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In Vitro Anticandidal and Antibiofilm Activities of *Capsella bursa pastoris* Root Against *Candida* Species

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Abstract

This research displays the anticandidal and antibiofilm activities of *Capsella bursa-pastoris* methanol and ethanol extracts against *Candida* species (*C. albicans, C. dupliniensis, C. parapsilosis, C. glabrata* and *C. tropicalis*). Methanol and ethanol extracts of *Capsella bursa-pastoris* were obtained by maceration method. The anticandidal activity of extracts was estimated by agar well diffusion and microdilution assays. The antibiofilm activity of extracts was determined with crystal violet (CV) assay. The root extract *of Capsella bursa pastoris* has an anticandidal activity for all *Candida* species. The flower extract of this plant has the anticandidal activity of *C. albicans*. Microdilution assays showed that plant extract was recorded as >250 mg/L. However, CV assay shows that increasing the concentration of root extracts significantly reduced biofilm formation. The minimum biofilm inhibitor concentration (MBIC) value against *C. tropicalis* was recorded as 64 mg/L. These results suggest that *Capsella bursa-pastoris* root extracts can be used as antibiofilm agents against *Candida* species.

Keywords: Capsella bursa-pastoris root, Anticandidal, Antibiofilm, Extraction, *Candida* species.

Introduction

Medicinal plants have been widely used in the treatment of many diseases throughout human history (1,2) and nowadays, plants are widely used in traditional medicine in Asia and Europe. According to the World Health Organization (WHO), 25% of the pharmaceutical products and drugs used today are obtained from medicinal plants (3). Nowadays, antimicrobial resistance development is dramatically increasing due to the misuse of antimicrobial drugs, biofilm formation, mutation, and gene transfer among bacteria and fungi (4,5). The discovery rate of new antimicrobial drugs is also quite slow (5,6). The most important fungal infection, *Candida* infection, is increasing rapidly all over the world. *Candida albicans* is the most common fungal pathogen. In addition to *C.*

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albicans, other agents such as bacteria, yeast, or fungi cause skin infections in humans and animals. *Candida* species normally live on the skin or inside the body without causing problems. Nevertheless, *Candida* overgrowth and biofilm formation cause infection (6).

Weed compounds can be anticandidal drug candidates due to the bioactive molecules they contain. Indeed, these examples were reviewed by Soliman et al, 2017 (7). Due to the lack of toxic and kinetic information about weed anticandidal products, no drugs are marketed or used in modern therapy yet (7,8). *Capsella bursa-pastoris*, one of the wild herbs, grows in Turkey and is used in traditional medicine (9). The previous report indicated that Capsella bursa-pastoris has antibacterial activity against Gram-positive and Gram-negative bacteria (10).

Notably, its anti-inflammatory, antioxidant and antiulcer activity has been demonstrated (11,12). To our best knowledge, there is no study on the anticandidal activity of *Capsella bursa-pastoris*. Therefore, in this study, we aimed to investigate the potential anticandidal effects of *Capsella bursa-pastoris* extracts against albicans and non-albicans *Candida* species.

Materials and Methods

***Candida* species and Culture Conditions:** In this study, 5 different *Candida* strains (*C. albicans* ATCC 90028, *C. dubliniensis* CBS 7987, *C. glabrata* ATCC 2001, *C. parapsilosis* ATCC 22019 and *C. tropicalis* KUEN 1025) were used. All strains were grown on potato dextrose agar (PDA, Oxoid) medium at 30℃ for 48 hours. Liquid cultures for microdilution and antibiofilm activity were performed in potato dextrose broth (PDB, Oxoid) medium at 30 ℃ and 150 rpm. All experiments were performed in at least two replicates.

Preparation of Ethanolic and Methanolic Extracts of *Capsella bursa pastoris:* *Capsella bursa pastoris* was obtained in the local market. About 10 g of the plant (flower, leaf and root, separately) were extracted with 200 mL of methanol and ethanol using the maceration technique (13) for 72 hours at 4℃. After filtration, extracts were evaporated in the device (Rotary Evaporator, Scilogex SCI100). All remaining materials were dissolved in dimethyl sulphoxide (DMSO, Isolab). All extraction was done separately to the flower, leaf and root parts of the plant.

Agar Diffusion Assay: To screen anticandidal compounds of *Capsella bursa pastoris* extract, agar well diffusion assay was performed (14). All *Candida* species, were inoculated in PDB medium at 30℃ and 150 rpm. After incubation final inoculum was adjusted to 0,12-0,15 optical density at 600 nm. PDA plates were lawn cultured and holes were bored with a cork borer (6 mm). Each well was filled with 200 µL of plant extract (1 mg/mL). DMSO was used as a negative control. All plates were incubated at 30℃, statically. After 24 hours of incubation periods, clear zones around the well were measured.

