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Antioxidant and anticandidal effects of *Pedicularis comosa* L. var. *acmodonta* Boiss.

Nagehan SALTAN[1], Zeynep GÜLCAN[*1], Gökalp İŞCAN[2], Yavuz Bülent KÖSE[1]
ORCID: 0000-0002-1207-909X; 0000-0002-4379-3253; 0000-0003-1210-0490; 0000-0002-3060-7271

[1] Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Eskişehir, Türkiye
[2] Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye

Abstract

The aim of this study is to measure the antioxidant and anticandidal efficiency of *Pedicularis comosa* var. *acmodonta* (Orobanchaceae). Using a spectrophotometric test on extracts from *P.comosa* var. *acmodonta* grown in Türkiye, the antioxidant efficiency of the plant extracts was estimated by measuring the violet coloration in a methanol solution containing 1,1-diphenylpyryl hydrazil (DPPH·). In addition, the results of the anticandidal effect tests using the CLSI M27-A2 method are also included in this study. The 70% ethanol extract of *P. comosa* var. *acmodonta* showed the highest antioxidant activity (0.143 mg/mL). According to the anticandidal activity test findings on five *Candida* species, it was determined that 70% ethanol extract of *P. comosa* var. *acmodonta* inhibited *C. utilis* at a dose of 31.25 µg/mL (MIC).

Keywords: anticandidal, antioxidant, *Pedicularis,* Orobanchaceae.

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Pedicularis comosa L. var. *acmodonta* Boiss. türünün antioksidan ve antikandidal etkilerinin belirlenmesi

Özet

Bu çalışmanın amacı, *Pedicularis comosa* var. *acmodonta* (Orobanchaceae) türünün antioksidan ve antikandidal aktivitesini değerlendirmektir. Türkiye'de yetişen *P. comosa* var. *acmodonta* ekstreleri üzerinde bir spektrofotometrik test kullanılarak, bitki ekstrelerinin antioksidan aktivitesi, 1,1-difenilpiril hidrazil (DPPH·**)** içeren bir metanol solüsyonundaki mor rengin açılması ölçülerek değerlendirilmiştir. Ayrıca CLSI M27-A2 yöntemi kullanılarak yapılan antikandidal etki testlerinin sonuçları da bu çalışmada yer almaktadır. *P. comosa* var. *acmodonta* 'nın %70 etanol ekstresi en yüksek antioksidan aktiviteyi (0.143 mg/mL) göstermiştir. Beş *Candida* türü üzerinde yapılan antikandidal aktivite testi bulgularına göre *P. comosa* var. *acmodonta* 'nın %70 etanol ekstresinin *C. utilis*'i 31.25 µg/mL (MIC) dozunda inhibe ettiği belirlenmiştir.

Anahtar kelimeler: antikandidal, antioksidan, *Pedicularis*, Orobanchaceae.

1. Introduction

The *Orobanchaceae* family consists mostly of parasitic plants. Currently, it is represented worldwide by 98 genera and 2233 species [1]. It is observed that ethnobotanical studies on parasitic plants are limited worldwide. O'Neill and Rana conducted ethnobotanical research on parasitic plants found in Nepal (Himalayas). As a result of the research, it was determined that more than 150 taxa are distributed in Nepal. Through interviews with 141 informants, it was observed that 43 parasitic plants were ethnobotanically used by the local population for medicinal, animal feed, food, religious ceremonies, and various materials [2, 3].

Pedicularis L. is a genus of typically grows to a height of up to 50 cm and is perennial herbaceous plant. *Pedicularis* species have historically been used for a very long time as folk medicines to cure tiredness and digestive problems, as well as to clear heat and remove toxins while preserving vitality and removing edema [4]. Different

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +902223350580-3704 Fax.: ++902223350580-3704; E-mail:zgulcan@anadolu.edu.tr
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therapeutic uses for *Pedicularis* species exist in Traditional Chinese Medicine [5]. For the treatment of collapse, weariness, and senility, several of them have roots and stems that are utilized in Chinese folk medicine and as heart tonics. Iridoids, phenylethanoids, phenylpropanoids, and flavonoids [6-9] all of which are produced by the genus *Pedicularis*, are known to have antioxidant effects.

