Antimicrobial activities of *Allium staticiforme* and *Allium subhirsutum*
Antimicrobial activities of *Allium staticifor me* and *Allium subhirsutum*

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**Abstract**

The antibacterial and antifungal activities of the bulb and flower of *Allium staticifor me* and *Allium subhirsutum* were investigated. In addition, DPPH radical scavenging activity and total phenolic contents were determined. The results show that methanolic extracts of *A. staticifor me* and *A. subhirsutum* had antifungal activities against *Candida albicans*, together with a less activity level against *Escherichia coli*, *Staphylococcus epidermidis*, *S. aureus*, *Enterococcus faecalis*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. The total phenolic contents of *A. staticifor me* leaf and bulb were determined as 17 and 2.4 mg of GAE/100 g, respectively. The IC₅₀ of methanolic extracts of *A. staticifor me* and *A. subhirsutum* were also determined. In conclusion, both *A. staticifor me* and *A. subhirsutum* have antifungal activities with weak antibacterial activities. These plants have DPPH radical scavenging activities.

**Materials and Methods**

**Materials**

*A. staticifor me* Sm. and *A. subhirsutum* L. were obtained from the Atatürk Horticul tual Central Research Institute, Yalova, Turkey in May 2017. The microorganism strains used in this study were *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Salmonella typhimurium* ATCC 14028 and *Candida albicans* ATCC 1029. All strains were provided from Microorganism Culture Collections Research and Application Center of Istanbul University and Microbiology Laboratory Culture Collection of Gebze Institute of Technology. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich. Folin-Ciocalteu’s phenol reagent, gallic acid, ascorbic acid and sodium carbonate were purchased from Merck.

**Extract preparation**

*A. staticifor me* and *A. subhirsutum* were divided into

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sections like flower, bulb and leaf. Each part was dried separately via lyophilization method which is based on the sublimation of ice crystal from frozen material. The dried parts were ground into the powder using an electric mill. The obtained powder of flower, leaf and bulb parts of A. staticiforme and A. subhirsutum were extracted using a soxhlet apparatus. Methanol was used as organic solvent for extracting the bioactive compounds from A. staticiforme and A. subhirsutum. 3 g of each part of the plant was placed to the soxhlet apparatus. The extraction was performed during 18 hours with 100 mL of solvent. Rotary evaporation under vacuum at 45°C for 10 min was carried out for removing the solvent. After these processes, the extracts were prepared at the determined concentration (6,400-3,200 µg/10 µL) by adding solvents that used in the extraction process.

**Determination of total phenolic content**

The total phenolic content of methanolic extract was determined by Folin-Ciocalteu procedure as described with minor modifications (Singleton and Rossi, 1965). The 100 µL of methanolic extract (1,000 µg/mL) was mixed with 200 µL of Folin-Ciocalteu (50%) and was kept waiting for 2 min. Then, 1 mL of 2% sodium carbonate solution was added and shaken well. The mixture was kept in a dark place for 1 hour. The absorbance of the mixture was measured at 760 nm by using a spectrophotometer (Shimadzu UV mini-1240). The total phenolic content values were determined from a calibration curve prepared with a series of gallic acid standards (50, 100, 200, 300, 400 mg/L). The results were expressed as mg of GAE/100 g.

**Determination of DPPH radical scavenging activity**

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by using the procedure reported with minor modifications (Blois, 1958). The methanolic extracts of A. staticiforme and A. subhirsutum were prepared in a range concentration of 250 to 1,750 µg/mL. Then, 1 mL of prepared extract was mixed with 1 mL of 0.04% DPPH solution. Each mixture was shaken vigorously and kept for 30 min in a dark place at room temperature. The absorbance of samples was measured at 517 nm by using a spectrophotometer (Shimadzu UV mini-1240). Methanol was used as blank and ascorbic acid was used as standard solution. A control including 1 mL of methanol and 1 mL of DPPH was also utilized. The inhibition percentage of the samples was calculated according to the following formula:

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

Where, \(A_{\text{control}}\) is the absorbance of mixture of methanol and DPPH solution without extract and \(A_{\text{sample}}\) is the absorbance of sample with DPPH solution.

The antiradical activity was stated as IC\(_{50}\) (µg/mL), indicating the extract concentrations scavenging 50% of DPPH radicals. The lower IC\(_{50}\) indicates a higher antioxidant activity of a compound.

