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Antioxidant and Anti-Obesity Potentials of Korean-Native Wild Vegetables (*Allium species*)

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Abstract: Four Korea-native wild vegetables belonging to the *Allium* genus (*Allium tuberosum* Rottl., *Allium senescens* L., *Allium thunbergii* G. Don., and *Allium sacculiferum* Maxim.) were investigated for their antioxidant and anti-obesity potential, in order to promote the utilization of wild edible plants for the development of potential new crops. In this study, we compared the antioxidant activity of the plant extracts by ABTS+ and DPPH scavenging method. We then compared the anti-adipogenic effects of the extracts in adipocytes. We quantified the phenolics in the extracts using high-performance liquid chromatography (HPLC) to identify the bioactive compounds. *A. tuberosum* and *A. sacculiferum* had a higher total phenolic content (19.0 ± 0.3 and 19.6 ± 0.7 mg GA/g extract, respectively) than *A. senescens* and *A. thunbergii* (16.0 ± 0.2 and 14.5 ± 0.7 mg GA/g extract). Caffeic acid, an antioxidant and anti-adipogenic compound, was detected in *A. tuberosum* and *A. sacculiferum*, but not in *A. senescens* and *A. thunbergii*. *A. tuberosum*, and *A. sacculiferum* showed superior antioxidative and anti-adipogenic activities compared to *A. senescens* and *A. thunbergii*. Therefore, *A. tuberosum* and *A. sacculiferum*, both of which contain antioxidant and anti-adipogenic phenolics, such as caffeic acid, present a therapeutic potential against obesity. Various phenolic compounds, which have been found to differ among *Allium* spp., may partially account for differences among the *Allium* spp. with respect to their antioxidant and anti-obesity effects.

Keywords: *Allium tuberosum* Rottl.; *Allium senescens* L.; *Allium thunbergii* G. Don.; *Allium sacculiferum* Maxim.; wild vegetable; phenolic; antioxidant; antiobesity

1. Introduction

Globally, obesity has emerged as a major cause of various metabolic diseases, such as diabetes, cardiovascular disease, and osteoporosis. It is known that high-fat or high-calorie diets can cause hyperlipidemia, obesity, increased oxidative stress, and increased inflammation [1]. Oxidative stress disrupts the balance between reactive oxygen species (ROS) and antioxidant defense systems [2]. Oxidative stress causes cellular DNA damage and the oxidation of enzyme cofactors, thus resulting in various diseases such as cancer, neurodegenerative diseases, and aging processes [3]. In addition, oxidative stress promotes adipogenesis through NADPH oxidase, a major source of intracellular lipid and ROS generation [4]. Oxidative stress, including free radicals and ROS, can be inhibited or eliminated by enzymatic and non-enzymatic antioxidants to maintain cell balance [5]. According to recent studies, maintaining hypoglycemia, regulating the body's antioxidant

system and insulin, inhibiting the differentiation and proliferation of pre-adipocytes, and suppressing adipogenesis are all thought to suppress obesity [6].

Plants generate specialized metabolites in order to protect themselves against insects, viruses, and dispersing animals [7,8]. These metabolites include alkaloids, glycosides, amines, steroids, flavonoids, and related metabolites [7,8]. They possess biological activities and are mostly found in the leaves of various plants [7]. Among plant metabolites, phenolics have various biological effects, such as antioxidant, anti-microbial, and anti-diabetic properties [9]. In particular, phenolics are compounds directly linked to non-enzymatic antioxidants because of their reactivity as hydrogen or electron donors and reactivity with other antioxidants [10]. Phenolics are useful natural compounds capable of preventing oxidative stress and are considered important in food, pharmaceutical, and cosmetic industries [11].

