



BJP

Bangladesh Journal of Pharmacology

Research Article

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

## Antimicrobial activities of *Allium staticiforme* and *Allium subhirsutum*

Alican Bahadır Semerci<sup>1</sup>, Dilek İnceçayır<sup>1</sup>, Vusale Mammadova<sup>1</sup>, Ayşegül Hoş<sup>2</sup> and Kenan Tunç<sup>1</sup>

<sup>1</sup>Department of Biology, Sakarya University, 54187, Sakarya, Turkey; <sup>2</sup>Department of Basic Pharmaceutical Sciences, School of Pharmacy, Istanbul Medipol University, Beykoz, Istanbul, Turkey.

### Article Info

Received: 22 August 2019  
Accepted: 5 January 2020  
Available Online: 9 March 2020  
DOI: 10.3329/bjp.v15i1.42373

### Cite this article:

Semerci AB, İnceçayır D, Mammadova V, Hoş A, Tunç K. Antimicrobial activities of *Allium staticiforme* and *Allium subhirsutum*. Bangladesh J Pharmacol. 2020; 15: 19-23.

### Abstract

The antibacterial and antifungal activities of the bulb and flower of *Allium staticiforme* 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. staticiforme* 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. staticiforme* leaf and bulb were determined as 17 and 2.4 mg of GAE/100 g, respectively. The IC<sub>50</sub> of methanolic extracts of *A. staticiforme* and *A. subhirsutum* were also determined. In conclusion, both *A. staticiforme* and *A. subhirsutum* have antifungal activities with weak antibacterial activities. These plants have DPPH radical scavenging activities.

## Introduction

A large number of plants have been reported with antimicrobial properties. Recent studies on several plants such as *Duranta erecta* (Donkor et al., 2019), *Frankenia hirsute* (Canlı et al., 2017), *Pinus coulteri* (Merah et al., 2018), *Syzygium cumini* (Oliveira et al., 2007), *Plectranthus glandulosus* (Egwaikhede et al., 2007), *Warburgia ugandensis* (Okello and Kang, 2019) show antimicrobial activities.

Louis Pasteur was the first to describe the antibacterial effect of onion (*Allium cepa*) and garlic (*Allium sativum*) juices (Durairaj et al., 2009). However, the antimicrobial activities of *A. staticiforme* and *A. subhirsutum* have not been published in scholarly journal.

Recent trends are to correlate the antimicrobial activity of a plant with the free radical scavenging properties.

In the present study, it is aimed to find out the antibacterial and antifungal activities of *A. staticiforme* and *A. subhirsutum*. Also, the DPPH radical scavenging activity and the total phenolic contents of *A. staticiforme* and *A. subhirsutum* were measured.

## Materials and Methods

### Materials

*A. staticiforme* Sm. and *A. subhirsutum* L. were obtained from the Atatürk Horticultural 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. staticiforme* and *A. subhirsutum* were divided into



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 anti-oxidant 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.*

### Box 1: Disc Diffusion Method

#### Principle

Antimicrobial activity of an extract was first screened for its inhibitory zone by the agar disc diffusion method.

#### Requirements

Amphotericin B, Densitometer, Gentamicin, Incubator, Mueller Hinton agar, Plant extracts (*A. staticiforme* and *A. subhirsutum*), Microorganisms (*S. epidermidis*, *B. subtilis*, *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. aureus*, *S. typhimurium*, *C. albicans*).

#### Procedure

*Step 1:* The bacterial strains to be used was activated with nutrient agar and *Candida albicans* was activated with Sabouraud dextrose agar.

*Step 2:* The overnight bacterial and fungal cultures were utilized to prepare the bacterial and yeast suspensions which were adjusted to 0.5 McFarland by using a densitometer.

*Step 3:* The sterile discs (6 mm in diameter) were impregnated

with the 10 µL of prepared extracts.

*Step 4:* The inoculations of density adjusted microorganism suspensions to Mueller Hinton agars were performed using sterile swabs.

*Step 5:* The impregnated discs were slightly placed to the inoculated Mueller Hinton agar.

*Step 6:* The incubation process was carried out at 37°C for 24 hours.

*Step 7:* After this procedure, the diameter of the inhibition zone was measured by a digital caliper.

*Step 8:* Solvent (methanol) impregnated discs were used as the negative control and commercial antibiotic discs (gentamicin 10 µg, amphotericin B 100 U) were utilized as the positive one.

*Step 9:* The antimicrobial activity test was performed three times under aseptic conditions and the diameter of inhibition zone measured was the average of the three replicates.

#### References (video)

Merah et al., 2018; Qaralleh, 2018



*staticiforme* and *A. subhirsutum* extracts had great potential as antifungal and antibacterial agents against the microorganisms (Table I). *C. albicans* showed maximum sensitivity ( $28.2 \pm 1.5$  mm zone of inhibition) to the methanolic extract (in 6,400  $\mu\text{g}/\text{disc}$  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  $\text{IC}_{50}$  values of *A. staticiforme* and *A. subhirsutum* were determined for leaf as 693 and 1086  $\mu\text{g}/\text{mL}$ , respectively (Figure 1). Also, the  $\text{IC}_{50}$  values of bulb part of *A. staticiforme* and *A. subhirsutum* were found as 1362 and 847  $\mu\text{g}/\text{mL}$ , 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).

