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The Study of the Antioxidant Activity and Phenolic Compounds of Different *Allium* Species

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Abstract

Allium species, widely known as food flavoring, vegetables, and folk medicine, have been extensively studied for their therapeutic and pharmacological effects. While the health benefits of onions have been well-documented, there is limited research on Mongolian onions. Previous studies have shown that their beneficial effects are associated with their total phenolic contents, especially quercetin derivatives. This study aims to determine the *in vitro* antioxidant activity of various *Allium* species cultivated in Mongolia and Taiwan region. Different onion species, including *Allium tagar*. L, *Allium fistulosum*. L and *Allium cepa*. L were collected in Mongolia, and yellow and red onions (*Allium cepa* L) were collected in Taiwan region. The total phenolic content (TPC) was measured by the Folin-Ciocalteu assay. Furthermore, the quercetin derivatives of TPC were measured by HPLC. The antioxidant activity of water and methanol extracts was determined via DPPH and FRAP assays. The results showed that the highest TPC was in the methanol extracts of *Allium tagar*. L (4.23 ± 0.03 mg GAE/g dry weight), *Allium fistulosum*. L (3.96 ± 0.08 mg GAE/g dry weight) in Mongolia, and the TPC following the addition of red and yellow onion in Taiwan region. Therefore, quercetin-4-O-glucoside is the dominant methanol extract of *Allium tagar*. L (1.37 ± 0.15 mg/g dry weight) had the highest content compared to other extracts—the methanol extracts of *Allium tagar*. L and red onion (*Allium cepa* L) showed the highest free radical scavenging activity in both the DPPH and FRAP assays (79.2% ± 4.37% DPPH inhibition, 63.7 ± 1.07 mg Trolox/g FW, and 86.7% ± 7.27% DPPH inhibition, 28.6% ± 1.0 mg Trolox/g FW). Our study found that onion species are good sources of phenolic compounds, including quercetin-4-O-glucoside, and methanol extracts of onions had higher content than water extract. Moreover, it positively affects antioxidant *in vitro* systems, DPPH radical scavenging ability, and FRAP antioxidant capacity.

Keywords

Total Phenolic Compounds, Antioxidant Capacity, and Onions

1. Introduction

Allium is the largest and most crucial representative genus of the *Alliaceae* family, comprising 700 species widely distributed worldwide. Among them, 37 species of *Allium* vegetables are harvested and mainly consumed. Onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) are the most commonly consumed vegetables and medical plants. Rich in vitamins, minerals, and antioxidants, onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) are vegetables that contribute to a balanced diet and have been linked to various health improvements. Thus, they are an essential part of both local and global food systems.

Regular intake of them decreases the risk of colorectal, lung, liver, brain, stomach, ovarian, prostate, and breast cancer [1]. Therefore, it has anti-diabetic, anti-obesity, and other health-beneficial effects. Thus, the antioxidant activities of different onion species were investigated in detail [2]. Tsai *et al.* reported that green onion and garlic have anti-oxidative and anti-inflammatory effects by their scavenging abilities of the cation radical ABTS+ and inhibitory effect of NO production in lipopolysaccharide (LPS) activated macrophages, respectively [3]. Many factors were reported to influence the antioxidant activity of onions, such as the genetic background, cultivating techniques, environmental influences, and storage conditions [4]-[8]. Differences in the metabolites in onion species may reflect their physiological adaptation to different ecological conditions during domestication [9] [10]. Moreover, high amounts of organosulfur compounds, polyphenols, and flavonoids were present. Its constituents, especially quercetin, are potential immunomodulatory therapeutic candidates for treating chronic disorders. Previous research showed that their health effects were associated with their compounds of phytonutrients, such as the content of flavonoids, oligosaccharides, thiosulfate, and other sulfur compounds [8]-[11]. Among them, phenolic compounds and quercetin derivatives (28.4 - 48.6 mg/100 g) are higher in onion species than in other *Allium* species [12]-[14]. Quercetin aglycone, quercetin-3,4'-O-diglucoside and quercetin-4'-O-glucoside are the predominant forms in onions [14]. Therefore, the number of phenolic compounds found in each variety varied significantly, e.g., gallic acid (9.3 - 354 μg/g), ferulic acid (13.5 - 116 μg/g), quercetin (14.5 - 5110 μg/g), protocatechuic acid (3.1 - 138 μg/g), and kaempferol (3.2 - 481 μg/g) [10] [11] [13]. Previous studies showed the four onion (*Allium cepa* L.) variants (violet, red, green, and white) for their compliance by the high-performance liquid chromatography (HPLC), and kaempferol, ferulic acid, quercetin, gallic acid, and protocatechuic acid were also identified [14] [15]. However, genotype, climatic conditions, and storage period significantly influence therapeutic effects. Bulbs differ in size, form, color, and species. At the same time, warmer temperatures are usually milder and sweeter than in other climates [16] [17]. Studies have shown the health functions of onions well, but there are limited studies about Mongolian onions. This study aims to examine the *in vitro* antioxidant activity of different *Allium* species grown in Mongolia and Taiwan region.