Wild vegetables and flowers of the *Allium* spp. belong to the *Amaryllidaceae* family, with approximately 977 different spp. of garlic, onions, chives, shallots, and leeks [12]. The most important *Allium* spp. contain flavonoids, organosulfur compounds, and saponin. *Allium tuberosum* (*A. tuberosum*) grows in the wild in the central and northern parts of Asia, such as Japan, China, Korea, India, Nepal, Thailand, and the Philippines. Of these countries, only China, Korea, and Japan use *A. tuberosum* as a food source [12]. In Korea, *A. tuberosum* grows in the mountains, and its industrial cultivation has increased due to the development of greenhouses, pest control technology, and pharmaceuticals. *A. tuberosum* is a green-yellow vegetable that contains many nutrients, such as carotene, vitamin B2, vitamin C, calcium, and iron. It is known to have anti-cancer [13], anti-bacterial [14], anti-diabetic, hepatoprotective [15], and anti-obesity activities [16]. Other *Allium* spp. that are cultivated in Korea include *A. anisopodium*, *A. cyanem*, *A. cyanem* var. *deltooides*, *A. deltoideifistulosum*, *A. senescens*, *A. thunbergii*, and *A. sacculiferum*. [12]. Of these, *A. senescens*, *A. thunbergii*, and *A. sacculiferum* have anti-obesity, anti-cancer, and anti-inflammatory activities [16–19]. Although the classification and growth among *Allium* spp. in Korea are being studied, studies on their biological activity and phenolic profiling are scarce [20,21].

In this study, we analyzed the general growth characteristics of four *Allium* spp. (*A. tuberosum*, *A. senescens*, *A. thunbergii*, and *A. sacculiferum*) in identical environmental conditions and determined their antioxidant and anti-adipogenic activities. We also investigated their bioactive components by total phenol (TP) content analysis and phenolic profiling.

2. Materials and Methods

2.1. Plant Material and Growth Characteristics

The plant samples of *Allium* spp. (*A. tuberosum*, *A. senescens*, *A. thunbergii*, and *A. sacculiferum*) were collected from Korea. Voucher specimens were deposited at the Korea Medicinal Resources Herbarium, Eumseong, Korea (Table 1).

Table 1. Collection sites, voucher numbers, local names, and parts used of *Allium* spp.

Plant	Collection Site	Voucher No.	Local Names	Parts Used
<i>A. tuberosum</i>	Mt.Obong, (Yangyang, Gangwon)	MPS001706	Buchu	Aerial part
<i>A. senescens</i>	Peak. Hyangro, (Yangyang, Gangwon)	MPS005298	Dume-buchu	Aerial part
<i>A. thunbergii</i>	Mt.Sobaek, (Danyang, Chungbuk)	MPS004662	San-buchu	Aerial part
<i>A. sacculiferum</i>	NIHHS (Eumseong, Chungbuk)	MPS005491	Chamsan-buchu	Aerial part

Allium spp. seeds were sown in 200-cell plug trays (60 cm × 41 cm × 5 cm) containing commercial medium (Baroker medium, SeoulBio, Eumseong, Korea) and were maintained at 20 ± 5 °C in a greenhouse. After 15 days, the seedlings were cultivated in the field.

Allium spp. were harvested after 2 months (15 June 2018), and their growth characteristics were examined (Figure 1). For subsequent experiments, 50 g of *Allium* spp. was extracted with water (500 mL), repeated 3 times, at room temperature for 24 h and concentrated using an evaporator system under reflux (BÜCHI, Meierseggrasse, Flawil, Switzerland). *Allium* spp. extracts were freeze-dried.

2.2. Cell Culture

The 3T3-L1 pre-adipocytes were purchased from the American Type Culture Collection (CL-173, ATCC, Manassas, VA, USA). 3T3-L1 preadipocytes were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Mississauga, Canada) containing $1 \times$ penicillin/streptomycin/glutamine (PSG, Gibco, Mississauga, Canada) and 10% bovine calf serum (Gibco, Mississauga, Canada) at 37 °C in a 5% CO₂ incubator. The medium was replaced every 2 days for subculture. Cells were used at passages 5–10 in all of the experiments.

