Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2022, 15(1), 135-143
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2022, 15(1), 135-143
DOI: 10.18185/erzifbed.995560
Research Article

# Enzyme Inhibition, Antimicrobial Potentials of *Saponaria prostrata* plant extracts

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Geliş / Received: 14/09/2021, Kabul / Accepted: 20/12/2021

**Abstract**

*Saponaria prostrata* is a medicinal plant that contains various secondary metabolites such as phenolic acid, flavonoids, triterpenoids, and fatty acids that are related to some biological activities. In this study, we evaluated the enzyme inhibitory, antimicrobial potentials of *S. prostrata*. The antimicrobial activity of *S. prostrata* was measured using three Gram-positive, four Gram-negative bacteria species, and three fungi species. The highest antibacterial activity was detected against the *Staphylococcus aureus* ATCC 25923 (13±0.81 mm inhibition zone). The enzyme inhibition effect ($IC_{50}$ values) of *S. prostrata* were calculated against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α-glycosidase (α-Gly) as 2.39 mg/mL, 3.69 mg/ml, and 2.48 mg/mL, respectively.

**Keywords:** *Cholinesterase, disc diffusion, α-glycosidase, Saponaria prostrata*

**Enzim İnhibisyonu, *Saponaria prostrata* bitki ekstraktının Antimikrobiyal Potansiyelleri**

**Öz**

Saponaria prostrata, bazı biyolojik aktivitelerle ilişkili fenolik asit, flavonoidler, triterpenoidler ve yağ asitleri gibi çeşitli ikincil metabolitleri içeren tıbbi bir bitkidir. Bu çalışmada S. prostrata'nın enzim inhibitör, antimikrobiyal potansiyellerini değerlendirdik. S. prostrata'nın antimikrobiyal aktivitesi, üç Gram pozitif, dört Gram negatif bakteri türü ve üç mantar türü kullanılarak ölçüldü. En yüksek antibakteriyel aktivite Staphylococcus aureus ATCC 25923'e (13 ± 0.81 mm inhibisyon bölgesi) karşı tespit edildi. S. prostrata'nın enzim inhibisyon etkisi (IC50 değerleri) asetilkolinesteraz (AChE), butirilkolinesteraz (BChE) ve α-glikosidaz (α-Gly)'ye karşı 2,39 mg/ml, 3,69 mg/ml ve 2,48 mg/ml olarak sırasıyla hesaplanmıştır.

**Anahtar Kelimeler:** Kolinesteraz, disk difüzyonu, α-glikosidaz, Saponaria prostrata

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## 1. Introduction

Medicinal plants are effective natural sources of healthcare for treatments of many ailments (Nascimento, Locatelli, Freitas, & Silva, 2000). Traditional medicine is a major part of human healthcare in many parts of the world including developed countries (Kartal, 2007). Owing to the resistance and the possible side effects of the microorganisms build against antibiotics used in the treatment, researchers' attention has been focused on plant extracts and bioactive compounds isolated from plant species (Essawi & Srour, 2000; Kokoska, Polesny, Rada, Nepovim, & Vanek, 2002). Recently, researchers seek for new ways to treat some diseases including metabolic inflammation (Kong et al., 2020), cancer metastasis (Pang et al., 2020), and cataract treatment (Jeevanandam, Madhumitha, & Saraswathi). The high majority of plants have been recognized as having commercial values (Van Wyk, 2008).

*S. prostrata* is an endemic and medicinal plant in the flora of Turkey (Davis, 1982a) that belongs to *Caryophyllaceae* family (ATAŞLAR, 2004). This plant species might be annual, biennial, or perennial plants that branched with decumbent prostrate or ascending branches with spreading white hairs (Davis, 1982b). Because of their natures, green plants, fruits, and vegetables are often being used as primary natural antioxidant sources (Aras, Dogru, & Bursal, 2016). Compare to synthetics, consumption of natural antioxidants is better for human health because synthetics have numerous carcinogenic effects (Maadane et al., 2015).

