BJP

Bangladesh Journal of Pharmacology

Research Article

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

A Journal of the Bangladesh Pharmacological Society (BDPS) Bangladesh J Pharmacol 2020; 15: 19-23
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088

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

Alican Bahadir Semerci[1], Dilek İnceçayır[1], Vusale Mammadova[1], Ayşegül Hoş[2] and Kenan Tunç[1]

[1]*Department of Biology, Sakarya University, 54187, Sakarya, Turkey;* [2]*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*, *Enterecoccus faecalis, Salmonella typhimurium* and *Pseudomanas 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_{50} 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* (Canli et al., 2017), *Pinus coulteri* (Merah et al., 2018), *Syzygium cumini* (Oliveira et al., 2007), *Plectranthus glandulosis* (Egwaikhide 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, *Enterecoccus faecalis* ATCC 29212, *Pseudomanas 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:

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

Where, $A_{control}$ is the absorbance of mixture of methanol and DPPH solution without extract and A_{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.*

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

Table I

Inhibition zone diameters of methanolic extract of *A. staticiforme* and *A. subhirsutum*

Extract (µg/disc)				Inhibition zone diameter (mm)							
				Escherichia coli	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Staphylococcus aureus*	*Enterecoccus faecalis*	*Salmonella typhimurium*	*Pseudomanas aeruginosa*	*Candida albicans*
A. staticiforme	Bulb	MeOH	6400	0	8.2 ± 0.1	8.2 ± 1.3	6.0 ± 0	8.5 ± 0.4	9.7 ± 1.1	8.8 ± 0.5	28.2 ± 1.5
			3200	0	6.0 ± 0	6.5 ± 0	0	6.0 ± 0	6.6 ± 0.5	7.0 ± 0.3	21.0 ± 1.6
	Flower	MeOH	6400	12.4 ± 2.2	11.5 ± 0.6	9.3 ± 0.5	0	10.1 ± 0.6	7.5 ± 0.6	7.4 ± 0.1	24.1 ± 1.2
			3200	6.0 ± 0	9.0 ± 0	0	0	0	0	0	13.4 ± 0.3
	Leaf	MeOH	6400	0	0	0	0	0	0	0	0
			3200	0	0	0	0	0	0	0	0
A. subhirsutum	Bulb	MeOH	6400	8.2 ± 0.2	13.1 ± 0.2	0	8.3 ± 0.1	8.2 ± 0.3	9.9 ± 1.5	9.7 ± 0.8	13.8 ± 1.2
			3200	0	0	0	0	0	0	0	10.7 ± 1.1
	Flower	MeOH	6400	8.2 ± 0.2	13.1 ± 0.2	0	8.3 ± 0.1	8.2 ± 0.3	9.9 ± 1.5	9.7 ± 0.8	20.5 ± 1.2
			3200	0	0	0	0	0	0	0	18.5 ± 1.1
	Leaf	MeOH	6400	6.5 ± 0	9.2 ± 0.7	0	6.5 ± 0.1	8.7 ± 0.5	9.3 ± 0.3	8.3 ± 0.5	8.6 ± 0.7
			3200	0	7.8 ± 0.3	0	0	0	7.5 ± 0.5	0	6.6 ± 0.5
Gentamicin (10 µg)				19	21	17	20	20	21	22	not tested
Amphotericin B (100 U)				not tested	not tested	not tested	not tested	not tested	not tested	not tested	16

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).

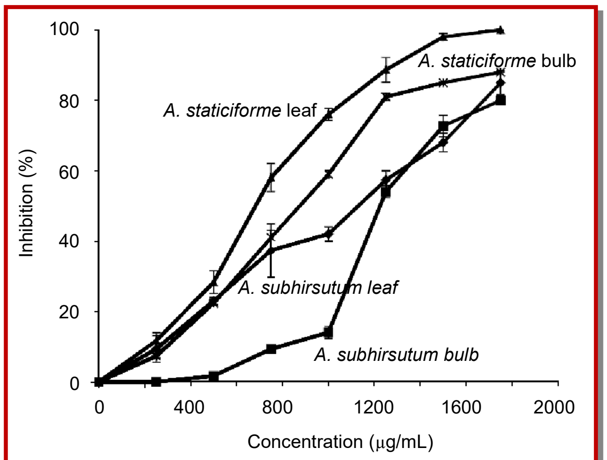

Figure 1: DPPH radical scavenging activity of *A. staticiforme* and *A. subhirsutum*

Each value was represented as mean ± SEM of three measurements

Table II

Total phenolic contents and IC_{50} values of *A. staticiforme and A. subhirsutum* methanolic extracts

Extract (µg/disc)	TPC (mg GAE/100 g)	IC_{50} (µg/mL)
A. subhirsutum bulb	4.8 ± 0.5^{b}	847 ± 6.8^{c}
A. subhirsutum leaf	17.5 ± 0.2^{d}	1086 ± 2.7^{d}
A. staticiforme bulb	2.4 ± 0.1^{a}	1362 ± 1.1^{e}
A. staiciforme leaf	17 ± 0.1^{c}	693 ± 2.3^{b}
Ascorbic acid	Not tested	5.65 ± 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 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* 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 thiosulfinates 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.

Financial Support

Sakarya University under Project No. BAPK 2017-02-20-006

Conflict of Interest

Authors declare no conflict of interest.

References

Aksoy L, Kolay E, Ağılönü Y, Aslan Z, Kargıoğlu 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 glandulosis* 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. Hortic Environ Bioechnol. 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 İndia. 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 β-lactamase and extended-spectrum β-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 hydro-alcoholic 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