2.3. Cell Viability

Cell viability was measured with CellTiter 96[®] AQueous One Solution according to the manufacturer's instructions (Promega, Madison, WI, USA) [22]. The 3T3-L1 pre-adipocytes were cultured in 96-well plates at 1×10^5 cells/mL for 24 h and were treated with *Allium* spp. extracts. After 24 h, the absorbance was measured at 490 nm using a multi-plate reader (Synergy H1, Biotek, Winooski, VT, USA).

2.4. Differentiation of Pre-Adipocytes

The 3T3-L1 preadipocytes were differentiated as previously described [23]. Briefly, confluent pre-adipocytes were differentiated using DMEM containing 10% fetal bovine serum (FBS, Gibco, Mississauga, Canada), $1 \times$ PSG, 1 mM insulin (Gibco, Mississauga, Canada), 0.5 mM isobutylmethylxanthine (Sigma-Aldrich, St. Louis, MO, USA), and dexamethasone (0.1 mM) (Sigma-Aldrich, St. Louis, MO, USA) for 3 days. DMEM media with 10% FBS, $1 \times$ PSG and 1 mM insulin was used until cells were completely differentiated for an additional 6 days.

2.5. ORO Staining

Cultures were fixed for at least 1 h with 10% formalin diluted in water, in a 37 °C incubator, then washed twice with water and stained with a working solution of ORO (Sigma-Aldrich, St. Louis, MO, USA). To determine the extent of adipose conversion, 100% isopropanol was added to the stained tissue [24]. The absorbance of the extracted dye was read at 510 nm using a microplate reader.

2.6. Phenolic Analysis and Antioxidant Activity

2.6.1. DPPH Free Radical Scavenging Assay

The antioxidant activity of *Allium* spp. was determined using a DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich, St. Louis, MO, USA) scavenging assay following the method of Nazlić with some modifications. Briefly, 40 µL of sample (0.05, 0.1, 0.2, 0.5, 1, and 2 mg/mL) was mixed with 0.2 mM DPPH (160 µL) [25]. After 30 min, the absorbance was measured at 517 nm using a multimode microplate reader. The DPPH radical scavenging activity was expressed as IC₅₀ value, a concentration that scavenges 50% of the free radicals.

2.6.2. ABTS Radical Scavenging Assay

The ABTS⁺ scavenging assay was performed as described by Tanruean et al., with slight modifications [26]. The ABTS⁺ solution was prepared with ABTS tablets (Sigma-Aldrich, St. Louis, MO, USA) and potassium persulfate (2.6 mM) at 4 °C for 16 h with the light blocked. Absorbance was measured at 734 nm using a multi-mode microplate reader. The radical scavenging activity was expressed as IC₅₀ value, a concentration that scavenges 50% of the radicals.

2.6.3. Determination of TP Content

The TP content was analyzed using the Folin-Denis method [27]. Briefly, 500 µL of each extract (3 mg/mL) was added to 50 µL of Folin–Ciocalteu reagent (Sigma-Aldrich, MO, USA). After 5 min, 100 µL of 20% sodium carbonate anhydrous saturated solution was added. After 1 h, the absorbance was measured at 725 nm using a multi-plate reader. The TP content was determined through a comparison with the standards. Gallic acid (Sigma-Aldrich, USA) was used as a standard, and the TP content (mg/g) was determined using the following equation ($R^2 = 0.999$):

$$Y = 0.0058X - 0.0165 \quad (1)$$

where Y is absorbance and X is measured concentration (mg/mL).