Plants' secondary metabolites such as phenolic structures have been stated as AChE inhibitors that can be used for AD treatment (Murray, Faraoni, Castro, Alza, & Cavallaro, 2013; Nuapia, Chimuka, & Cukrowska, 2018). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are two main forms of cholinesterase enzymes and are characterized in muscles, nerve cells, erythrocytes, brain, and placental tissue at high concentrations. As a nervous system member AChE catalyzes the hydrolysis of acetylcholine and terminates its neurotransmitter activity (Nogrady & Alai, 1983). It has been accepted that BChE inhibition is a standard approach in the treatment of neurodegenerative diseases (Zengin et al., 2019). The insufficiency of the AChE activity may harm the equilibrium, locomotion, escape, feeding, and reproductive behaviors of the subjected organisms (dos Santos Miron et al., 2005; Z. Yan et al., 2019). AChE inhibitors including galantamine, donepezil, and rivastigmine are the present pharmacological agents to change the clinical symptom of Alzheimer's disease (WIDERA & Covinsky, 2013). The α-Glycosidase enzyme, another enzyme of the study, has been recorded as a particular concern in pharmaceutical research. By way of α-glycosidase inhibition, the lag of glucose absorption and the liberalization of the glucose molecule from complex carbohydrate compounds can be performed (Taslimi et al., 2018).

In this study, we determined AChE, BChE, and α-Gly enzyme inhibitions effects and antimicrobial properties of *S. prostrata*.

## 2. Experimental

### 2.1. Plant material

The aerial parts of *Saponaria prostrata* WILLD *subsp. prostrata (S. prostrata)* were collected from their natural habitats rocky slopes of Haserek mountain at 1800-1850 m altitude from Bingol, Turkey by Dr. Ömer Kılıç (herbarium number: 4764). The taxonomic description was made according to a previous study (Davis, 1975).

### 2.2. Extracts preparation

*S. prostrata* water and ethanol extracts were prepared considering previous work (Bingol & Bursal, 2018). For this purpose, 20 g air-dried plant leaves were powdered and mixed with 200 mL distilled water or ethanol (1/10:w/v), respectively. The mixtures were homogenized (12 h) and filtered. The frozen water solvent was lyophilized at 5 mm Hg at -50 °C. The ethanol solvent was evaporated with an evaporator.

### 2.3. Antimicrobial activity

For antimicrobial activity, *Saccharomyces cerevisiae*, *Candida albicans* ATCC 10231, *Yarrowia lipolytica* fungi species, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeroginosa* ATCC 9027, *Escherichia coli* ATCC 11229, gram-negative bacteria and *Bacillus megaterium* DSM 32, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 gram-positive bacteria were used. As positive controls ampicillin/sulbactam (SAM-20 µg), rifampicin (RD-5 µg), and amikacin (AK-30 µg), erythromycin (E-15 µg), were used.

The antimicrobial capacity of the *S. prostrata* plant samples was determined using the disc diffusion technique. Initially, 30 µL, 60 µL, and 90 µL of ethanol extract were absorbed into sterile disc (8 mm diameter). For inoculating, 1 % rate of each microorganism from 106 -107 CFU/mL suspension was added to 15 mL sterile media (Muller-Hinton agar) for bacteria and (Sabourand 2 % Glucose agar) for yeast. The inoculated media were poured into petri dishes (9 cm) and left to 4 °C for 1 h. The petri dishes were incubated at 37 °C for 18-24 h, except for *Candida albicans, Yarrowia lipolytica, and Saccharomyces cerevisiae* which were incubated at 27 °C. Inhibition zones have calculated a caliber and recorded as the mean diameter of 3 replications in mm (Aras et al., 2018; Bukhari et al., 2020; Turan, Savci, Buldurun, Alan, & Adigüzel, 2016).