**Statistical analysis**

Statistical analysis was performed using SPSS, version 20.0. Group comparisons were performed using One-way analysis of variance (ANOVA) followed by Duncan test. P value less than 0.05 was considered to be statistically significant.

**Results**

The results of the present study indicated that A.
<table>
<thead>
<tr>
<th>Extract (µg/disc)</th>
<th>Inhibition zone diameter (mm)</th>
<th>Escherichia coli</th>
<th>Staphylococcus epidermidis</th>
<th>Bacillus subtilis</th>
<th>Staphylococcus aureus</th>
<th>Enterococcus faecalis</th>
<th>Salmonella typhimurium</th>
<th>Pseudomonas aeruginosa</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. staticiforme</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulb MeOH 6400</td>
<td></td>
<td>0</td>
<td>8.2 ± 0.1</td>
<td>8.2 ± 1.3</td>
<td>6.0 ± 0</td>
<td>8.5 ± 0.4</td>
<td>9.7 ± 1.1</td>
<td>88.0 ± 0.5</td>
<td>28.2 ± 1.5</td>
</tr>
<tr>
<td>3200</td>
<td></td>
<td>0</td>
<td>6.0 ± 0</td>
<td>6.5 ± 0</td>
<td>0</td>
<td>6.0 ± 0</td>
<td>6.6 ± 0.5</td>
<td>7.0 ± 0.3</td>
<td>21.0 ± 1.6</td>
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<tr>
<td>Flower MeOH 6400</td>
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<td>12.4 ± 2.2</td>
<td>11.5 ± 0.6</td>
<td>9.3 ± 0.5</td>
<td>0</td>
<td>10.1 ± 0.6</td>
<td>7.5 ± 0.6</td>
<td>7.4 ± 0.1</td>
<td>24.1 ± 1.2</td>
</tr>
<tr>
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<td>9.0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13.4 ± 0.3</td>
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<td>Leaf MeOH 6400</td>
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<td>0</td>
<td>0</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>A. subhirsutum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulb MeOH 6400</td>
<td></td>
<td>8.2 ± 0.2</td>
<td>13.1 ± 0.2</td>
<td>0</td>
<td>8.3 ± 0.1</td>
<td>8.2 ± 0.3</td>
<td>9.9 ± 1.5</td>
<td>97 ± 0.8</td>
<td>13.8 ± 1.2</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10.7 ± 1.1</td>
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<td>Flower MeOH 6400</td>
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<td>8.2 ± 0.2</td>
<td>13.1 ± 0.2</td>
<td>0</td>
<td>8.3 ± 0.1</td>
<td>8.2 ± 0.3</td>
<td>9.9 ± 1.5</td>
<td>97 ± 0.8</td>
<td>20.5 ± 1.2</td>
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<td>0</td>
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<td>9.2 ± 0.7</td>
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<td>8.7 ± 0.5</td>
<td>9.3 ± 0.3</td>
<td>83.0 ± 0.5</td>
<td>8.6 ± 0.7</td>
</tr>
<tr>
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<td></td>
<td>0</td>
<td>7.8 ± 0.3</td>
<td>0</td>
<td>0</td>
<td>7.5 ± 0.5</td>
<td>0</td>
<td>6.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Gentamicin (10 µg)</td>
<td></td>
<td>19</td>
<td>21</td>
<td>17</td>
<td>20</td>
<td>20</td>
<td>21</td>
<td>22</td>
<td>not tested</td>
</tr>
<tr>
<td>Amphotericin B (100 U)</td>
<td></td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
<td>16</td>
</tr>
</tbody>
</table>
staticiforme and A. subhirsutum extracts had great potential as antifungal and antibacterial agents against the microorganisms (Table I). C. albicans showed maximum sensitivity (28.2 ± 1.5 mm zone of inhibition) to the methanolic extract (in 6,400 µg/disc concentration) of A. staticiforme bulb. However, the methanolic extract of A. subhirsutum flower had shown strong antifungal activity with 20.5 mm inhibition zone diameter against C. albicans. The methanolic extract of flower part of A. staticiforme had antibacterial activity against the test microorganisms. The level of antimicrobial activity of A. subhirsutum and A. staticiforme has been evaluated to be as follows: bulb>flower>leaf.

The IC$_{50}$ values of A. staticiforme and A. subhirsutum were determined for leaf as 693 and 1086 µg/mL, respectively (Figure 1). Also, the IC$_{50}$ values of bulb part of A. staticiforme and A. subhirsutum were found as 1362 and 847 µg/mL, respectively (Table II). The total phenolic contents of the leaf part of A. staticiforme and A. subhirsutum were measured as 17 and 17.5 mg GAE/100 g, respectively (Table II).