2.6.4. Phenolics Analysis Using HPLC

The Agilent 1200 series HPLC was used (Agilent Technologies, Santa Clara, CA, USA) and samples were analyzed through a reversed-phase using a synergy fusion RP column (250 × 4.6 mm, 4 µm; Phenomenex, Torrance, CA, USA). All analysis conditions were performed as previously described [28]. Phenolic standards such as homogentisic acid, protocatechuic acid, gentisic acid, chlorogenic acid, caffeic acid, phloretic acid, p-coumaric acid, ferulic acid, veratric acid, cinnamic acid, quercetin, and naringenin (Sigma-Aldrich, St. Louis, MO, USA) were prepared and filtered using 0.45 µM polyvinylidene difluoride (PVDF; Pall Co., Port Washington, NY, USA).

2.7. Statistical Analysis

All experimental results are presented as the mean ± standard deviation (SD) of three independent experiments. The statistical significance of the differences was calculated using a one-way analysis of variance (ANOVA) using Duncan's test, and correlations were calculated using Pearson's correlation coefficient (Statistical Package for the Social Sciences, ver. 21.0 for Window).

3. Results

3.1. Growth Characteristic of *Allium* Spp.

In this study, the growth characteristics of four *Allium* spp. (*A. tuberosum*, *A. senescens*, *A. thunbergia*, and *A. sacculiferum*) were measured immediately after cultivation (Figure 1 and Table 2). The total length was similar among the *Allium* spp., at 212–244 mm, except for *A. sacculiferum*, which was 110.8 mm in length. The stem diameter and root length differed considerably between *A. tuberosum* and *A. sacculiferum*, but the number of leaves did not show a significant difference among species.

Table 2. Plant height, stem diameter, length of root, and number of leaves according to *Allium* spp.

Plant	Plant Height (Mm)	Stem Diameter (Mm)	Length of Longest Root (Mm)	Number of Leaves
<i>A. tuberosum</i>	212.5 ± 4.3 ^b	0.4 ± 0.0 ^c	78.7 ± 3.5 ^b	4.0 ± 1.4 ^a
<i>A. senescens</i>	242.0 ± 9.5 ^a	46.7 ± 2.9 ^a	114.8 ± 10.9 ^a	3.7 ± 0.6 ^{ab}
<i>A. thunbergii</i>	244.5 ± 11.0 ^a	35.0 ± 10.6 ^b	124.0 ± 17.7 ^a	2.3 ± 0.0 ^b
<i>A. sacculiferum</i>	110.8 ± 18.7 ^c	2.0 ± 0.7 ^c	40.0 ± 4.2 ^c	3.3 ± 0.0 ^{ab}

All values are means ± SD, and values with different superscript letters on the same column indicate a statistically significant difference determined by Duncan's test ($p < 0.05$).

Figure 1. General images of *A. tuberosum* (a), *A. senescens* (b), *A. thunbergii* (c), and *A. sacculiferum* (d).

3.2. Antioxidant Activities of *Allium Spp.*

The antioxidant activity of *Allium spp.* were evaluated using DPPH and ABTS free radical scavenging methods, which are common and rapid methods for in vitro antioxidant assay [29]. The IC₅₀ values of *Allium spp.* for DPPH and ABTS + scavenging activity varied from 694.2 to 980.0 and from 44.0 to 396.2 µg/mL, respectively (Table 3). The extracts of *A. tuberosum* (694.2 ± 10.0 µg/mL) and *A. sacculiferum* (723.1 ± 12.3 µg/mL) showed higher scavenging activity for DPPH free radical without significant differences between them. The extract of *A. sacculiferum* showed the highest ABTS⁺ scavenging (44.0 ± 0.7 µg/mL). The highest yield of *Allium spp.* was approximately 55% and was consistent with all spp. except for *A. tuberosum*.

Table 3. Anti-oxidative activities of *Allium spp.* water extracts.

