion. These results are in agreement with our observations, which showed that the antioxidant activity of *A. fistulosum* stem extracts was higher than that of *A. sativum* extracts.

3.5. Correlation Analysis of the DPPH IC₅₀ Value, Total Phenolic Contents or TEAC with Antioxidant Properties

Correlation analysis was used to determine any possible relation between the DPPH IC₅₀ value, the total phenolic contents, or the TEAC assay of the antioxidant activity of different extracts from various parts of *Allium* spp. Statistical analysis revealed that the DPPH IC₅₀ value, the total phenolic contents, as well as the TEAC assay significantly correlated in the *Allium* extracts (**Figure 3**). As the results show in **Figure 3(a)**, statistical analysis revealed that the total phenolic contents in the *Allium* extracts and the DPPH IC₅₀ value were inversely correlated; indicating that higher phenolic content leads to stronger antioxidant capacity in the *Allium* extracts. Correlation between the DPPH IC₅₀ of the extracts obtained from various tissues of *Allium* spp. and the total phenolic content was significant ($r^2 = 0.92$). The antioxidant activities measured in the TEAC assay also significantly correlate with the DPPH IC₅₀ value ($r^2 = 0.92$), as shown in **Figure 3(b)**. This finding, which indicated that both the DPPH IC₅₀ value and the TEAC assay could be used simultaneously to determine the antioxidant activities of *Allium* extracts, is supported by the findings of many other similar experiments [9,14,22,23].

In addition, the correlation between the total phenolic content and the Trolox equivalent antioxidant capacity of the extracts from various parts of *Allium* spp. was showed in **Figure 3(c)**. Correlation between the total phenolic content and the Trolox equivalent antioxidant capacity of *Allium* spp. extracts was significant ($r^2 = 0.95$). The anti-