![Figure 1: DPPH radical scavenging activity of A. staticiforme and A. subhirsutum](image)

Each value was represented as mean ± SEM of three measurements.

### Table II

<table>
<thead>
<tr>
<th>Extract</th>
<th>TPC (mg GAE/100 g)</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. subhirsutum bulb</td>
<td>4.8 ± 0.5$^b$</td>
<td>847 ± 6.8$^c$</td>
</tr>
<tr>
<td>A. subhirsutum leaf</td>
<td>17.5 ± 0.2$^d$</td>
<td>1086 ± 2.7$^d$</td>
</tr>
<tr>
<td>A. staticiforme bulb</td>
<td>2.4 ± 0.1$^d$</td>
<td>1362 ± 1.1$^d$</td>
</tr>
<tr>
<td>A. staticiforme leaf</td>
<td>17 ± 0.1$^c$</td>
<td>693 ± 2.3$^c$</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Not tested</td>
<td>5.65 ± 0.1$^a$</td>
</tr>
</tbody>
</table>

Different letters symbolized significant differences (p<0.05) by mean of the ANOVA Duncan-test; TPC means total phenolic contents.

**Discussion**

The most species of Allium have antimicrobial activity and the maximum level is reached on the mushrooms. In a study made by Iwalokun et al. (2004) the extracts of A. sativum produces an average inhibition zone diameter of 29.8 mm for various 10 Candida sp. In another work (Shirani et al. 2017), it has been stated that the extract obtained from Allium tripepaldé produces an inhibition zone diameter of 21 mm. This work supports the result that the Allium species show very high antifungal activity against the well-known fungi. It was found that the methanolic extracts of bulb and flower of A. staticiforme and A. subhirsutum have highly strong antifungal activities against C. albicans. Therefore, the activity of A. staticiforme and A. subhirsutum on C. albicans is striking. In this study, it was also determined that the flower and bulb section of A. staticiforme possess antibacterial activity against E. coli, S. epidermidis, B. subtilis, S. aureus, E. faecalis, T. typhimurium and P. aeruginosa. Furthermore, A. subhirsutum have shown antibacterial activity against E. coli, S. epidermidis, S. aureus, E. faecalis, T. typhimurium and P. aeruginosa.

In this work, the IC$_{50}$ values of the extracts are in between 693-1362 µg/mL. The antioxidant activity of the extracts has been evaluated to be less with respect to ascorbic acid. It was found that A. staticiforme leaf possesses higher antioxidant activity than the bulb of the plant. On the other hand, it was determined that the bulb part of A. subhirsutum has higher antioxidant activity than the leaf part of the plant. Discrepancies in extract activities might be attributed to the joint influences of both genetic factors as well as the growing conditions. Genotypic and environmental factors are found to affect the antioxidant activities in onions (Kaur et al., 2009; Ghahremanimajd et al., 2012). There are several works on the relation between the antioxidant activity and the phenolic contents. Some authors have found a correlation between the phenolic content and the antioxidant activity, while others found no such relationship (Ismail et al., 2004; Aksoy et al., 2013). In this work, we have found no relation between the two. For example, for the leaf part of A. staticiforme has higher phenolic content with respect to its bulb part, whereas the inverse is true i.e., its antioxidant activity is higher in the bulb.

Antimicrobial compounds of Allium vary depending on procedure, for example various thiosulfinites occur when freshly crushed; dialk(en)yl sulfides are present when crushed and stored; ajoene is revealed when macerated in oil; heterocyclic sulfur compounds, allyl alcohol and 3-(allyltrisulfanyl)-2-aminopropanoic acid occur when heated at 121°C (Kyung, 2012).

The investigation of antimicrobial properties of plant extracts attracts great attention in the food industry owing to their potential use in natural additives.
biological activities of plants are important for the pharmaceutical industry. From this point of view, the results of the antibacterial, the antifungal and the DPPH radical scavenging activities of *A. staticiforme* and *A. subhirsutum* reported in the present study might be beneficial for the food industry and the pharmaceutical applications.

**Conclusion**

*A. staticiforme* and *A. subhirsutum* have antifungal activities with weak antibacterial activities. Both have DPPH radical scavenging activities.

**Conflict of Interest**

Authors declare no conflict of interest.

**Acknowledgement**

This work was supported by Sakarya University under Project No. BAPK 2017-02-20-006.

**References**