Plants	Antioxidant Activity ¹		TP ²	Yields ³
	Dpph	Abts ⁺		
<i>A. tuberosum</i>	694.2 ± 10.7 ^b	100.2 ± 1.7 ^b	17.6 ± 0.3 ^a	29.7
<i>A. senescens</i>	880.0 ± 51.4 ^a	101.1 ± 3.7 ^b	16.0 ± 0.2 ^c	55.3
<i>A. thunbergii</i>	980.0 ± 5.1 ^a	396.2 ± 2.6 ^a	14.5 ± 0.7 ^d	55
<i>A. sacculiferum</i>	723.1 ± 12.3 ^c	44.0 ± 0.7 ^c	17.3 ± 0.1 ^b	53.1
Ascorbic acid	55.34 ± 2.08 ^d	31.29 ± 1.47 ^d	-	-

¹ Values are indicated as IC₅₀ values (µg/mL). ² Total phenolic acid (mg GA/g extract). ³ Extract yield (%). All values are means ± SD, and values with different superscript letters on the same column indicate a statistically significant difference determined by Duncan's test ($p < 0.05$).

3.3. Cell Viability of *Allium Spp.*

The viability of 3T3-L1 cells, when treated with the *Allium spp.* Extract, was investigated using the MTS assay, which is commonly used for cell viability analyses by measuring the activity of mitochondrial dehydrogenase (Figure 2) [30]. There was no difference observed in the viability of 3T3-L1 cells when treated with 25–200 µg/mL (≥100%) of *Allium spp.* extract. It has been reported that cells are considered highly viable if the viability is more than 90% [31]. Thus, extracts of *Allium spp.* of concentrations up to 100 µg/mL (≥90%) were chosen for subsequent experiments.

Figure 2. Effect of *Allium* spp. extracts (25–200 µg/mL) on viability of 3T3-L1 cell, analyzed by MTS assay. All values are means $\pm$ SD ($n = 3$). The statistically significant difference was determined by Duncan's test (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). Conc., concentration.

3.4. Effect of The Differentiation Inhibition of *Allium* Spp. by ORO Staining

Adipogenesis has been reported to contribute to the differentiation of pre-adipocytes into adipocytes, lipid accumulation, and growth arrest [32]. Lipid droplet accumulation in adipocytes is known to be an indicator of adipogenesis [33]. Therefore, the anti-adipogenic effect of *Allium* spp. extracts, with a media of differentiation (MDI), were evaluated using ORO in 3T3-L1 cells (Figure 3A). Microscopy revealed that the MDI positive control had more stained lipid droplets than in the undifferentiated negative controls. The amount was also significantly increased in the differentiated controls, to 2.01-fold higher than the undifferentiated control. Cells treated with extracts of *A. tuberosum* (50 and 100 µg/mL; 0.82 and 0.74-fold vs. MDI control, $p < 0.05$) and *A. sacculiferum* (50 and 100 µg/mL; 0.69 and 0.57-fold vs. MDI control, $p < 0.05$) had markedly decreased lipid droplets, as shown in Figure 3B.

Figure 3. Cont.

Figure 3. Inhibition of lipid accumulation by *Allium* spp. extracts, as analyzed by microscopy (A) and quantifications (B). Differentiated 3T3-L1 cells were treated every 2 days with extracts (50 and 100 µg/mL) for 8 days in adipocyte-induction media. All values are means $\pm$ SD ($n = 3$). Values with different superscript letters indicate a statistically significant difference determined by Duncan's test ($p < 0.05$). MDI, media of differentiation; Conc., concentration.

3.5. Profiling of Phenolics in *Allium* Spp.

Chromatographic techniques are necessary for identifying bioactive compounds [34]. Thus, we performed a high performance liquid chromatography (HPLC) analysis to determine the phenolics present in *Allium* spp. Homogentisic acids were detected in all *Allium* spp. Additionally, caffeic acid and ferulic acid were detected in *A. tuberosum* and *A. sacculiferum*. Veratric acid and quercetin were detected in *A. senescens*, and ferulic acid, veratric acid, and quercetin were detected in *A. thunbergii* (Figure 4).

