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Investigation of antioxidant and antibacterial activities, phenolic contents of *Allium staticiforme* bulb fractions in different polarities

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In this study, it was aimed to evaluate the antioxidant and antimicrobial activities of *Allium staticiforme* bulb fractions (petroleum ether, chloroform, methanol and acetone) *in vitro*. Fractions were made by Soxhlet method, antibacterial activity was evaluated using disc diffusion method, antioxidant activity was evaluated using DPPH radical scavenging detection and reduction power test. At the same time, the chemical content was determined by examining TLC and total phenolic matter. The concentrations reducing the DPPH radical of the fractions by 50% were determined to be 283.1 µg/mL for methanol, 291.5 µg/mL for petroleum ether, 346 µg/mL for chloroform and 533.6 µg/mL for acetone, respectively. It was observed that the total phenolic substance amounts were in the order (from the higher to the lower): petroleum ether, methanol, chloroform and acetone fractions. The reduction power was directly proportional to the increase in the concentration for all fractions. The highest antibacterial effect was found on the petroleum ether fraction (12 mm) on *S. aureus*. It was determined that the petroleum ether fraction showed a broad spectrum of antibacterial activity. In the current study the antibacterial and the antioxidant activity of *Allium staticiforme* bulb fractions (with different polarity) have been investigated for the first time and shown that the solution used in extract preparation is important in revealing the bioactive molecules.

Keywords: *Allium staticiforme*, Antibacterial activity, Antioxidant activity, Solvent

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Allium is a genus that includes more than 800 species in the world and most of them are distributed in the Northern hemisphere. Shallots, garlic, chives, onions, leeks, scallions are most commonly used *Allium* vegetables throughout the globe in different delicacies. The genus *Allium* L. was traditionally affiliated to tribe Allieae under Liliaceae. In recent studies, the genus *Allium* is included in the Amaryllidaceae or the Alliaceae family because flower state is umbella and has spata at its base[1-3]. *Allium* spp. (Onion, Garlic and Ginger) types are used as medicinal and aromatic plants in traditional medicine as well as vegetables and spices[4,5].

Aromatic and medicinal plants are known to produce some bioactive molecules that show antimicrobial activity. These substances, which have little toxicity to cells and are able to inhibit pathogens, are considered candidates for the development of new antimicrobial drugs[6,7].

Aromatic herbs are also frequently used in food supplements as a natural source of antioxidants. In addition, antioxidants play an important role in preventing various lifestyle-related diseases and aging. Due to the increased safety concerns associated with the use of synthetic antioxidants, the search for cheaper and safer sources of antioxidants from natural sources has increased. Herbal compounds responsible for antioxidant activity are polyphenols. Polyphenols have antioxidant properties due to their redox properties such as adsorption or neutralization of free radicals, decomposition of peroxides, and metal chelation[8-11]. *Allium* plants contain a wide variety of sulfur compounds that have a characteristic taste, eye-watering odor, and show strong antioxidant properties[12].

In this study, the fractions prepared using different solvents from *Allium staticiforme* bulbs were evaluated by two complementary methods to in vitro antioxidant activities: reduction power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical tests. In addition, antibacterial activities and total phenolic content of the fractions were investigated.

Materıal and Methods

Allium staticiforme were obtained from Atatürk Horticultural Central Research Institute, Yalova,

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Turkey in May 2020. All chemicals used in the study were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemie (Steinheim, Germany).

Extract preparation

The bulbs were dried in the lyophilization device for 24 h. The dried bulbs were pulverized by an electric grinder. 30 g of bulb sample was extracted for 8 h using petroleum, chloroform, methanol and acetone solutions in the Soxhlet apparatus, respectively. Thus, 4 different fractions in different polarity solvents were obtained. Solvent (petroleum ether, chloroform, methanol and acetone) was removed by rotary evaporation for 10 min at 45°C. At the end of the extraction process, the stock extracts were prepared at a concentration of 1 mg/mL by using each fraction's own solution.

Thin layer chromatography

Thin layer chromatography was used to show the chemical contents of the *A. staticiforme*. TLC was carried out on TLC sheets silica gel (MERCK). It was run by loading 15 µL of TLC from each fraction at different concentrations (10:90, 50:50, 90:10 (v: v)) of the petroether-methanol mixture. Drying silicas were imaged at a UV light of 254 nm.

