

Investigation of Antifungal and Antioxidant Properties of *Capparis ovata* Methanolic Extracts

Capparis ovata Metanolik Ekstraktının Antifungal ve Antioksidan Özelliklerinin Araştırılması

 Oktay ÖZKAN¹,  Dinçer ERDAĞ²,  Gözde ATİLA³,  Kemal METİNER⁴,  Baran ÇELİK⁵,  Asım KART^{6*},  Hamit USLU⁷,  Fatma Esin KIRIK⁸

¹ Niğde Ömer Halisdemir University, Faculty of Medicine, Department of Internal Medicine, Niğde

² Kafkas University, Atatürk Health Vocational Higher School, Health Care Services Department, Kars

³ Kafkas University, Faculty of Veterinary Medicine, Department of Basic Sciences, Kars

⁴ Istanbul University, Faculty of Veterinary Medicine, Department of Preclinical Sciences, Istanbul

⁵ Istanbul University, Faculty of Veterinary Medicine, Department of Preclinical Sciences, Istanbul

^{6*} Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Burdur

⁷ Kafkas University, Atatürk Health Vocational Higher School, Health Care Services Department, Kars

⁸ Niğde Ömer Halisdemir University, Faculty of Medicine, Department of Basic Medical Sciences, Niğde

Abstract: Caper plant belonging to the genus *Capparis* (family *Capparaceae*) is a plant used in traditional medicine to cure various illnesses since ancient times. Studies have shown significant medicinal properties of various *Capparis* species. This study was designed to examine *in vitro* antifungal and antioxidant activity of methanolic extracts of *Capparis ovata* buds. It was determined that *C. ovata* inhibited the NO radical in a dose-dependent manner and exhibited reducing power activity. According to the standard pyrocatechol graph, 1 mg of *C. ovata* contains 19.64 µg of phenolic equivalent of pyrocatechol. *In vitro* antifungal susceptibility testing of *C. ovata* methanolic extract against eight different fungal strains was determined by broth macrodilution method. According to MIC values of *in vitro* antifungal activity testing *C. ovata* has moderate antifungal activity. In conclusion, *C. ovata* was found to have a significant antifungal and antioxidant potential. Hence, this plant could have the potential to be used against fungal and oxidative stress related many disease conditions.

Keywords: *Capparis ovata*, Antifungal, Antioxidant, *In vitro*

Öz: *Capparis* genusuna (*Capparaceae* familyası) bağlı kapari bitkisi eski çağlardan beri geleneksel tıpta çeşitli hastalıkların tedavisinde kullanılmaktadır. Çeşitli *Capparis* türlerinin önemli tıbbi etkilerini ortaya çıkaran çalışmalar bulunmaktadır. Bu çalışmada *C. ovata* tomurcukları metanol ekstraktının *in vitro* antioksidan ve antifungal aktivitesi çalışılmıştır. *Capparis ovata*'nın doza bağımlı olarak NO radikallerini inhibe ettiği ve indirgeyici güç aktivitesi gösterdiği tespit edilmiştir. Standart pirokatekol çizelgesine göre 1 mg *C. ovata* 19,64 µg pirokatekole eş fenolik bileşik içermektedir. *Capparis ovata* metanol ekstraktı broth makrodilüsyon metodu ile sekiz farklı fungus suşuna karşı antifungal etkisi test edilmiştir. *In vitro* antifungal aktivite testi MIC verilerine göre *C. ovata* ılımlı antifungal etkiye sahiptir. Sonuç olarak *C. ovata* önemli derecede antifungal ve antioksidan potansiyele sahip bulunmuştur. Bu nedenle, bu bitki, mantar ve oksidatif stresle ilişkili birçok hastalık durumlarına karşı kullanıma potansiyeline sahip olabilir.