A quantitative analysis using each calibration curve showed that homogentisic acid was highest in *A. senescens* (75.7 ± 1.6 µg/g d.b. extract), followed by that in *A. sacculiferum* (63.0 ± 0.5 µg/g d.b. extract), *A. tuberosum* (50.8 ± 0.7 µg/g d.b. extract), and *A. thunbergii* (47.8 ± 0.5 µg/g d.b. extract) (Table 4). Caffeic acid was only detected in *A. thunbergii* (3.9 ± 0.1 µg/g d.b. extract) and *A. sacculiferum* (3.6 ± 0.1 µg/g d.b. extract). Ferulic acid was highest in *A. tuberosum* (15.9 ± 0.1 µg/g d.b. extract), with *A. thunbergii* (0.6 ± 0.2 µg/g d.b. extract) and *A. sacculiferum* (0.3 ± 0.1 µg/g d.b. extract) having smaller amounts. Veratric acid and quercetin were only detected in *A. senescens* (58.9 ± 1.3 and 53.8 ± 0.6 µg/g d.b. extract) and *A. thunbergii* (54.4 ± 1.0 and 44.0 ± 1.0 µg/g d.b. extract).

Based on our results, a correlation analysis was performed between biological activities and phenolics (Table 5). The lipid accumulation correlated with TP (-0.826), DPPH (0.867), ABTS (0.784), and caffeic acid (-0.889). These results show that *Allium* spp. have a significant correlation with the antioxidant activity of caffeic acid, which inhibits lipid accumulation.

Figure 4. Chromatogram of phenolic compounds present in water extract of *Allium* spp., as analyzed by HPLC. Peak identification: 1. homogentisic acid, 2. Protocatechinic acid, 3. Gentisic acid, 4. Chlorogenic acid, 5. Caffeic acid, 6. Propionic acid, 7. Coumaric acid, 8. Ferulic acid, 9. Veratric acid, 10. Cinnamic acid, 11. Quercetin, 12. Naringenin, and 13. Hesperidin. Chromatogram of *A. tuberosum* (A), *A. senescens* (B), *A. thunbergii* (C) and *A. sacculiferum* (D).

Table 4. Quantitative analysis of phenolic compounds in *Allium* spp. water extracts.

Phenolics	<i>A. tuberosum</i>	<i>A. senescens</i>	<i>A. thunbergii</i>	<i>A. sacculiferum</i>
Homogentisic acid	50.8 ± 0.7 ^c	75.7 ± 1.6 ^a	47.8 ± 0.5 ^d	63.0 ± 0.5 ^b
Caffeic acid	3.9 ± 0.1 ^a	N.D. ¹	N.D.	3.6 ± 0.1 ^b
Ferulic acid	15.9 ± 0.1 ^a	N.D.	0.6 ± 0.2 ^b	0.3 ± 0.1 ^c
Veratric acid	N.D.	58.9 ± 1.3 ^a	54.4 ± 1.0 ^b	N.D.
Quercetin	N.D.	53.8 ± 0.6 ^a	44.0 ± 1.0 ^b	N.D.

¹ Values are indicated as content (µg/g d.b. extract). N.D.; not detected. Values are shown as mean ± SD (*n* = 3). Values with different superscript letters in the same row indicate statistically significant differences as determined by Duncan's test (*p* < 0.05).

Table 5. Correlation analysis with biological activity and phenolics in *Allium* spp. extracts.

Factors	TP ¹	Homogentic Acid	Caffeic Acid	Ferulic Acid	DPPH ²	ABTS ³	Lipid Accumulation
TP		−0.373	0.932	0.464	−0.796 **	−0.439	−0.826 ***
Homogentic acid			−0.238	−0.474	−0.024	−0.587 *	−0.120
Caffeic acid				0.618 *	−0.931	−0.633 *	−0.889 ***
Ferulic acid					−0.595 *	−0.231	−0.248
DPPH						0.811 **	0.867 ***
ABTS							0.784 **
Lipid accumulation							

¹ Total phenolic acid (mg GA/g extract). ^{2,3} Values are indicated as IC₅₀ values (μg/mL). Values are shown as mean ± SD (n = 3). Statistically significant differences determined using Pearson's correlation coefficient (* p < 0.05, ** p < 0.01, and *** p < 0.001).

