KSÜ Tarım ve Doğa Derg 25 (1): 83-87, 2022
KSU J. Agric Nat 25 (1): 83-87, 2022
https://doi.org/10.18016/ksutarimdoga.vi.880151

Biological Activities of Wild Poisonous Mushroom *Entoloma sinuatum* (Bull.) P. Kumm (Boletales)

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ABSTRACT

In many studies conducted in recent years, it has been found that the beneficial natural ingredients found in mushrooms are very important in curing various diseases and eliminating disease factors. It has been reported that fungi that spread in different countries have biological activities. In this study, antioxidant level (TAS), oxidant level (TOS), and antibacterial and antifungalactivity of *Entoloma sinuatum* (Bull.) P. Kumm were determined. Ethanol extract of the mushroom was extracted in a Soxhlet apparatus. Antioxidant and oxidant levels were determined using Rel Assay TAS and TOS kits. Also, antimicrobial activity was tested against bacterial and fungal strains using the agar dilution method. As a result of the studies, the TAS value of the mushroom was determined 2.64±0.15, the TOS value was 6.58±0.23, and the OSI (Oxidative stress index) value was 0.25±0.02. In addition, it was determined that the ethanol extract of the mushroom was effective against bacteria at 200 and 400 µg/mL, and against fungi at 50 µg/mL concentrations. As a result, it was determined that *E. sinuatum* is a natural antioxidant and antimicrobial agent.

Research Article

Article History
Received : 14.02.2021
Accepted : 06.05.2021

Keywords
Antibacterial
Antifungal
Antioxidant
Entoloma sinuatum,
Medicinal mushroom

Yabani Zehirli Mantar *Entoloma sinuatum* (Bull.) P. Kumm 'un (Boletales) Biyolojik Aktiviteleri

ÖZET

Son yıllarda yapılan birçok çalışmada mantarlarda bulunan faydalı doğal bileşenlerin çeşitli hastalıkları iyileştirmede ve hastalık etkenini ortadan kaldırmada çok önemli olduğu bulunmuştur. Farklı ülkelerde yayılış gösteren mantarların biyolojik aktivitelerinin olduğu bildirilmiştir. Bu çalışmada Türkiye'de Entoloma sinuatum (Bull.) P. Kumm'un antioksidan seviyesi, oksidan seviyesi ve antimikrobiyal aktivitesi belirlendi. Mantarın etanol özütü soxhlet cihazında çıkarıldı. Antioksidan ve oksidan seviyeleri Rel Assay TAS ve TOS kitleri kullanılarak belirlendi. Ayrıca antibakteriyel ve antifungal aktivite agar dilüsyon metodu kullanılarak bakteri ve fungus suşlarına karşı test edildi. Yapılan çalışmalar sonucunda mantarın TAS değeri 2.64±0.15, TOS değeri 6.58±0.23 ve OSI (Oksidatif stres indeksi) değeri 0.25±0.02 olarak belirlendi. Ayrıca mantarın etanol özütünün bakterilere karşı 200 ve 400 µg/mL, funguslara karşı 50 µg/mL konsantrasyonlarda etkili olduğu belirlendi. Sonuç olarak E. sinuatum'un doğal antioksidan ve antimikrobiyal ajan olduğu belirlendi.

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 14.02.2021
Kabul Tarihi : 06.05.2021

Anahtar Kelimeler
Antibakteriyel
Antifungal
Antioksidan
Entoloma sinuatum
Tıbbi mantarlar

To Cite : Bal C, Baba H, Akata I, Sevindik M, Selamoğlu Z, Akgül H 2022. Biological Activities of Wild Poisonous Mushroom *Entoloma sinuatum* (Boletales). KSU J. Agric Nat 25 (1): 83-87. https://doi.org/10.18016/ksutarimdoga.vi.880151.

INTRODUCTION

In terms of nutritional values of mushrooms, it has been utilized in the treatment of cancer, cholesterol-lowering, stress, insomnia, asthma, allergies, and diabetes, and it has been included in studies that it is effective. Besides, mushrooms contain various

secondary metabolites, including phenolic compounds, polyketides, terpenes, and steroids that act as antioxidants (Ahmed et al., 2015; İnci and Kırbağ, 2018; Krupodorova and Sevindik, 2020). In studies on natural mushrooms in different parts of the world, it has been reported that mushrooms have different biological activities such as antioxidant, antimicrobial, anticancer, antitumor, antiproliferative, anti-inflamatuvar, DNA protective, anti-aging, and antiallergic (Bae et al., 2007; Bal et al., 2017; Garcia-Lafuentea, et al., 2010; Osman and Toliba, 2019; Sevindik et al., 2017; Zhang et al., 2019; Umaña et al., 2020). In this context, the discovery of new natural products has been inevitable, especially in recent years, due to the interest of people in natural products and the possible side effects of synthetic drugs. In this study, wild poisonous mushroom *Entoloma sinuatum* (Bull.) P. Kumm was used as a material.