2. Materials and Methods

2.1. Experimental Reagents

Folin-Ciocalteu, 2,2-diphenyl-picrylhydrazyl (DPPH), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), hydrochloric acid (HCl), ferric chloride ($FeCl_3$), methanol, and gallic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). All reagents and solvents used were of analytical grade.

2.2. Preparation of Samples

Different onion species, including *Allium tagar*. L, *Allium fistulosum*. L and *Allium cepa*. L were collected from the market in Ulaanbaatar, Mongolia, and onions (*Allium* cepa. L) were purchased from the market in Taipei, Taiwan region. After peeling and cleaning, the whole vegetables were chopped and homogenized in a high-speed blender, followed by lyophilization (Freeze-dryer model FD24-6P-D5P). The dry samples were used for further extraction of compounds. The major bioactive compounds in *allium* vegetables are organosulfur and phenolic; the extracts rich in these compounds were isolated by Lu [4] and Wetli [12], respectively. Briefly, the dry powder of onions was extracted with water and methanol overnight and centrifuged at 3000 rpm for 15 min at 4˚C. The supernatant was collected, and the solvents were evaporated on a rotary evaporator at 40˚C, 90 rpm, re-dissolved, and analyzed for antioxidant activity.

2.3. Determination of Total Phenolic Contents (TPC)

TPC in the different extracts was determined by the Folin-Ciocalteu methods according to the process by Lu [4] with minor modifications. Briefly, Folin-Ciocalteu reagent was diluted 10-fold with water, mixed with different extracts, and incubated for 10 min at room temperature. Then, 2% sodium carbonate (w/v) was added and incubated in the dark for 45 min, followed by measuring the absorbance at 765 nm. Results were expressed as mg of gallic acid equivalents per g fresh weight (mg GAE/g FW).

2.4. Analysis of Quercetin Derivatives Content in Different Extracts by High-Performance Liquid Chromatography (HPLC)

To identify the active compounds of extract containing quercetin and their glucoside were examined by high-performance liquid chromatography (HPLC) (TSP, Germany). The HPLC conditions for the quercetin derivatives were as follows: C18 column (Vercopak, ODS-3, 4.6 mm × 250 mm); UV absorbance: 374 nm; analyzing temperature 30˚C; flow rate: 1 ml/min; gradient mobile phase system: solvent A, 0.05% phosphoric acid in water, solvent B, 0.05% phosphoric acid in methanol following method described by Lombard [5].

2.5. Antioxidative Activities of Onion Extracts

The total antioxidant capacity (TAC), the measurement of free radical scavenging activity of the onion and garlic extracts, was measured using a DPPH method as modified from Lu [4] and Sun [6]. The extracts were added to the free radical 2,2-diphenyl-picrylhydrazyl (DPPH) solution, and the absorbance of the DPPH was determined at 515 nm. The following equation expressed the results:

$$\text{Scavenging activity } (\%) = \frac{A_c - A_s}{A_c} \times 100$$

where Ac was the absorbance of DPPH without the sample, and As was that of the sample with DPPH.

The antioxidant activity was measured by a ferric-reducing antioxidant power (FRAP) assay [17]. The samples were mixed with the FRAP reagent, and the increase in absorbance at 593 nm due to the formation of tri-pyridyl-S-triazine complexes with Fe^{2+} [TPTZ-Fe(II)] was determined [4]. The samples' results were expressed as μmol Trolox equivalents/g fresh weight sample (μmol Trolox/g FW).

2.6. Statistical Analysis

All tests were performed three times, and the results were expressed as mean value and standard deviation. The statistical analyses were performed with the aid of SPSS software version 24. Differences between onion species were considered significant at $p < 0.05$.

3. Results and Discussions

This study determined the total phenolic contents and antioxidant activity of different onion species. Solvents with different polarities, including water, methanol, ethanol, and ethyl acetate, were used to extract bioactive components from onion and garlic. Screening results of onion species showed that those with higher phenolic contents were selected for further study, using their water and methanol extracts.