Total phenolic content (TPC)

The total phenolic content of the fractions at a concentration of 1000 µg/mL was determined by the Singleton and Rossinin method (1965) with minor modifications[13]. 100 µL fractions were mixed with 200 µL Folin-Ciocalteu (50%) and rested for 3 min. 1 mL of 2% Na_2CO_3 was added and mixed. It was then kept at room temperature for 1 h in the dark. The absorbance of the mixture was read at 760 nm by using a spectrophotometer. TPC was determined as mg GA/g using the gallic acid standard curve (50, 100, 200, 300, 400 mg/L).

Determination of DPPH radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was studied by modifying the method described by Blois[14]. The fractions of bulb of *A. staticiforme* were worked in a range concentration of 100-500 µg/mL. In summary, the same volume (1 mL) of 0.04% DPPH solution was added onto the prepared extract. The stirred mixtures were stored for 30 min at room temperature in the dark. At the end of the period, samples were read in a spectrophotometer at 517 nm. Ascorbic acid was used as standard antioxidant and ethanol was used as a blind. A control including 1 mL of ethanol and 1 mL of DPPH was also utilized. Percent DPPH scavenging activity was calculated by the formula below.

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Reducing power

Reducing power of the fraction was quantified according to Oyaizu[15]. Phosphate buffer of 2500 µL and potassium ferricyanide of 1% were added onto the fractions of 1000 µL with the concentration of 100-1000 µg/mL and this mixture has been incubated at 50°C for 20 min. 2500 µL of 10% trichloroacetic acid was then added to the mixture, and then centrifuged at 2500 rpm for 10 min. After these processes, 1000 µL of supernatant was taken from the mixture and 2500 µL of water and 500 µL of 1% (w/v) $FeCl_3$. $6H_2O$ were added. Ascorbic acid at the same concentration was used as a standard. The absorbance value was measured at 700 nm and distilled water was used as blank.

Antibacterial activity

The bacterial strains used in the study were obtained from Sakarya University, Department of Biology. Antibacterial activity of the fractions was evaluated by the disc diffusion method. Bacterial suspensions of 0.5 Mcfarland density were obtained from activated fresh cultures. Bacterial suspensions were inoculated into Müeller Hinton Agar medium with a sterile swab and discs (6 mm) previously soaked with 15 mL of plant extract were placed in the cultivated petri dishes. Then, they were incubated at 37°C for 24 h. The solvent from which the fraction was prepared (petroleum ether, chloroform, methanol and acetone) was used as negative control, and gentamicin loaded disks were used as positive control. The zones formed at the end of the incubation were measured with the aid of a digital caliper.

Statistical Analysis

All experiments were done in triplicate and results were averaged. Statistical analyses were made with the program SPSS 20.0. Statistical analyzes of the data were performed with one-way ANOVA and Duncan test ($P < 0.05$).

Results and Dıscussıon

Yields, TPC

The biological activities of plant extracts differ significantly depending on the solvent used, temperature and pressure. The structure of isolated

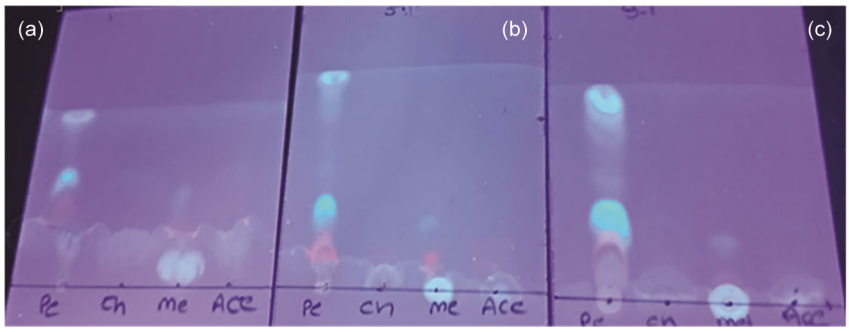

Fig. 1 — TLC images of *A. staticiforme* bulb fractions [petroleum ether/methanol ratio (v:v)=A) 1:1, B) 5:1, C) 9:1] Pe: Petroleum ether, Ch: Chloroform, Me: Methanol, Ac: Acetone