Anahtar Kelimeler: *Capparis ovata*, Antifungal, Antioksidant, *In vitro*

*Corresponding author : Asım KART

e-mail : akart@mehmetakif.edu.tr

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Introduction

The caper plant is locally called with different names as bubu, gebere, gabar, kapari, keper and

kebere in Turkey. It is generally consumed as pickles due to economical and nutritional

properties (Duman and Ozcan, 2014; Belviranlı *et al.*, 2016). This plant belongs to the family of Capparaceae and to the genus *Capparis* with 250 and 400 species. They are grown generally in tropical and subtropical regions but some also are in temperate regions in the Mediterranean including Turkey (Lansky *et al.*, 2013). *Capparis ovata* have wide natural distribution, and it is cultivated in large parts of Turkey (Arslan *et al.*, 2010; Haciseferogulları *et al.*, 2011).

Different parts of capper plant have been used since ancient times for cosmetic, nutritional and medicinal purposes. In traditional medicine *Capparis* species were used to treat some disorders including rheumatic diseases, stomach problems, headache and toothache (Lansky *et al.*, 2013; Tlili *et al.*, 2011). Caper plant has different pharmacological activities and its various parts are used for pharmaceutical purposes (Arslan *et al.*, 2010; Kondawar *et al.*, 2011). The Capparaceae family generally contains glucosinolates, alkaloids and flavonoids, but the phytochemical composition of the contents obtained from different plant parts varies (Arslan *et al.*, 2010; Tlili *et al.*, 2011). A number of studies have shown that *capparis* species have significant immunostimulant, antitumoral, antidiabetic, antisclerosis, antimicrobial, antioxidative, antiinflammatory, immunomodulatory and antiviral activities. However, additional studies are needed to validate the use of *Capparis* species in medical treatment (Tlili *et al.*, 2011; Zia-Ul-Hag *et al.*, 2011; Mishra *et al.*, 2007).

Studies indicate that the incidence and prevalence of some mycoses remains to be a public health challenge. Increased use of antifungal drugs has led to the resistance problems in today's world. In addition, due to increased number of multidrug-resistant fungal strains and limited number of antifungal agents, it is necessary to discover new antifungals from natural products (Aqil *et al.*, 2010).

Some medicinal plants possess potentially great antioxidant activity. Antioxidants are capable of preventing or protecting from the oxidative stress

in cells. Hence, they have beneficial effects in the treatment of many diseases. Medicinal plants are a good source of natural antioxidants, which are alternative ways to use of synthetic antioxidants. They are also very good candidate for side effect free alternatives in comparison to synthetic antioxidants to be used in food industry and in preventive medicine (Ali *et al.*, 2008; Krishnaiah *et al.*, 2011).

Therefore, it is aimed to investigate the antioxidant and antifungal activities of *C. ovata* species in this study.

Materials and Methods

Preparation of Plant Material and Extracts

The buds of *C. ovata* were obtained from a local herbal shop in Kayseri Province (Gül Gıda ve Tarım Ürünleri) Turkey. Methanolic extracts of the buds were prepared according to the method described by Özkan *et al.* (2013) were stored at -25 °C until used in the experiments.

Antifungal Activity Testing

Test Microorganisms:

The antifungal activities of *C. ovata* methanolic extracts were evaluated against different types of standard fungal strains including yeasts and filamentous fungi. *Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC22019), *Candida krusei* (ATCC 6258), *Malassezia pachydermatis* yeast strains and *Microsporium canis*, *Microsporium gypseum*, *Microsporium nanum* and *Trichophyton mentagrophytes* filamentous fungi strains were used as test microorganisms and provided from the culture collection of Istanbul University Faculty of Veterinary Medicine Department of Microbiology.

In Vitro Antifungal Susceptibility Testing:

Reference methods were used according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) for *in vitro* antifungal

susceptibility testing. Broth macrodilution assay was performed for determination of minimum inhibitory concentration (MIC) values of the methanolic extract of *C. ovata* buds using the guidelines CLSI M27-A3 for yeasts and CLSI M38-A2 for filamentous fungi (CLSI, 2008a; CLSI, 2008b). Broth macrodilution assay was also performed for Amphotericin B as a reference antifungal agent.