Microdilution Assay: Microdilution test was performed by the extracts with positive results in agar diffusion according to EUCAST protocol with some modification (15). Briefly, overnight yeast culture was prepared in PDB medium at 30 ℃ and 150 rpm. After incubation final inoculum was adjusted to 0,08-0,1 optical density at 600 nm. In a 96-well plate, 100 µL of the extract with increasing concentration (0.5-128 mg/L) was added and 100 µL of yeast culture was added. The final volume was adjusted to 200 µL. PDB medium was used as a negative control. Eventually, the plates were incubated statically at 30°C for 24 hours. At the end of the period, the concentration with no yeast growth was determined as MIC value.

Antibiofilm Activity: For the antibiofilm assay, CV assay was used with some modifications (16). Firstly, yeast culture was started in a similar way to the microdilution test as described above in the 96-well plate. After 48 hours of incubation, all free cell contents were removed and the wells were washed with sterile water. Then, 0,1% crystal violet (CV) dye was added in

each well for 20 min. After that, CV dye was removed. All wells were washed with tap water. Finally, 30% acetic acid was added to each well, and absorbance was measured at 590 nm with a spectrophotometer (Thermo, Multiscan).

Results

In the current study, three different parts (flower, leaf and root) of *Capsella bursa pastoris* were extracted separately. The anticandidal activity of the extracts against *Candida* species was determined by agar well diffusion and microdilution assays. In the agar diffusion test performed for flower extracts, it was observed that a zone was observed in the ethanol flower against *C. albicans* (15 mm). In the leaf extract, no results were obtained. On the other hand, for *Capsella bursa pastoris* root extract, zone formation was observed against all *Candida* species (15-18 mm) Figure 1 shows images of selected Petri dishes. Ultimately, we may say that the root extract of *Capsella bursa pastoris* has anticandidal properties against albicans and non-albicans species.

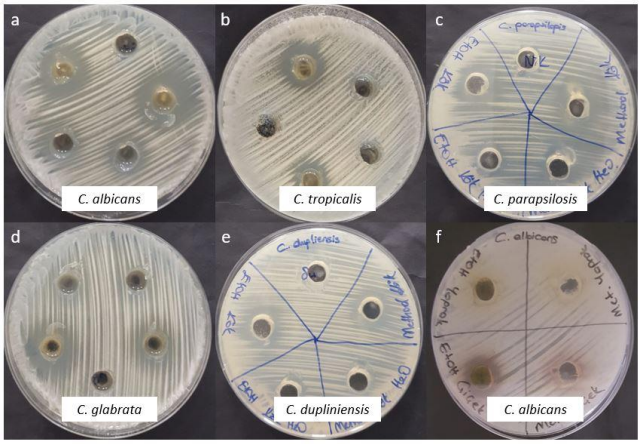

Figure 1: Shows images of selected Petri dishes

The microdilution test was performed to determine the minimum inhibitory concentration (MIC). Growth was found to decrease with increasing concentrations within the concentrations we studied. However, MIC values were not observed and recorded as >250 mg/L. Although the MIC value could not be determined, increasing the concentration of root extracts significantly reduced biofilm formation. Strikingly, the root of *Capsella bursa pastoris* showed antibiofilm activity against *C. tropicalis* and inhibited biofilm formation by 64 µg/mL. The root extract also significantly inhibited *C. albicans* biofilm formation at a value of 256 µg/mL. Therefore, these values were recorded as MBIC values. All in all, Figure 2 shows the CV assay results. Similar results were not obtained in the leaves and flowers of the *Capsella bursa pastoris* weed.

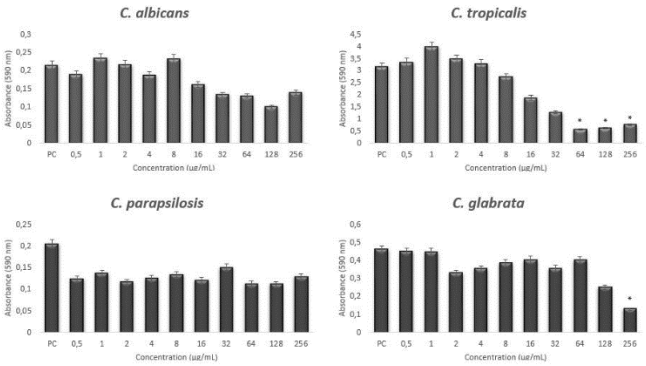

Figure 2: Shows the CV assay results

Discussion

Medicinal plants are used to treat many diseases such as infections and cancer. In particular, herbal treatments for infectious diseases have been applied since ancient times (1-3). In addition, medicinal plants are also the natural material of many drugs.