### 2.4. Enzyme inhibitions

α-Gly inhibition of *S. prostrata* was calculated using *p* nitrophenyl-D-glucopyranoside (*p*-NPG) as the substrate. This technique was used as presented in a previous study (Turkan, Cetin, Taslimi, & Gulçin, 2018). The absorbances and $IC_{50}$ values were calculated at 405 nm.

BChE and AChE activities were measured according to Ellman's procedure(Adiguzel, Esener, Ergin, Aktan, & Sekerci, 2011) as described previously (Bursal et al., 2019). Butyrylthiocholine iodide BChI/acetylcholine iodide (AChI) and 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) were used for the determination of BChE/AChE activities. In brief, 100 µL buffer (Tris–HCl, 1.0 M, pH 8.0) and 10 mL of sample solutions with different concentrations were dissolved in deionized water. Then, 50 µL BChE/AChE (5.3210−3 EU) solution was added, and the resulting solution was incubated for 10 min at 25°C. After incubation, a portion of DTNB (50 µL, 0.5 mM) was added. Finally, the reaction was started by the addition of 50 mL BChI/AChI (10/10 mM). The enzymatic hydrolysis of both substrates was determined spectrophotometrically at 412 nm from the formation of the yellow 5-thio-2-nitrobenzoate anion. Negative control contains all solutions for enzymatic activity determination except for AChE/BChE. Tacrine (TAC) was used as a standard compound for both BChE and AChE enzymes.

## 3. Results and Discussion

### 3.1. Antimicrobial activity

The measurement of the antimicrobial activity of the plant-based material has been increased due to the increase of infectious diseases in the last decades. Seconder metabolites of plants, especially phenolic compounds have some effects on pathogens and antibacterial properties (Kossah, Zhang, & Chen, 2011). *S. prostrata* extract antimicrobial activity was calculated against three gram-positive, four gram-negative bacteria, and three fungi species. The zones of inhibition were determined for each concentration to detect the inhibition of bacteria growth. The reference antibiotics were also analyzed for comparison (Table 1).

**Table 1.** Antifungal and antibacterial properties of *S. prostrata* and standard antibiotic discs

| **Microorganisms** | | ***S. prostrata*** (20 mg/mL ethanol extract) | | | **Antibiotic Discs** | | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 µL | 60 µL | 90 µL | Erythromycin | Ampicillin/ sulbactam | Amikacin | Rifampicin |
| Gram (+) | *B.subtilis* | -[1] | - | 10±0.47 | 20±1.24 | 14±0.47 | 11±1.24 | 21±1.24 |
| | *S. aureus* | 10±0.00 | 12±0.81 | 13±0.81 | 21±0.00 | 10±0.81 | 9±0.00 | 18±1.69 |
| | *B. megaterium* | -[1] | 9±0.47 | 10±0.47 | 25±1.69 | -[1] | 10±0.81 | 16±1.24 |
| Gram (-) | *E. aerogenes* | -[1] | 9±0.00 | 10±0.81 | 27±1.24 | 10±0.47 | 9±0.00 | 16±0.47 |
| | *E. coli* | -[1] | -[1] | -[1] | 19±0.00 | 13±1.24 | 13±0.81 | 18±1.24 |
| | *P. aeroginosa* | -[1] | -[1] | -[1] | 19±1.69 | -[1] | 14±0.00 | 8±0.00 |
| | *K. pneumoniae* | -[1] | 9±0.00 | 9±0.47 | 19±0.47 | 16±1.69 | 10±0.47 | 19±1.69 |
| Fungus | *Y. lipolytica* | -[1] | -[1] | -[1] | -[1] | -[1] | -[1] | -[1] |
| | *C. albicans* | -[1] | -[1] | -[1] | -[1] | -[1] | -[1] | -[1] |
| | *S. cereviciae* | -[1] | -[1] | -[1] | -[1] | -[1] | -[1] | -[1] |