Antioxidant Activity Testing

NO Radical Scavenging Activity

Nitric oxide (NO) radical scavenging activity of *C. ovata* extract was measured according to the methods of Badami *et al.* (2003) and Kumar *et al.* (2005) with slight modification. The reaction is based on production of NO by sodium nitroprusside at physiological pH. Nitric oxide

interacts with oxygen to generate nitrite. Nitrite ions form a colored product by Greiss reaction, which is read at 548 nm to determine the NO level (Green *et al.*, 1982).

Reducing Power Capacity

The reducing power of *C. ovata* extract was measured by the method of Oyaizu (1986).

Determination of Total Phenolic Compounds

Total soluble phenolic content of *C. ovata* extract was measured using Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977).

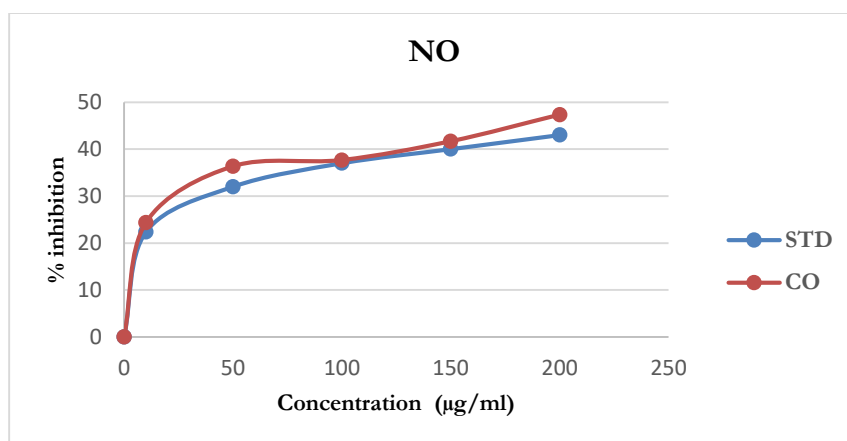


Figure 1. Inhibition of nitric oxide (NO) radicals by *Capparis ovata* and Rutin standard.

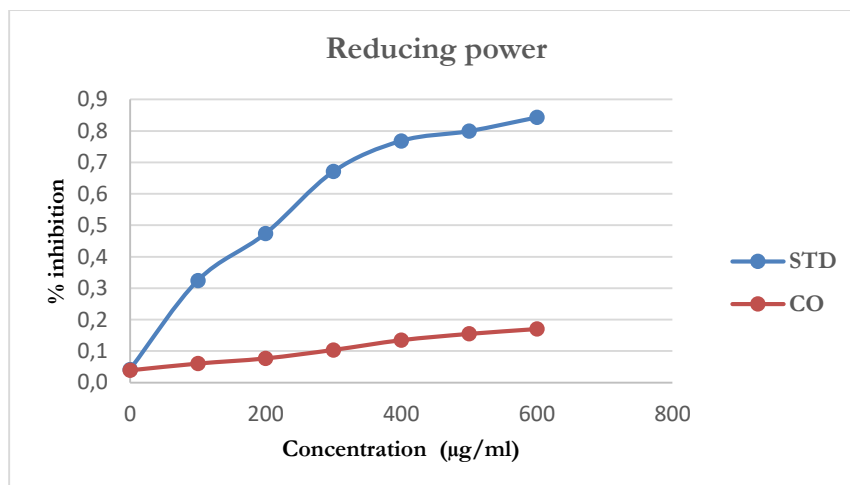


Figure 2. Reducing power of *Capparis ovata* and butylated hydroxyl toluene (BHT).

Results

The *C. ovata* methanolic extract at different amounts was compared with Rutin standard for NO radical scavenging activity (Figure 1).

Figure 2 illustrates the reducing power capacity of *C. ovata* extract in comparison to butylated hydroxyl toluene (BHT). The reducing power of *C. ovata* methanol extract increased with the increasing amounts of sample.