E. sinuatum (livid entoloma, livid agaric, livid pinkgill, leaden entoloma, and lead poisoner) is a poisonous fungus that has been detected in many regions of the world. Fruiting bodies of the mushroom appear in late summer and autumn (Zeitlmayr, 1976). It is seen in deciduous woodlands covered with clay and calcareous soils, usually Oak and Beech, rarely in birch or in nearby park areas. Sometimes it meets in the form of a fairy ring. It can be seen alone or in groups and most members of the genus are saprotrophic. In young form, it can be mixed with the edible *Calocybe gambosa* or *Clitopilus prunulus*. Also, an ordinary observer might confuse *E. sinuatum* with the meadow mushroom *Agaricus campestris* (Lamaison and Polese, 2005). For this reason, it has played a leading role in many mushroom poisoning cases in Europe. *E. sinuatum* poisoning is generally not fatal. Following consumption of mushrooms, the gastrointestinal syndrome is observed. Depression and delirium are rare sequelae (Bastida et al., 1987). In this study, the antioxidant, oxidant, and antimicrobial activities of wild poisonous mushroom *E. sinuatum* were determined.

MATERIALS and METHODS

Sample extraction

Mushroom samples were collected from the Belgrad forest (41°10'56.63"N- 28°59'5.91"E, 145m) (Istanbul/Turkey). After the samples were collected, they were dried in a dryer (Profilo, PFD2350W) for about 12 hours at 45 °C and 10 g of each mushroom sample was taken and ground into powder (WANXINGBO, Grinder). Powder samples were extracted with 200 mL of ethanol for 6 h in the Soxhlet apparatus then obtained by filtration through filter paper (ISOLAB, 125mm) and the solvent was removed on a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator) at 40 °C. Crude extracts were stored at + 4 ° C until further conducting experiments.

Antibacterial activities

The antimicrobial activity tests of ethanol extract of the mushroom sample were determined by the agar dilution method. The lowest concentration of the extract preventing the growth of microorganisms was determined as the MIC (Minimum Inhibitor Concentration) value. Extract concentrations were set at 6.25 to800 µg/mL in distilled water (CLSI, 2002; CLSI, 2003). *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as test bacteria. Bacteria were pre-cultured in Muller Hinton Broth medium. Amikacin, Ampicillin, and Ciprofloxacin were used as positive controls (Hindler et al., 1992).

Antifungal activities

Candida albicans (ATCC 10231) and *C. tropicalis* (ATCC 13803) were used as test fungi. Fungi were pre-cultured in RPMI 1640 Broth medium. Fluconazole and Amphotericin B were used as positive controls (Hindler et al., 1992).

Antioxidant activities

The total antioxidant status, total oxidant status, and oxidative stress index of ethanol extracts of mushrooms were evaluated using Rel Assay TAS and TOS kits. Trolox was utilized as a calibrator in antioxidant studies. Hydrogen peroxide was used as a calibrator in oxidant studies (Erel, 2004; Erel, 2005). The OSI (Oxidative stress index) value is determined by proportioning the TAS value to the TOS value. The following formula was used for the OSI value (Sevindik, 2018).

RESULTS and DISCUSSION

Antibacterial and Antifungal activities

Antibacterial and antifungal drugs used today are inadequate due to the antibiotic resistance that microorganisms have gained in recent years. Moreover, people turned to natural products due to the possible side effects of antibacterial and antifungal drugs (Liu et al., 2017; Abdalla et al., 2020). This trend has made the discovery of new antibacterial and antifungal natural products. In this study, the antibacterial and antifungal activities of the ethanol extract of *E. sinuatum* was determined. The findings obtained were shown in Table1.

In previous studies on *Entoloma* species, the methanol extract of *Entoloma speculum* was reported to be effective against *Xanthomonas campestris, Pseudomonas syringae, Agrobacterium tumefaciens, Klebsiella pneumonia, Escherihia coli, Salmonella typhi, P. aeruginosa, Staphylococcus aureus, Streptomyces pneumoneae, Candida albicans,*

Chrysosporium merdarium, Trichophyton rubrum, Chrysosporium keratinophilum, Fusarium solani, Penicillium chrysogenum, Aspergillus flavus and *A. niger* in different concentrations (Kodiyalmath and Krishnappa, 2018). In another study, ethanol extract of *Entoloma nubigenum* was reported to be effective against *Bacillus subtilis, Escherichia faecalis, Staphylococcus aureus, Penicillium notatum,* and *Ceratocistys pilifera* (Reinoso et al., 2013). In our study, the antibacterial and antifungal activities of ethanol extract of *E. sinuatum* was determined. As a result of this, it has been determined to be effective against *S. aureus* and *P. aeruginosa* in 400 µg/mL concentration, *E. faecalis* and *E. coli* at 200 µg/mL concentration, *C. albicans* and *C. tropicalis* at 50 µg/mL concentration. In other words, the antifungal activity of the mushroom extract appears to be higher. It was also determined that the fungus can be used as a natural antibacterial and antifungal source.