[1]:No inhibition zone,

[2]:Inhibition zone (mm)

Herein, the highest antibacterial activity was detected against the *S. aureus* (13±0.81 mm inhibition zone). The extract showed low antibacterial activity against *B. megaterium, E. aerogenes,* and *K. pneumoniae*. Nevertheless, the extract did not demonstrate any antimicrobial activity against *E. coli,aeroginosa, C. albicans, Y. lipolytica,* and *S. cereviciae.*

The antibacterial activity level changed with increasing concentrations. Increasing 1–2 mm was observed regarding concentrations increased by 30 µL, 60 µL, and 90 µL, respectively. The antibacterial activity of *S. prostrata* was also compared with the standard antibiotics. The extract showed equipotent activity with ampicillin/sulbactam (SAM-20 mg) and amikacin (AK-30 mg), in contrast, erythromycin (E-15 mg) and rifampicin (RD-5 mg) showed low activity as shown in Table 2.

Various biological activities of *Saponaria* plant species have been studied and reported in the literature. *Saponaria officinalis* methanol extract had antimicrobial activity against 19 of 27 microorganisms (6-23 mm inhibition zone). However, aqueous extracts of *S. officinalis* showed only weak antimicrobial activity against 5 of 27 microorganisms (7-11 mm inhibition zone) (Sengul et al., 2011). In another study, extracts *of S. officinalis* showed greater activity on gram-negative organisms compared to gram-positive organisms. The extracts have been reported to show more activity on E. coli and *S. typhimurium,* while lower activity on *C. sporogenes* and *S. pneumoniae.* It was determined that the methanol extract showed better activity than ciprofloxacin (Veda, Mallikarjuna, & Ganga, 2017). In the disk diffusion method, hydroalcoholic extracts of *S. officinalis* formed a 16-19 mm inhibition zone depending on the concentration. This extract has been reported to show better activity than standard antibiotics (Nabinejad, 2013). No antimicrobial activity was found in the literature of the *S. prostrata* species we used in our study. When our results were compared with the species in the genus *Saponaria,* it was determined that there was lower activity. At the same time, it was determined that our extract showed less activity than standard antibiotics.

### 3.2. Enzyme inhibition

AChE and BChE have been detected and identified in neurofibrillary knots and amyloid plaques (Li et al., 2018). In a normal brain, AChE is detected as a predominant enzyme, as well as BChE is considered to have a relation with a secondary role for regulation of acetylcholine level (Chen et al., 2017). As a progressive, neurodegenerative disorder, and age-related Alzheimer's disease is marked by traceable memory loss or cognitive impairment. AChE is one of the crucial cholinergic system components in the peripheral nervous system, that thought to have the ability to hydrolyze the acetylcholine (X. Yan, Chen, Zhang, & Du, 2018). A remarkable increase in the AChE activity is observed in the early stage of AD, while the activity of BChE generally increases in the later stages of AD. Therefore, these enzymes are important therapeutic targets for the recovery of the loss of cholinergic and are considered

to be the hallmark of AD (Zengin et al., 2018). In addition, α-Gly inhibitors are involved in the diagnosis and treatment of diabetes (Türkan, Atalar, Aras, Gülçin, & Bursal, 2020).