4. Discussion

A. tuberosum is widely consumed as a traditional ingredient in Korea and has been used as an herbal medicine for a long time [12]. However, the biological activity of leeks grown in Korea is only known for only few of the *Allium* spp. There have been many studies analyzing the functional activity of *Allium* spp. methanol extracts in preventing obesity [16,17,19]. However, methanol extracts cannot be used as food. We selected three types of *Allium* spp. with anti-obesity effects, and *A. thunbergii*, which is often used interchangeably with *A. sacculiferum* although it was found to be an independent spp. In a recent study [35]. In this study, we investigated the growth characteristics, secondary metabolites, and beneficial effects of *Allium* spp. In Korea for use as food, and the correlation between them.

The antioxidant effect of plants is known to be beneficial not only for plants but also for humans. For example, various phenolic substances have been reported to have an excellent free radical scavenging ability. Free radicals in vivo affect aging and disease in living tissue. Plant phenolic compounds are known to exhibit physiologically active functions by binding phenolic hydroxyl groups to macromolecules, such as enzymes [36]. TP is known to have a high antioxidant activity, which is because the phenolic hydroxyl group (OH) of the compound binds to proteins and polymers. In general, it has been reported that as the content of TP increases, physiological activities such as antioxidant activity increase [37]. DPPH and ABTS radicals are relatively stable free radicals and are reduced by sulfur-containing amino acids such as cysteine, GSH, ascorbate, and butylated hydroxyanisole. Accordingly, DPPH and ABTS are widely used to analyze the in vitro antioxidant activity of various extracts [38]. On comparing the antioxidant effect of all *Allium* spp., *A. sacculiferum* was identified to have the highest radical scavenging effect and TP content.

Adipogenesis is similar to the development of obesity in humans, and the excessive differentiation of 3T3-L1 cells into pre-adipocytes can induce obesity; therefore, these cells have been widely used in studies that assess the characteristics of adipogenesis and obesity [39]. A correlation between antioxidants and adipogenesis has been reported to promote the conversion of pre-adipocytes to mature adipocytes [24]. Thus, to investigate the inhibitory effect of *A. tuberosum* and *A. sacculiferum* extracts, which were found to have high antioxidant activity, ORO staining was performed. They had an inhibitory effect on the formation of lipid droplets. Therefore, *A. tuberosum* and *A. sacculiferum* extracts, which have been confirmed to have antioxidant activity, can be utilized for their anti-adipogenic effects.

According to a number of recent studies, plant-derived phenolic compounds inhibit adipogenesis [40]. Phenolic profiling was performed utilizing an HPLC analysis to determine the component responsible for the inhibitory action of *Allium* spp. In our study, caffeic acid was detected in *A. tuberosum* and *A. sacculiferum*. Caffeic acid has been reported to be an anti-obesity agent [41]. Using our data, an analysis was conducted to determine the correlation between phenolics and the biological activities of *Allium* spp. (Table 5). The

anti-adipogenic and antioxidant effects of *A. tuberosum* and *A. sacculiferum* may be due to its caffeic acid content.

Collectively, these results suggest that *Allium* spp. have potential antioxidant and anti-adipogenic effects, which derive from the presence of phenolic compounds, particularly caffeic acid.

5. Conclusions

Among *Allium* spp., *A. tuberosum* and *A. sacculiferum* have the antioxidant potential of scavenging free radicals and also inhibit adipogenesis in 3T3-L1 cells by decreasing lipid accumulation. The properties of these species were found to be correlated with their phenolic content, specifically caffeic acid. Consequently, various metabolites and phenolic compounds were found to differ according to *Allium* spp., thus showing differences among the *Allium* spp., with respect to their beneficial biological effects.

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