The absorbance value of 1 mg of *C. ovata* extract was determined to be 0.031 at 760 nm. It was

found that 1 mg of *C. ovata* contains 19.64 µg of phenolic equivalent of pyrocatechol.

The results of broth macrodilution assay for antifungal susceptibility testing of *C. ovata* methanolic extract and the reference antifungal drug amphotericin B against fungal test strains are shown in Table 1. All of the tested fungal strains were sensitive to *C. ovata* methanolic extract. *Candida krusei* ATCC22019, *Candida parapsilosis* ATCC 6258 and *Microsporum nanum* strains were more sensitive to *C. ovata*. However, MIC values of *C. ovata* extracts against tested fungal strains were significantly higher than amphotericin B.

Table 1: *In vitro* antifungal susceptibility of *Capparis ovata* methanolic extract and amphotericin B against fungal test strains.

Fungal Test Strains	MIC values (mg/ml)	
	<i>Capparis ovata</i> methanolic extract (mg/ml)	Amphotericin B (mg/ml)
<i>Candida albicans</i> ATCC 90028	200	0.00024
<i>Candida krusei</i> ATCC22019	100	0.00195
<i>Candida parapsilosis</i> ATCC 6258	100	0.00195
<i>Malassezia pachydermatis</i>	200	0.00024
<i>Microsporum canis</i>	200	0.00024
<i>Microsporum gypseum</i>	200	0.00024
<i>Microsporum nanum</i>	100	0.00195
<i>Trichophyton mentagrophytes</i>	200	0.00024

Discussion

Previous studies indicate that the antioxidant activity of the *C. ovata* and other capparid species have high anti-oxidant activity (El-Ghorab *et al.*, 2007; Naziroğlu *et al.*, 2011; Unver *et al.*, 2009; Duman *et al.*, 2013; Naziroğlu *et al.*, 2013). In this study, the results of radical scavenging activity of the *C. ovata* extract and the reducing power and phenolic compound content indicates that this plant is a potential antioxidant. Our results are similar to previous works studying other Capparid species. Zia-Ul-Haq *et al.* (2011) reported that *C. decidua* extracts have prominently high levels of phenolic compounds and showed potent antioxidant activity and reduced different types of radicals. El-Ghorab *et al.* (2007) showed that the methanolic extract of *Capparis spinosa* has a strong antioxidant activity. The ethyl acetate and aqueous extract of *C. spinosa* was reported to have DPPH scavenging activities (Yang *et al.*, 2010). Due to these properties, *C. ovata* has the potential to be used as a natural antioxidant source in both pharmaceutical and food industry. Several studies indicate that many *Capparis* species have the antifungal activities against several fungal pathogens (Naziroğlu *et al.*, 2011; Aslam *et al.* 2010; Sharma *et al.*, 2009; Keymanesh *et al.*, 2009; Malabadi *et al.*, 2007; Buwa *et al.*, 2006; Anywar *et al.*, 2014; Rathee *et al.*, 2013). However, there is lack of studies about antifungal activity of the species *C. ovata*. There are the reports that other Capparid species have antifungal activity. *Capparis spinosa* was assayed for antifungal activity toward the phytopathogenic fungi *Valsa valis* by Lam and Tzi-Bun Ng (2009). According to these results of research *C. spinosa* inhibited the reproduction of this fungus. Aslam *et al.* (2010) have tested the *Capparis decidua* against different fungus strains. In their study, they found that this herb is effective against to *Rhizoctonia solani*. According to *in vitro* antifungal susceptibility testing results of this study, *C. ovata* has moderate antifungal activity. In conclusion, this study indicates that *C. ovata* has an antioxidant and antifungal effect and is worth

considering in therapeutic formulations to cure several diseases. However further studies are recommended to verify the role of this plant in herbal medicine for discovering new natural bioactive pharmaceuticals.

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