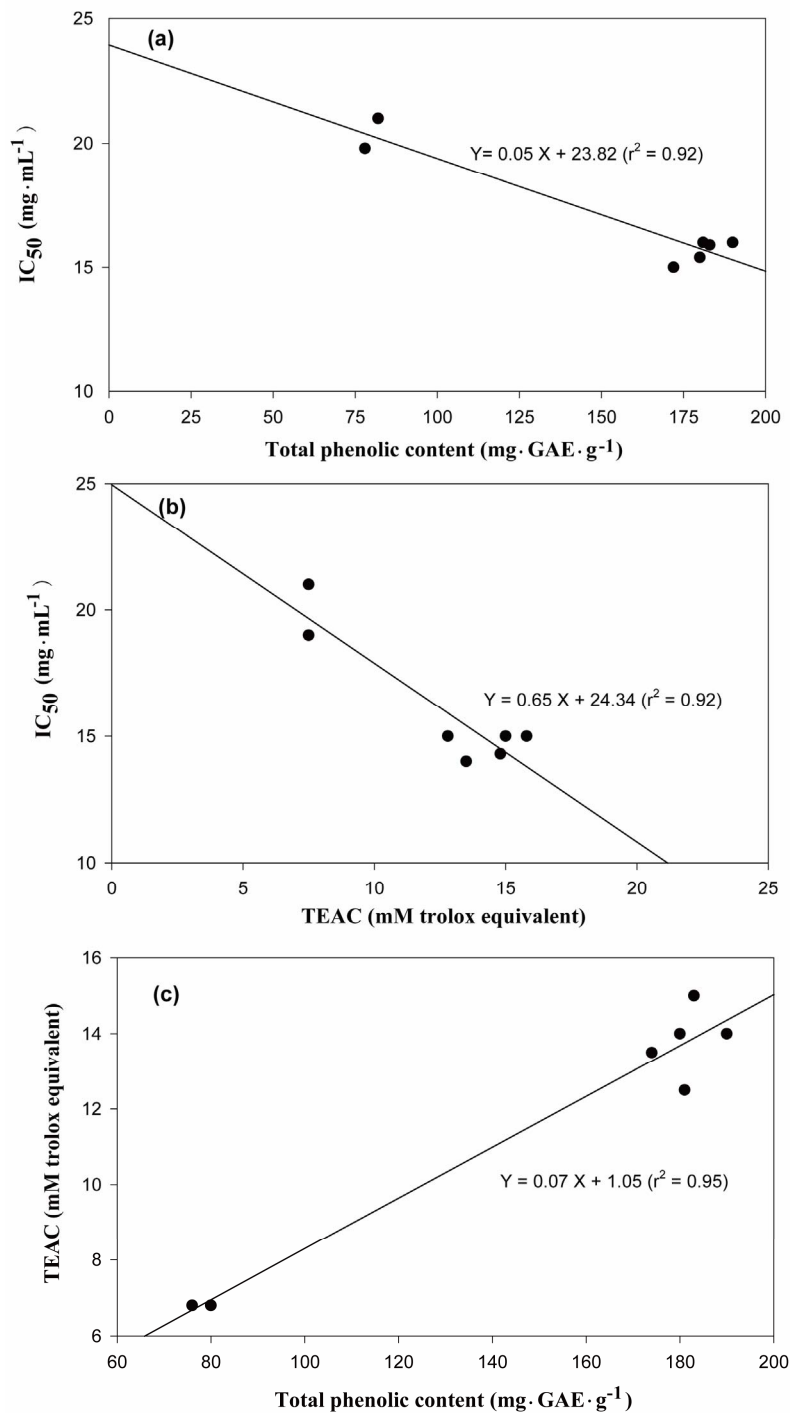


Figure 3. Correlation analysis of the DPPH IC_{50} value, total phenolic contents or TEAC with antioxidant properties. (a) Linear correlation of total phenolic contents with respect to the DPPH IC_{50} , (b) Linear correlation of the TEAC with respect to the DPPH IC_{50} and (c) Linear correlation of total phenolic contents with respect to the TEAC, of the tissues from various parts of the *Allium* spp. Data are expressed as the average of three determinations.

oxidant activity exhibited by the *Allium* extracts may be due to high levels of phenolic compounds. Therefore, it might be concluded that the total phenolic content could be used as an index for free radical-scavenging and anti-

oxidant capacity in the *Allium* extracts. These results, which proved that higher phenolic content could elucidate stronger antioxidant capacity, were consistent with those of other studies [13,15,20,23].

3.6. Antimicrobial Activity of *Allium* Extracts

The antimicrobial activity of *Allium* extracts was tested *in vitro* by using the disc diffusion and broth dilution method. According to the results given in **Table 2**, most *Allium* extracts exhibited antimicrobial activity against all tested microorganisms. While the results indicated that AFR and ASR extracts displayed no inhibition activity against all tested microorganisms, AFS, AFL, ASB, ASS and ASL obviously inhibited growth of the tested microorganisms. The current study revealed that the extract of ASB was more active against *S. aureus*, with an MIC and minimal bactericidal concentration (MBC) of 0.2 mg·mL⁻¹ and 0.4 mg·mL⁻¹, respectively (**Table 3**). The extract of AFS was more active against *B. cereus*, with an MIC and MBC of 0.2 and 0.4 mg·mL⁻¹, respec-

tively. Among these various extracts, only ASB was more effective in inhibiting the growth of *E. coli*, with an MIC of 0.4 mg·mL⁻¹ and an MBC of 0.8 mg·mL⁻¹. According to the results of this study, the extracts from various parts of *Allium* spp. also exhibited strong inhibitory activity against the tested bacteria. However, the *Allium* extracts showed higher inhibitory responses towards *B. cereus*, *S. aureus* and *P. aeruginosa*, but they were less effective in inhibiting *E. coli*. The mechanisms as how natural compounds in green onion and garlic exert their function have been previously discussed and the antimicrobial activity of the allicin contained in the garlic and the onion has been proposed in many studies [6, 24-26]. The sulfur-containing compounds, such as allicin contained in green onion and garlic, could be responsible for the antimicrobial potential. The allicin exhibited

Table 2. Antimicrobial activities of various *Allium* extracts, penicillin and tetracycline.