3.1. Total Phenolic Contents (TPC) in Onion Species

The total phenolic compounds ranged from 2.03 ± 0.04 mg GAE/g dry weight to 2.57 ± 0.05 mg GAE/g dry weight in the water extracts. There were no differences between the Mongolian and Taiwanese species in Taiwan region. The Mongolian tagar onion (*Allium tagar* L.) had the highest phenolic contents (**Table 1**). The results showed that water extracts of tagar onion (*Allium tagar* L.) had 2.57±0.05 mg GAE/g dry weight, while the methanol extracts had 4.23 ± 0.03 mg GAE/g dry weight. Wild onion (*Allium fistulosum*. L) had 2.5 ± 0.03 mg GAE/g dry weight in the water extracts and 3.96 ± 0.08 mg GAE/g dry weight in the methanol extracts. Yellow onion (*Allium cepa* L.) had 2.03 ± 0.04 mg GAE/g dry weight in the water extracts and 2.82 ± 0.06 mg GAE/g dry weight in the methanol extracts.

Table 1. Total phenolic contents of onion species.

Samples	Total phenolic contents, mg GAE/g DW		
	Samples	Water extracts	Methanol extracts
Mongolian onion species	Tagar onion (*Allium tagar*. L)	2.57 ± 0.05	4.23 ± 0.03*
	Wild onion (Allium *fistulosum*. L)	2.50 ± 0.03	3.96 ± 0.08*
	Yellow onion (*Allium cepa*. L)	2.03 ± 0.04	2.82 ± 0.06
Taiwanese onion species	Yellow onion (*Allium cepa*. L)	2.22 ± 0.08	3.74 ± 0.09
	Red onion (*Allium cepa*. L)	2.24 ± 0.06	3.88 ± 0.04

Values were expressed as the mean ± SD from three measurements; data with different symbols (*) indicated the significant difference between the solvents.

Sharma *et al.* and Santas *et al.* showed that the methanol extract of Spanish white onion (*Allium cepa* L.) contained 6.33 ± 0.3 mg GAE/g DW, while Spanish calcot onion (Allium cepa L.) had 2.58 ± 0.16 mg GAE/g DW TPC [16] [18] [19]. Moreover, Lu *et al.* determined that the aqueous methanol extract of white, yellow, red, and sweet onions had 2.69 ± 0.2 mg/g, 1.64 ± 0.14 mg/g, 4.28 ± 0.28 mg/g, and 1.42 ± 0.08 mg/g TPC, respectively [4]. Our results demonstrated that Mongolian onions had a higher TPC than others in similar amounts.

3.2. Determination of Quercetin Derivates

Figure 1 and **Figure 2** show that the phenolic compounds in onion extract were quercetin derivatives, of which Q4G was dominant. Other studies have supported this result, for instance, the two significant glucosides of quercetin in onions are quercetin-3,4'-O-diglucoside and quercetin-4'-O-monoglucoside, which represent approximately 80% of the total flavonol content of onion [16] [17]. Furthermore, the content of the phenolic compound quercetin in different extracts of onion species, as quantified by HPLC (**Table 2**), indicated that the methanol extract of Mongolian tagar onion (*Allium tagar* L.) contained the highest amount of Q4G among those in the other extracts (1.37 ± 0.15 mg/g FW).

Our result showed that the Q4G contents in water and methanol extracts of onion species were the same as reported previously; the methanol extracts of tagar onion had a higher content of 1.37 ± 0.15 mg Q4G per g. The methanol extracts of red onion had 0.93 ± 0.28 mg Q4G per g. The lowest content of phenolic compounds was found in the water extracts of onions, including wild onion (*Allium* *fistulosum*. L) with 0.19 ± 0.14 mg Q4G per g and yellow onions (*Allium cepa*. L) with 0.19 ± 0.27 mg Q4G per g.