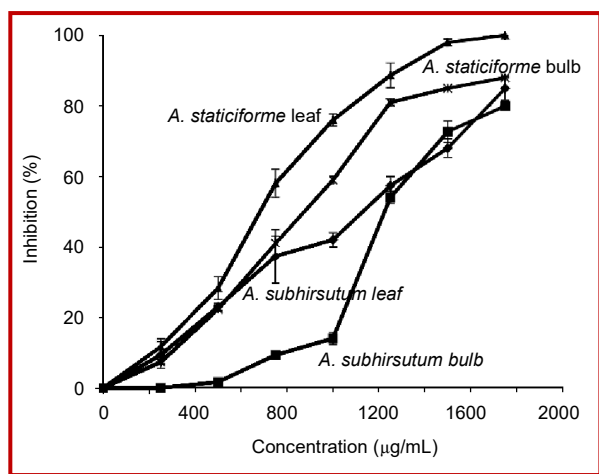


Figure 1: DPPH radical scavenging activity of *A. staticiforme* and *A. subhirsutum*

Each value was represented as mean  $\pm$  SEM of three measurements

Table II		
Total phenolic contents and $\text{IC}_{50}$ values of <i>A. staticiforme</i> and <i>A. subhirsutum</i> methanolic extracts		
Extract ( $\mu\text{g}/\text{disc}$ )	TPC (mg GAE/100 g)	$\text{IC}_{50}$ ( $\mu\text{g}/\text{mL}$ )
<i>A. subhirsutum</i> bulb	$4.8 \pm 0.5^b$	$847 \pm 6.8^c$
<i>A. subhirsutum</i> leaf	$17.5 \pm 0.2^d$	$1086 \pm 2.7^d$
<i>A. staticiforme</i> bulb	$2.4 \pm 0.1^a$	$1362 \pm 1.1^e$
<i>A. staticiforme</i> leaf	$17 \pm 0.1^c$	$693 \pm 2.3^b$
Ascorbic acid	Not tested	$5.65 \pm 0.1^a$

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 tripedale* 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*, *S. typhimurium* and *P. aeruginosa*. Furthermore, *A. subhirsutum* have shown antibacterial activity against *E. coli*, *S. epidermidis*, *S. aureus*, *E. faecalis*, *S. typhimurium* and *P. aeruginosa*.

In this work, the  $\text{IC}_{50}$  values of the extracts are in between 693-1362  $\mu\text{g}/\text{mL}$ . 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* have 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 anti-oxidant 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 thiosulfonates 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. The

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

- Aksoy L, Kolay E, Ağılönü Y, Aslan Z, Kargioğlu M. Free radical scavenging activity, total phenolic content, total antioxidant status of endemic *Thermopsis turcica*. Saudi J Biol Sci. 2013; 20: 235-39.
- Blois MS. Anti-oxidant determinations by the use of a stable free radical. Nature 1958; 181: 1199-200.
- Canli K, Simsek O, Yetgin A, Altuner E. Determination of the chemical composition and antimicrobial activity of *Frankenia hirsuta*. Bangladesh J Pharmacol. 2017; 12: 463-69.
- Donkor S, Larbie C, Komlaga G, Emikpe BO. Phytochemical, antimicrobial, and antioxidant profiles of *Duranta erecta* L. Parts. Biochem Res Int. 2019; 2019.
- Durairaj S, Srinivasan S, Lakshmanaperumalsamy P. *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. Electron J Biol. 2009; 5: 5-10.

- Egwaikhide PA, Gimba CE. Analysis of the phytochemical content and antimicrobial activity of *Plectranthus glandulosus* whole plant. Middle-East J Sci Res. 2007; 2: 135-38.
- Ghahremanimajd H, Dashti F, Dastan D, Mumivand H, Hadian J, Esna-Ashari M. Antioxidant and antimicrobial activities of Iranian Mooseer (*Allium hirtifolium* Boiss.) populations. Horticult Environ Biotechnol. 2012; 53: 116-22.
- Ismail A, Marjan ZM, Foong CW. Total antioxidant activity and phenolic content in selected vegetables. Food Chem. 2004; 87: 581-86.
- Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J. *In vitro* antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and *Candida* species from Nigeria. J Med Food. 2004; 7: 327-33.
- Kaur C, Joshi S, Kapoor HC. Antioxidants in onion (*Allium cepa* L.) cultivars grown in India. J Food Biochem. 2009; 33: 184-200.
- Kyung KH. Antimicrobial properties of *Allium* species. Curr Opin Biotechnol. 2012; 23: 142-47.
- Merah S, Dahmane D, Krimat S, Metidji H, Nouasri A, Lamari L, Dob T. Chemical analysis of phenolic compounds and determination of antioxidant, antimicrobial and cytotoxic activities of organic extracts of *Pinus coulteri*. Bangladesh J Pharmacol. 2018; 13: 120-29.
- Okello D, Kang Y. Ethnopharmacological potentials of *Warburgia ugandensis* on antimicrobial activities. Chinese J Integr Med. 2019, 2019.
- Oliveira GF, Furtado N, Filho AA, Martins JK, Cunha WR, Silva ML. Antimicrobial activity of *Syzygium cumini* (Myrtaceae) leaves extract. Br J Microbiol. 2007; 38: 1517-25.
- Qaralleh H. Chemical composition and antibacterial activity of *Origanum ramonense* essential oil on the  $\beta$ -lactamase and extended-spectrum  $\beta$ -lactamase urinary tract isolates. Bangladesh J Pharmacol. 2018; 13: 280-86.
- Shirani M, Samimi A, Kalantari H, Madani M, Zanganeh AK. Chemical composition and antifungal effect of hydroalcoholic extract of *Allium tripedale* against *Candida* species. Curr Med Mycol. 2017; 3: 6-12.
- Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16: 144-58.

### Author Info

Kenan Tunç (Principal contact)  
e-mail: ktunc@sakarya.edu.tr