Pedicularis, known as “lousewort” or “wood concrete” in the literature, is popularly known as 'bitotu sormuk, sorma, sormuk otu' in Türkiye [10, 11]. The plant is used as a registered food in Eastern Anatolia due to the nectar in its flowers. It is one of the most commonly used classes of medicinal herbs and has many therapeutic benefits on fatigue, spontaneous perspiration, digestion issues, and heart issues. Additionally, some species of this genus have been found to exhibit hepatoprotective, anticancer, anti-oxidative, anti-haemolysis, and antibacterial properties [5, 6, 10]. In Flora of Turkey *Pedicularis* is represented by 11 species, 2 of which are endemic.

Due to the theuropatic benefits on digestion issues in the traditional use of the *Pedicularis* species, the antioxidant and anticandidal effects of *Pedicularis comosa* var. *acmodonta* extracts are emphasized in this study. To the best of our knowledge, methanolic extract of antibacterial efficacy was proven in earlier studies however there has been no research done on *Pedicularis* anticandidal effects [12].

2. Materials and methods

2.1. P. comosa L. var. acmodonta Boiss.

The plant was picked up from Han/Eskişehir in Türkiye on 26.05.2021 (Figure 1). Sample has been kept at the Herbarium in the Anadolu University, Eskisehir, Türkiye (ESSE 15818).

Figure 1. *Pedicularis comosa* var. *acmodonta*

2.2. Plant extract

The aerial parts were used. For using both polar and nonpolar solvents, 70% ethanol and *n*-hexane were used. Samples were held to macerate for 48 hours at 24°C temperature in an orbital shaker at 150 rpm. The solvents from the macerated were eliminated with a rotary evaporator under lower pressure after they had been filtered through filter paper. By lyophilizing, a 70% ethanolic extract was produced. The extracts were kept at +4 ºC after being released from their solvents until usage.

2.3. Antioxidant activity

The DPPH radical assay was put to use to evaluate the antioxidant properties of plant extracts made from the aerial parts using the techniques developed by Kumarasamy et al. with some modifications [13]. For standard matter, Gallic acid made used. All determinations were carried out in triplicate.

2.4. Antimicrobial activity

P. comosa var. *acmodonta* extracts were tested for their capability to inhibit the upgrowth of five different strains of *Candida*, including *C. utilis* (NRRL Y- 900), *C. albicans* (ATCC 90028), *C. tropicalis* (ATCC 750), *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258). Standard method (CLSI M27-A2) was used to identify the minimum inhibitory (MIC) concentrations of the extracts. Dilutions of the extracts were prepared between the concentration of 8000 to 15.6 µg/mL while the standard antifungals were 16 to 0.003 µg/mL. Minimal fungicidal concentration (MFC) was determined for the most active extract and the candida. A loop of medium from all clear wells including MIC was transferred into fresh PDA plates. After 24h incubation MFC was determined.

3. Results

Free radical scavenging activity of the 70% ethanol and *n*-hexane extracts were assessed spectrophotometrically by use of DPPH•. The *in vitro* antioxidant and anticandidal activities of the extracts obtained from the plant are presented in Tables 1 and 2. The results in Table 1 reveal that 70% ethanol extract (IC_{50} 0.143 mg/mL) was more capable of scavenging DPPH radical.

Table 1**.** DPPH• radical sweeping activity of the extracts (mg/mL)

Extracts	**DPPH test IC_{50} (mg/mL)**
70% ethanol	0.143 ±0.046
n-hexane	0.835 ±0.088
Gallic acid	0.002 ±0.001