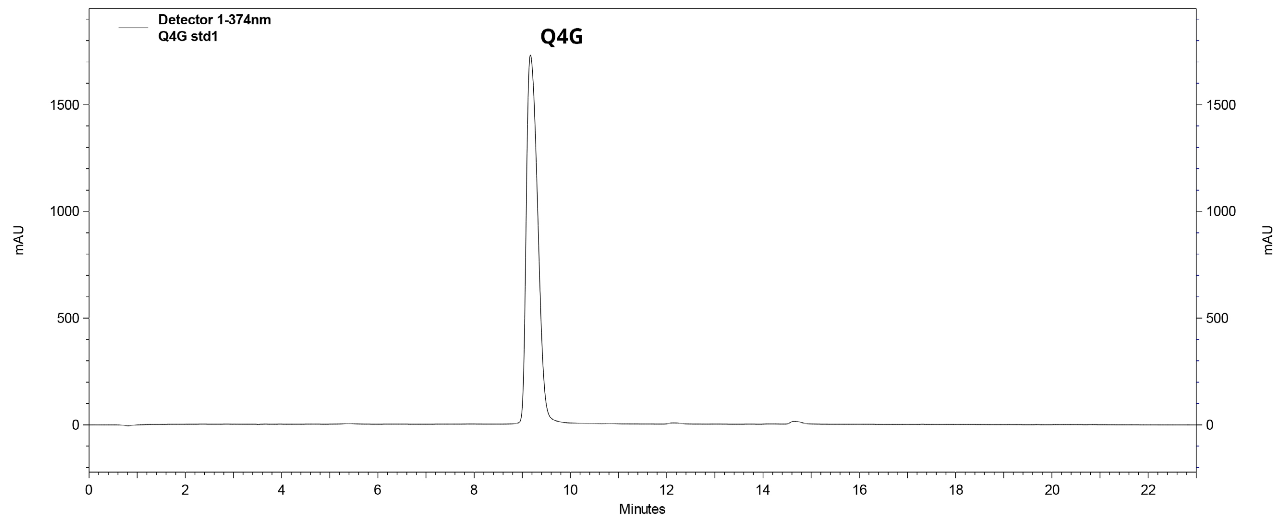

Figure 1. Typical chromatograms of Quercetin-4-O-glucoside (Q4G), standard solutions.

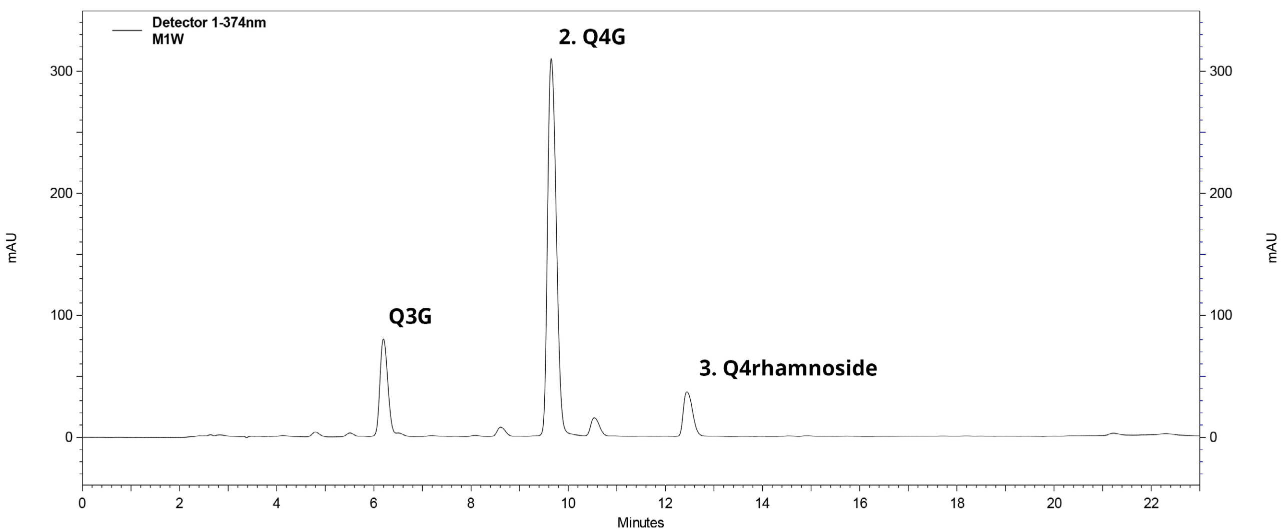

Figure 2. Water extract of onion samples: 1. Quercetin-3-O-glucoside (Q3G), 2. Quercetin-4-O-glucoside (Q4G), 3. Quercetin-3-O-rhamnoside (Q3rhamnoside).

Table 2. Q4G content of different onion extracts.