metabolites determines their solubility in solvents of different polarity. Thus, the solvent and isolation technique used can have a significant effect on the extraction yield of plant secondary metabolites and the activity of the extracts prepared[16,17]. Therefore, in the current study, the extraction yields, the antioxidant and the antibacterial activities and the total phenolic content of fractions prepared from *A. staticiforme* bulbs with different solvents were evaluated. Silica TLC separations of *A. staticiforme* bulb fractions containing phenolic compounds are shown in Figure 1. Separation of the petroleum ether fraction over 6 cm in petroleum ether-methanol (9: 1, v:v) resulted in 6 bands. In fractions the best separation occurred in the solvent at a concentration of 9: 1, with the fraction rich in content determined to be the petroleum ether then methanol.

Review of the literature has shown that the extraction efficiency of plant material increases with increasing solvent polarity[18,19]. In our study, the highest yield was obtained from the methanol fraction. This is followed by petroleum ether, acetone and chloroform, respectively (Table 1). In a study examining extracts obtained with different solvents from *Allium roseum* L. species, it was reported that the highest yield was the extract prepared with methanol solution, followed by petroleum ether ethyl acetate and chloroform, respectively, in parallel with our study[20].

All *A. staticiforme* fraction contained phenolic compounds in their composition and the solvent used was identified as an important factor on the total phenol content ($p<0.05$). When the TPC values of *Allium* fractions were examined, it was observed that the highest values were 91.3 mg GA/g for petroleum ether, 28.6 mg GA/g for methanol, 25.1 mg GA/g for chloroform and 22.1 mg GA/g for acetone, respectively. It may have contained higher TPC than other fractions, since petroleum ether was used first in the preparation of the fractions.

Table 1 —Yields, TPC and IC 50 (50% scavenging of DPPH radical) value of *A. staticiforme* bulb fractions

Fraction	Yield (%) (w/w)	TPC (mg GA/g)	IC50 (µg/mL)
Petroleum ether	2.5^{b}	91.3 ± 0.9^{d}	291.54 ± 1.4^{c}
Chloroform	1.8^{a}	25.1 ± 1.6^{c}	346.1 ± 1.5^{d}
Methanol	12.6^{c}	28.6 ± 0.3^{b}	283.2 ± 0.6^{b}
Acetone	$2.1^{a,b}$	22.1 ± 0.01^{a}	533.6 ± 1.2^{e}
Ascorbic acid	-	-	12.6 ± 0.6^{a}

The results are given as the mean±SD of 3 replicates.Values with the different letters in the same row are significantly different ($p<0.05$).

Antioxidant activity

Plants rich in secondary metabolites such as phenolics, tannins and sulfur compounds have antioxidant activity owing to their redox properties and chemical structures. DPPH radical is widely used due to easy and fast results in free radical scavenging analysis of the plant extracts[21]. The percentage DPPH scavenging rates in the 100-500 µg/mL concentration range of the fractions used in our study are shown in Figure 2.

The methanol fraction showed a rapid increase in percent DPPH scavenging, while the acetone fraction showed a more steady increase in the concentration range studied. The fractions were determined to have a DPPH 50% scavenging value (IC50) in the fractions, for methanol as 283.2 µg/mL, petroleum ether as 291.1 µg/mL, chloroform as 346.1 µg/mL and acetone as 533 µg/mL ($P<0.05$), respectively. Also, the use of different solvents (of different polarities) can be used to explain the change in IC_{50} concentrations between fractions. When fractions were compared with the standard antioxidant ascorbic acid, it was observed that the fractions scavenge the DPPH radical significantly.

In the study, when we have evaluated the antioxidant activities of acetone, methanol and ethyl