The literature records indicate that the chemical compositions and biological activities of many *Pedicularis* species have been studied [14, 15, 16, 17, 18, 19]. Antioxidant activity of *P. sibthorpii* and *P. wilhelmsiana* were determined between the range of 0.01-0.7 mg/mL and 0.01-1.02 mg/mL by Khodaie et al. (2012). In a different investigation, the DPPH· radical scavenging and ABTS assays revealed that *P. mexicana* was active (0.883 0.032 mg/mL; 1.242 0.020 mg/mL) [20]. The DPPH radical photometric test was used in a study to evaluate antioxidant activity, and it was revealed that *P. comosa* L. var. *sibthorpii* (Boiss.) Boiss extracts had a very high ability to scavenge free radicals (IC_{50} <12.5 µg/mL) [21]. In a study evaluating the antidiabetic and antioxidant effect of the ethanol extract of *P. longiflora* Rudolph, it was reported that the increase in antioxidants and decrease in oxidants in the test groups may be due to the antioxidant features of the plant [22]. It was shown that the total polyphenol content of plants in water and methanol extracts ranged between 4.6 and 183.8 and 8.2 and 270.1 mg/g, respectively, and that *P. resupinata* had a high level of radical scavenging action against DPPH (IC_{50} <25 µg/mL) radical in Korea [23]. According to DPPH radical scavenging activity, extracts and various fractions from *P. longiflora* var. *tubiformis* (Klotzsch) P. C. Tsoong were concentrated and screened by Lan et al. It was shown that the 40% aqueous ethanol fraction demonstrated a remarkable antioxidant efficiency. The application of six major constituents (boschnaloside, alyssonoside, leucosceptoside A, isoverbascoside, leucosceptoside B and verbascoside) with high content revealed a clear antioxidant ability towards the DPPH radical [24].

Five *Candida* species were used for anticandidal assay according to the CLSI M27-A2 method. As regards the study results given in Table 2, it was determined that *C. utilis* (MIC 31.25 µg/mL) was quite sensitive to the ethanol extract prepared from the aerial parts of the plant.

Table 2. Anticandidal effect of *P. comosa* var. *acmodonta* extracts MIC (µg/mL)

Candida	**70% ethanol**	***n*-hexane**	**Amp-B**	**Keto**
C. utilis NRRL Y-900	31.25*	1000	1	0.5
C. albicans ATCC 90028	2000	4000	1	0.5
C. tropicalis ATCC 750	500	500	2	0.25
C. parapsilosis ATCC 22019	1000	1000	2	0.125
C. krusei ATCC 6258	500	500	1	1

*MFC: Minimum fungicidal concentration =1000 µg/mL
AMP-B: Amphotericin B, Keto: Ketoconazole

Studies on the antimicrobial effects of *Pedicularis* species primarily focus on bacteria. To the best of our knowledge, there is no study on the anticandidal effect of the species that is the subject of the study.

Opportunistic infections of *Candida* species are an important threat to public health such as systemic infections, vaginitis, oral and cutaneous candidiasis, and candidemia. To overcome the problem, it is a big need to find new natural substances with antifungal potential, more potent and less harmful.

In a previous study disc diffusion method was carried out to evaluate the antibacterial effect of the water and methanol extracts of *P. comosa* var. *sibthorpii* against ten pathogenic bacteria by Turker et al. (2021). It has been reported that plant extracts generally show antibacterial activity against *S. aureus*, *S. epidermidis* and *S. pyogenes* [21]. In another study, methanol extract of *P. sibthorpii* Boiss. was found to be effective against *P. aeruginosa*, *S. aureus* and *S. epidermidis*, however not sensitive to *C. albicans*. Moreover, methanolic extract from *P. wilhelmsiana* showed inhibitory activity against *P. aeroginosa, S. epidermidis*, *S. aureus* and *M. luteus*, whereas it did not show any inhibitory effect against *E. coli, S. paratyphi* and *B. cereus* [12]. Dulger and Ugurlu reported that the antifungal effect of *P. olympica* Boiss. extracts is less than that of standard antifungal antibiotics [25].

4. Conclusions and discussion

The biological activity data of the *P. comosa* var. *acmodonta*, revealed for the first time, is important to highlight our assumption that the plant can be exploited as a potential antioxidant, as well as contain positive components to ameliorate the negative effects of *Candida* spp. In this study, *P. comosa* var. *acmodonta* was evaluated in terms of anticandidal and antioxidant potential. As far as we know, the absence of anticandidal and antioxidant activity studies on *P. comosa* var. *acmodonta* increases the originality of this study. According to the activity results, the substances responsible for the effect are thought to be phenolic compounds [12]. It is foreseen that this study will constitute a source for comprehensive studies to be done in the future.

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