Samples	mg Q4G/ g FW, $r^2 = 0.97$		
	Samples	Water extracts	Methanol extracts
Mongolian onion species	Tagar onion (*Allium tagar*. L)	0.76 ± 0.34*	1.37 ± 0.15*
	Wild onion (*Allium fistulosum*. L)	0.19 ± 0.14	0.68.8 ± 0.06
	Yellow onion (*Allium cepa*. L)	0.60 ± 0.03	0.55 ± 0.22

Continued

Taiwanese onion species	Yellow onion (*Allium cepa*. L)	0.19 ± 0.27	0.52 ± 0.21
	Red onion (*Allium cepa*. L)	0.28 ± 0.02	0.93 ± 0.28*

Values were expressed as the mean ± SD from three measurements; data with different symbols (*) indicated the significant difference between the solvents.

Soininen *et al.* determined the Q4G of methanol extracts from Finnish yellow and red onions (*Allium cepa* L.) to be 260 ± 142.4 mg/kg and 249.1 ± 78.4 mg/kg, respectively [20] [21]. This suggests that TPC varies among onion varieties based on their origin. The study reported a variety of flavonoids in several onion varieties, including quercetin-4-O-monoglucoside, isorhamnetin 3,4-O-diglucoside, quercetin-3,4-O-diglucoside, quercetin aglycon, quercetin-3-monoglucoside, delphinidin 3,5-diglycosides, quercetin 3-glycosides, quercetin 7,4-O-diglucoside, quercetin 3,7,4-O-triglucoside, and quercetin-3-4-O-diglucoside [22] [23], among others. Compared to apples (50 mg/kg), broccoli (100 mg/kg), and blueberries (40 mg/kg), onions exhibited 5 to 10 times higher quercetin content (300 mg/kg).

3.3. Determination of Antioxidant Activities

The DPPH radical scavenging assay is one of the few stable and commercially available assays used to evaluate antioxidant potential, including hydrogen-donating ability *in vitro*. The DPPH scavenging capacity of water and methanol extracts of onion species was determined in terms of equivalent concentrations of Trolox ($r = 0.99$).

Table 3. DPPH, free radical scavenging ability in onion species.

Samples	DPPH, inhibition (%)		
	Samples	Water extracts	Methanol extracts
Mongolian onion species	Tagar onion (*Allium tagar*. L)	63.5 ± 5.39	79.2 ± 4.37
	Wild onion (*Allium fistulosum*. L)	69.5 ± 4.2	77.8 ± 16.6
	Yellow onion (*Allium cepa*. L)	64.6 ± 4.98	69.4 ± 6.28
Taiwanese onion species	Yellow onion (*Allium cepa*. L)	63.5 ± 5.39	79.2 ± 4.37
	Red onion (*Allium cepa*. L)	69.5 ± 4.2	77.8 ± 16.6

DPPH is the free radical scavenging activity of different extracts of onion species cultivated in Mongolia and Taiwan region. Values are expressed as the mean ± SD from three measurements; data indicated a significant difference between solvents among one species ($p < 0.05$).

Table 3 illustrates that the highest inhibition of DPPH radical scavenging was found at 86.7% ± 7.27% in red onion. The methanol extract of onion showed greater DPPH inhibition, while the water extract of garlic showed higher activity than that of the methanol extract. Similarly, the methanol extract of onion and the water extract of garlic showed the highest inhibition of DPPH radical scavenging activity. These results may be correlated with their TPC.

Table 4. FRAP, a marker of total antioxidant capacity in onion species.

Samples	FRAP, mg Trolox per g FW		
	Samples	Water extracts	Methanol extracts
Mongolian onion species	Tagar onion (*Allium tagar.* L)	1.85 ± 0.16	63.7 ± 1.07
	Wild onion (Allium *fistulosum.* L)	1.20 ± 0.62	50.8 ± 1.30
	Yellow onion (*Allium cepa.* L)	0.57 ± 0.73	32.3 ± 1.36
Taiwanese onion species	Yellow onion (*Allium cepa.* L)	2.06 ± 0.52	28.6 ± 1.0
	Red onion (*Allium cepa.* L)	1.14 ± 0.37	18.8 ± 1.49

FRAP is a marker of the total antioxidant capacity of different onion and garlic extracts cultivated in Mongolia and Taiwan region. Values are expressed as the mean ± SD from three measurements; data indicated a significant difference between solvents among one species ($p < 0.05$).