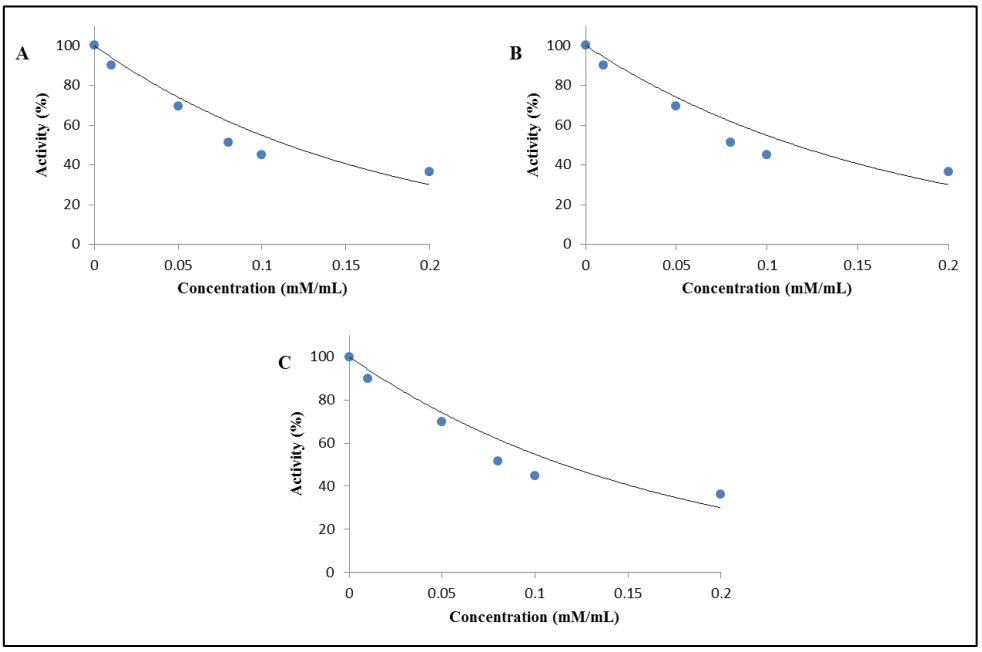


**Figure 1.** Enzyme inhibition of *S. prostrata* against AChE (A), BChE (B), and α-Gly (C).

Herein, each used enzyme was remarkably inhibited by *S. prostrata* plant extracts as shown in Figure 1 and Table 2. Tacrine as a positive standard is a reversible inhibitor of cholinesterase enzymes (BChE, AChE) and it was the first drug for AD treatment. The $IC_{50}$ values for *S. prostrata* on metabolic enzymes were obtained 3.69 mg/mL for AChE, 2.48 mg/mL for BChE, and 2.39 mg/mL for α-Gly. The $IC_{50}$ values of tacrine were determined as 12.36 mg/mL and 19.11 mg/mL, for AChE and BChE enzymes. Also, acarbose was used as a positive standard inhibitor for the α-Gly enzyme. The $IC_{50}$ value of acarbose was determined as 22.8 mg/mL.

**Table 2.** The $IC_{50}$ values of *S. prostrata* extract against the enzymes used

| | **AChE** | | **BChE** | | **α-Gly** | |
|---|---|---|---|---|---|---|
| **Compounds** | **$IC_{50}$ (mg/mL)** | **$R^2$** | **$IC_{50}$ (mg/mL)** | **$R^2$** | **$IC_{50}$ (mg/mL)** | **$R^2$** |
| ***S. prostrata*** | 3.69 | 0.9725 | 2.48 | 0.8568 | 2.39 | 0.9493 |
| **Tacrine** [a] | 12.36 | 0.958 1 | 19.11 | 0.981 | - | - |
| **Acarbose** [b] | 22.8 | - | - | - | - | - |

[a] used as a positive control for AChE and BChE enzymes

[b] used as a positive control for α-glycosidase (Tao, Zhang, Cheng, & Wang, 2013)

## 4. Conclusions

In this paper, we investigated and reported enzyme inhibitions that have roles in diabetes, Alzheimer's disease. Also, we analyzed the antimicrobial potential of *S. prostrata*. This study is a pioneer detailed study in evaluating some important chemical and biological properties of *S. prostrata*. The results highlighted the potential role of *S. prostrata* to prevent the oxidation process and help to figure out enzyme inhibition related to some diseases. This plant could be taken into consideration as a useful natural source in biomedical applications particularly for the treatment of diabetes and Alzheimer's disease.

**Conflict of Interest:** None

**Ethics in Publishing**

There are no ethical issues regarding the publication of this study.

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