Capsella bursa-pastoris is a widespread weed belonging to the family Brassicaceae. It has adapted to cold environments and has a short life span. It grows in many regions of Turkey (8). This weed is used in solving reproductive and menstrual problems among people. Taken together, many studies have verified that *Capsella bursa-pastoris* has antioxidant, anti-inflammatory, anticancer and antibacterial effects. *Capsella bursa-pastoris* is used as a wound-healing agent in Korean medicine (11).

Tatçi (1999) reported that extracts of *Capsella bursa-pastoris* prepared with chloroform and hexane showed

antibacterial activity against *Staphylococcus aureus* ATCC 28212 strain (22). In another study, it was reported that water, methanol and ethanol extract of *Capsella bursa-pastoris* had antibacterial activity on *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* (24). On the other hand in a different study reported that ethanolic extract of *Capsella bursa-pastoris* did not show any antibacterial activity against Pseudomonas aeruginosa isolates (25).

Candidal infections have been among the main causes of infections in recent years due to the use of broad-spectrum or combined antibiotics and suppression of the immune system by chemotherapeutic drugs. These infections can be endogenous or exogenous sources of transmission. *Candida* species rank fourth among bloodstream infections occurring in hospitals and intensive care units. They are responsible for 80% of fungal infections occurring in hospitals. The common species encountered in this 80% part is *C. albicans*. Along with the infections that have started to increase in recent years, there is a significant increase in infections caused by non-albicans *Candida* species (17,18).

More than 80% of infections are caused by biofilms. Candida species have the ability to form biofilms, indeed, it is clear that inhibiting *Candida* biofilms is also very important. These biofilms are composed of yeast and hyphae cells and an extracellular matrix. These can colonize mucosal surfaces such as oral and vaginal surfaces (19).

To our knowledge, there is no study on the effect of *Capsella bursa-pastoris* on *Candida* species and Candidal biofilms. We tested this weed's different parts (flower, leaf, root) separately on five different *Candida* species. According to our results, *Capsella bursa-pastoris* showed anticandidal activity against both albicans and non-albicans species. Soleimanpour et al. in their study in 2013, showed the effectiveness of *C. bursa-pastoris* extract against oral pathogens such as *Streptococcus mutans, Actinomyces viscosus, Streptococcus sanguis, and Enterococcus faecalis*. (20). In this study, MIC value was taken for ethanolic extracts. Similarly, in our study, we observed inhibition against *Candida* in ethanolic extracts by agar diffusion test.

In general, the flower and leaf parts of weeds are examined in order to determine bioactive molecules (7). We also included the root part to investigate anticandidal activity in our study. Eventually, we observed anticandidal and antibiofilm activity in root segments in the agar diffusion and CV assay. Taken together, concentration-dependent antibiofilm activity was also observed.

Based on our results, it was verified that *Capsella bursa-pastoris* root extract contains potential compounds against candidal infections. A study supporting this situation was published in 2000 by Park et al (21). Two antimicrobial peptides, shepherin I and shepherin II, were isolated from *Capsella bursa-pastoris* roots. These peptides show antibacterial and antifungal activity against *C. albicans*, *Cryptococcus neoformans*, *Aspergillus flavus* and *Fusarium culmorum*. As this previous study showed, we may have detected anticandidal activity originating from these antimicrobial peptides in the roots of *Capsella bursa-pastoris* collected in Turkey. Another suggestion might be that *Capsella bursa-pastoris* produces chemical compounds with different anticandidal properties. The fact that the leaves and root of *Capsella bursa-pastoris* contain neutral lipids (fatty acids), glucose and phospholipids also support this situation (23). In another study, it was reported that the root parts of this plant are rich in palmitic acid (26). Interestingly, the study published by Prasath et al in 2020 revealed that palmitic acid inhibited the *C. tropicalis* biofilm (27). Collectively, these studies support the anticandidal and antibiofilm activity of *Capsella bursa-pastoris*'s root and inhibition of *C. tropicalis* biofilm.

As a result, this study showed the first time *Capsella*

bursa-pastoris root was shown anticandidal and antibiofilm activity. However, the compounds that cause this activity should be characterized in future studies.

Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

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