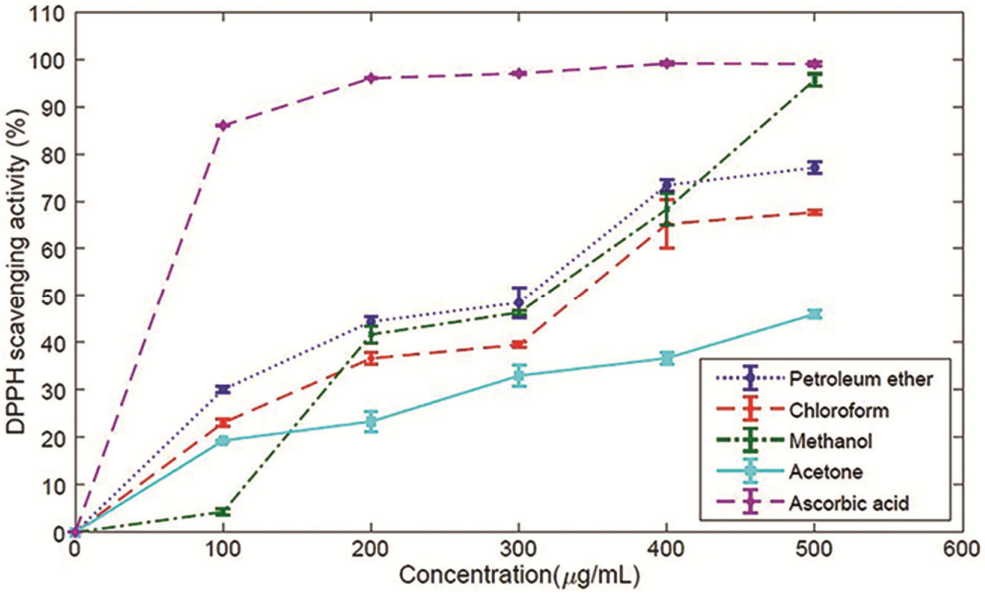

Fig. 2 — DPPH% scavenging rates of fractions in 100-500 µg/mL

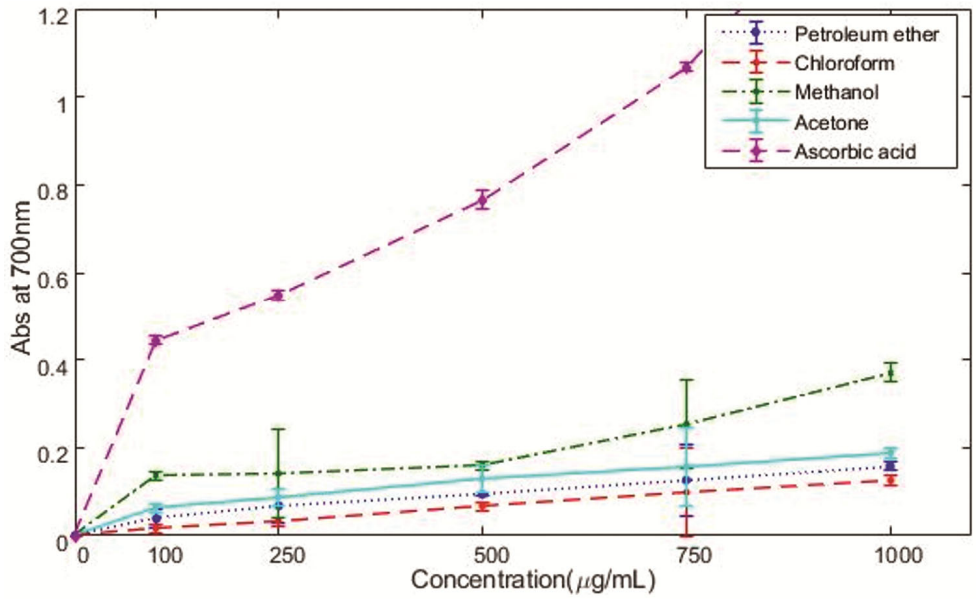

Fig. 3 — Reduction power of *A. staticiforme* bulb fractions

acetate extracts obtained from *A. flavum*, the highest DPPH scavenging activity was found to be in the following order: acetone, methanol and ethyl acetate[22]. In another study, IC_{50} values of ethanolic and aqueous extracts obtained from *Allium hookeri* bulbs were reported to have the values of 485 µg/mL and 1828 µg/mL[23]. It is seen from our study and the studies in the literature that the solvent used in the preparation of extracts is important in revealing the antioxidant activity. Although *Allium* species show high activity mostly in methanol due to the sulfur compounds they contain, the activity studies of the extracts of these plants obtained with different solvents need to be investigated more.

The reducing power analysis reflects the electron donation of antioxidant compounds. In this study, the ability of extracts to reduce Fe^{3+} to Fe^{2+} was determined[24]. In all fractions of *A. staticiforme* ampoule, the iron reducing activities increased with the increase in concentration (Fig. 3). As in the literature studies on *Allium* species, in our results, reduction tests were found to support antioxidant activity[25,26].