Therefore, DPPH radical-scavenging ability and the methanol extracts of onion (*Allium cepa* L.) are a positive correlation ($r = 0.655$). DPPH radical scavenging activity, iron chelating activity, and superoxide anion radical scavenging activity increased in a dose-dependent manner at concentrations of 0.5-2.0 mg/mL, indicating that the methanol extract had the highest antioxidant action *in vitro* [24]. The antioxidant properties, including the OH radical scavenging effects of quercetin, isorhamnetin-3-glucoside, dipropyl disulfide, and dipropyl sulfide extracted from the methanol extract of onion (*Allium cepa* L.), have also been demonstrated [25].

In a previous study, onion and garlic water extracts showed the highest inhibition of DPPH free radicals. However, the methanolic extract of garlic showed the highest FRAP, indicating its total antioxidant capacity (**Table 4**). This suggests that the antioxidant activities of onion species depend on their respective TPC ($r = 0.655$, $r = 0.284$). Santas *et al.* showed that the methanol extract of Spanish white onion (*Allium cepa* L.) had 24.9 ± 2.4 μM Trolox/g DW, while Spanish calcot onion (*Allium cepa* L.) had 12.7 ± 0.29 μM Trolox/g DW [19].

Temperature effects on plant growth and development depend upon plant species. Under an increasing climate change scenario, air temperatures are more likely to exceed the optimum range for many species [22]. Genetic and environmental influences determined the bulb shape of onions [26]. Thus, the bulb size may be determined mainly by the length of the growing season, temperature, light levels, bulb maturity, and planting density, among other environmental factors [27].

Previous studies have shown that *allium* vegetables, including onion and garlic, grown at colder temperatures may have higher amounts of specific bioactive compounds than those grown under warmer conditions; the relationship between temperature and bioactive compound content is complex and can vary depending on various factors. Several studies have investigated the effect of temperature on the content of bioactive compounds in onion and garlic plants.

These studies generally found that colder temperatures can increase the levels of specific bioactive compounds. Antioxidant compounds such as phenolics and flavonoids are associated with plant resistance [28] and are considered bulb onions' most significant health-related nutrients. Genetic and agronomic or environmental factors play crucial roles in the phenolic compositions and, thus, the nutritional quality of onions [16] [17].

Similarly, garlic grown at cooler temperatures contained 0.5 - 1.2 times higher amounts of allicin, a bioactive compound responsible for its characteristic odor and potential health benefits [28]. However, the specific mechanisms underlying this effect of temperature remain unclear. Cold temperatures may stimulate the production of bioactive compounds as a defense mechanism against environmental stressors. It's worth noting that while colder temperatures can enhance the bioactive compound content in onions and garlic, other factors such as soil conditions, cultivar selection, and plant maturity also play essential roles in determining the final composition of bioactive compounds. Available evidence indicates that onions and garlic grown at colder temperatures may contain higher amounts of bioactive compounds; however, temperature is just one of several factors influencing the content of these compounds.

Our results support the hypothesis that onion species grown in Mongolia may contain higher amounts of bioactive compounds than those grown in Taiwan region. These regions have distinct climatic characteristics owing to their geographical locations and topography. Mongolia has a continental climate characterized by sharp seasonal variations in temperature. The country experiences long, cold winters and short, hot summers. In winter, temperatures can drop below freezing, with average lows reaching −30˚C (−22˚F) in some areas. Summers are relatively short but can be hot, with temperatures averaging around 20˚C - 30˚C (68 - 86˚F) and occasionally exceeding 35˚C (95˚F) in the southern regions. Precipitation is generally low throughout the year, with most of it falling during the summer months. Taiwan region has a subtropical climate influenced by the East Asian monsoon system. The island experiences mild winters and hot, humid summers. Winters are generally pleasant, with temperatures ranging from 15˚C -20˚C (59 - 68˚F) in the northern areas and slightly warmer in the south. Summers are hot and humid, often exceeding 30˚C (86˚F) and high humidity. Overall, Mongolia has a more continental and extreme climate with harsh winters and hot summers, while Taiwan region has a subtropical climate with milder winters and hot, humid summers.

4. Conclusion

This paper presented the different onion species are good sources of total phenolic compounds and their derivatives, including *Allium tagar*. L and red onion *Allium cepa*. L had a higher content of them. This study found that the methanol extract of onion species had a higher content of TPC, and Q4G were more dominant compounds than others. Therefore, onion positively affects antioxidants *in vitro* systems, including DPPH radical scavenging ability and FRAP antioxidant capacity. Their antioxidant activities positively correlate with their TPC content due to the methanol extract of onion species. Also, Mongolian onions had a higher content of bioactive compounds than Taiwanese onions, due to, we suppose, their environmental conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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