Extracts	Inhibition zone (mm)			
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
AFR	(-) ^{e*}	(-) ^e	(-) ^d	(-) ^c
AFS	12.3 ^b ± 0.3 ^d	18.4 ± 2.1 ^{bc}	11.5 ± 0.2 ^{bc}	12.0 ± 0.5 ^b
AFL	14.1 ± 0.9 ^{cd}	12.5 ± 0.5 ^d	8.2 ± 0.1 ^c	16.5 ± 0.8 ^a
ASB	20.3 ± 0.5 ^b	15.7 ± 1.2 ^{cd}	13.6 ± 1.1 ^b	14.0 ± 0.5 ^{ab}
ASR	(-) ^e	(-) ^e	(-) ^d	(-) ^c
ASS	16.3 ± 0.5 ^c	14.6 ± 0.2 ^{cd}	12.8 ± 0.1 ^{bc}	13.8 ± 0.9 ^{ab}
ASL	15.0 ± 1.5 ^{cd}	11.5 ± 1.0 ^d	9.3 ± 0.7 ^{bc}	13.4 ± 0.3 ^{ab}
Penicillin ^{**}	24.5 ± 0.8 ^a	26.9 ± 1.5 ^a	10.2 ± 0.3 ^{bc}	11.5 ± 0.8 ^b
Tetracycline ^{***}	12.2 ± 1.5 ^d	22.3 ± 3.3 ^{ab}	25.4 ± 3.6 ^a	16.5 ± 2.3 ^a

The inhibition zone of respective bacteria is expressed as the average of three determinations ± SD. Data with different superscript lower-case letters in the individual column are significantly different at $p < 0.05$, according to the Scheffe's test. *No activity detected. **The concentration of penicillin was 10 µg·mL⁻¹. ***The concentration of tetracycline was 30 µg·mL⁻¹.

Table 3. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of various *Allium* extracts.

Microorganisms	<i>Staphylococcus aureus</i>		<i>Bacillus subtilis</i>		<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Extracts								
AFR	ND [*]	ND	ND	ND	ND	ND	ND	ND
AFS	0.4 ^{**}	0.8	0.2	0.4	0.8	1.0	0.4	0.8
AFL	0.4	0.8	0.4	0.8	1.0	>1.0	0.4	0.8
ASB	0.2	0.4	0.4	0.8	0.4	0.8	0.4	0.8
ASR	ND	ND	ND	ND	ND	ND	ND	ND
ASS	0.4	0.4	0.4	0.8	0.8	1.0	0.4	0.8
ASL	0.4	0.8	0.8	1.0	0.8	1.0	0.4	0.8

*Not determined. **The concentrations of various *Allium* extracts (mg·mL⁻¹).

dose-dependent antimicrobial activity against both *S. aureus* and *E. coli*, where the concentration of the allicin was from 5 to 20 mM; yet, *E. coli* was less sensitive to allicin than *S. aureus* was [6]. However, the antimicrobial mechanism of the *Allium* extracts has not been clearly elucidated and analyses of the active compounds which inhibit the growth of tested bacteria were under the way in our laboratory.

4. Conclusion

This paper presented the potential use of the antioxidant and antimicrobial properties of the ethanolic extracts of *Allium* spp. In our studies, it has also been noted that the *Allium* extracts show high radical-scavenging activity (63% - 90%) and can be considered a good source of natural antioxidants and antimicrobials. It was also found that the DPPH IC₅₀ value, the total phenolic contents, and the TEAC assay were strongly correlated. Evaluation of these three variables and their relationships could be used as an index for measuring the net effective antioxidant activity in *Allium* extracts. Furthermore, the results from the *Allium* extracts could be used in the development of food preservatives and antioxidants obtained from natural sources that are effective against foodborne pathogens.

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