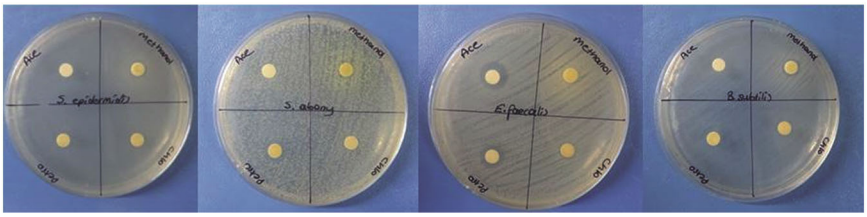

Fig. 4 — Photos showing antibacterial activity (diameter of the inhibition zone) of the tested fractions Petro: Petroleum ether, Chlo: Chloroform, Ac: Acetone

Antibacterial activity

To control various health complications, a large variety of antibacterial drugs are used that prevent or alleviate symptoms caused by microbes. These agents, together with the harmful microorganisms in the body, damage the beneficial flora and even increase the resistance of microorganisms when used in excessive. Undoubtedly, nature provides a cure for almost every disease. It is known that natural bioactive compounds, which are biodegradable and hydrolytic, have lower toxicity and high efficacy, and therefore, the scientific world is increasingly turning to new natural antimicrobial sources[27,28].

In this study, the antibacterial activity of *Allium staticiforme* bulb fractions on *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228, *S. typhimurium* ATCC 14028, *S. abony* NCTC 6017, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 strains was evaluated and the results were given in Table 2.

It was determined that petroleum ether fraction obtained from *A. staticiforme* showed broad spectrum antibacterial activity on test microorganisms. Possibly this effect could be related to the high phenolic content. The acetone fraction showed moderate antibacterial activity on *E. faecalis* with an inhibition diameter of 11 mm, while the chloroform fraction showed low antibacterial activity on *E. coli* only (Fig. 4). Have solely shown the fraction obtained from petroleum ether showed antibacterial activity on *S. abony, S. typhimurium* and *S. aureus* bacteria.

It was reported that the ethanolic extract obtained in a study on *Allium sativum* bulbs created an inhibition diameter of 11.5 mm on *E. coli* and 9 mm on *S. aureus*[29]. It was determined that *A. saralicum* bulb ethanolic extract (2 mg/mL) created 10 mm inhibition zone diameter on *S. aureus*[30]. It has been observed in studies that many *Allium* species consumed as spices and foods such as *Allium jesdianum, Allium cepa, Allium tuncelianum* show antimicrobial activity[31-35]. The antibacterial effects of fractions with different polarity obtained from *A. consanguineum* species produce different results. Our study and studies in the literature reveal the importance of the solution used in isolation of compounds obtained from the plants.

Table 2 — Antibacterial activity of *Allium staticiforme* bulb fractions on test microorganisms

Test bacteria	Inhibition Zone Diameter (mm)					
	Fractions (1000 µg/mL)					
	Petroleum ether	Chloroform	Methanol	Acetone	GC	Nc
B. subtilis	10.5±0.3	0	9±0.1	0	21	0
E. coli	10.5±0.7	8±0.1	9±0.2	0	17	0
E. faecalis	9±0.1	0	0	11±0.1	19	0
S. abony	9±0.6	0	0	0	19.5	0
S. aureus	12±0.1	0	0	0	22	0
S. epidermidis	0	0	8±0.6	0	21	0
S. typhimurium	10±0.5	0	0	0	20	0

GC: Gentamicin, Nc: negative control

Conclusions

The current study can be considered as a starting point for further examinations. *A. staticiforme* and bulb extracts are rich in phytochemical components that have antimicrobial and antioxidant activity. In this study, it was determined that it has a significant ($p<0.05$) effect on the solvent type (petroleum ether, chloroform, methanol and acetone), extraction yield, total phenolic content, antioxidant and antibacterial activity. As a potential antioxidant and antimicrobial source, *A. staticiforme* can be an alternative to synthetic antioxidants and preservatives in the food industry.

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Conflict of Interest

There is no conflict of interest.

Author Contributions

ABS; KT conceived and designed the study. ABS performed the experiments and analyzed the data; ABS; MS; KT participated in the figures production and drafted the paper. ABS; MS; KT revised the manuscript.

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