Table 1. Antibacterial and antifungal activities of ethanol extract of *E. sinuatum*
Çizelge 1. E. sinuatum'un etanol ekstresinin antibakteriyel ve antifungal aktiviteleri

	Antibacterial (µg/mL)				Antifungal (µg/mL)	
Sample	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*C. tropicalis*
Entoloma sinuatum	400	200	200	400	50	50
Flukonazole	-	-	-	-	1.56	3.12
Ampicillin	3.12	1.56	3.12	-	-	-
Ciprofloxacin	0.78	0.78	1.56	3.12	-	-

Antioxidant activities

Antioxidant compounds are found in many natural products including mushrooms that spread in many different ecosystems. Mushrooms are very vital sources of antioxidants with their numerous antioxidant-effective enzymes and phenolic compounds (Lu et al., 2018; İnci et al., 2019).

Table 2. TAS, TOS and OSI Values of ethanol extract of *E. sinuatum*
Çizelge 2. E. sinuatum'un etanol ekstresinin TAS, TOS ve OSI Değerleri

Sample	TAS (mmol/L)	TOS (µmol/L)	OSI
E. sinuatum	2.64±0.15	6.58±0.23	0.25±0.02

Values are presented as mean±S.D

Many studies have shown that mushrooms have high antioxidant activities. The total antioxidant and total oxidant status of used in our study were determined for the first time. In previous TAS and TOS studies on wild mushrooms, TAS value of *Tricholoma virgatum* was reported as 3.754, TOS value was 8.362 and OSI value was 0.223 (Selamoğlu et al., 2020). The TAS value of *Cerioporus varius* was reported as 2.312, TOS value was 14.358 and OSI value was 0.627 (Sevindik, 2019). TAS value of *Suillus granulatus* was reported as 3.143, TOS value was 18.933 and OSI value was 0.603 (Mushtaq et al., 2020). The TAS value of *Lactifluus rugatus* was reported as 3.237, the TOS value was 8.178 and the OSI value was 0.254 (Sevindik, 2020). The TAS value of *Infundibulicybe geotropa* was reported as 1.854, TOS value was 30.385 and OSI value was 1.639 (Sevindik et al., 2020). The TAS value of *Lepista nuda* was reported as 3.102, the TOS value was 36.920, and the OSI value was 1.190 (Bal et al., 2019). Compared to these studies, the TAS value of *E. sinuatum* was found to be higher than *I. geotropa* and *C. varius*, lower than *L. rugatus, L. nuda, S. granulatus* and *T. virgatum*. TAS value shows the whole of the antioxidant compounds produced within the mushroom (Krupodorova and Sevindik, 2020). The difference in TAS values determined in studies on different mushrooms draws attention. The difference in TAS value between species is due to the difference in the potential of fungi to produce compounds with antioxidant properties.

When TOS values were examined, *E. sinuatum* was determined to be lower than *C. varius, L. rugatus, I. geotropa, L. nuda* and *T. virgatum*. The TOS value indicates the whole of the oxidant compounds produced by the fungus as a result of environmental effects metabolic activities (Krupodorova and Sevindik, 2020). TOS value of *E. sinuatum* used in our study was generally found to be low. In addition, the OSI value shows how much the oxidant compounds produced in the mushroom's body are suppressed by the endogenous antioxidants (Krupodorova and Sevindik, 2020). *E. sinuatum* suppressed better than *I. geotropa, L. rugatus, S. granulatus, L. nuda,* and *C. varius*, less than *T. virgatum*. Determining the oxidative stress index is important in determining the antioxidant potential of the mushroom. The OSI value shows the success of suppressing the oxidant compounds produced by the fungus by the antioxidant defence system. In this context, it is seen that the antioxidant defence system of *E. sinuatum* is successful in suppressing oxidant compounds. As a result, it was determined that *E. sinuatum* has antioxidant potential.

CONCLUSION

In this study, the antioxidant, oxidant, and antimicrobial potential of wild poisonous mushroom *E. sinuatum* was determined. As a result, it was found that the antioxidant potential of the mushroom was at

normal levels. It was also found that the oxidant level was low, yet, fungi were more effective against fungi. Besides, it was determined that *E. sinuatum* could be a natural antioxidant and antimicrobial source.

ACKNOWLEDGEMENTS

We thanks to Osmaniye Korkut Ata University, Central Research Laboratory for their support.

Researchers Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

The article authors declare that they do not have any conflict of interest.

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