



Antioxidant and Antimicrobial Activities of Ethanol Extract of *Lepidium spinosum*

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ABSTRACT

In this study, the antioxidant and antimicrobial potentials of the ethanol extract of the aerial parts of *Lepidium spinosum* Ard., which is distributed in many regions of the world, were determined. The aerial parts of the plant were dried, powdered and extracted with ethanol. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the plant extract were determined using Rel Assay Diagnostics kits. Antimicrobial activities of the plant extract were determined against standard bacterial and fungal strains by agar dilution method. As a result of the studies, the TAS value of the plant extract was determined as 4.550 ± 0.132 , the TOS value as 12.610 ± 0.221 , and the OSI value as 0.277 ± 0.007 . In addition, it has been found to be effective at 25-200 $\mu\text{g/mL}$ against standard bacterial strains and 100-200 $\mu\text{g/mL}$ concentrations against fungal strains. As a result, it was determined that *L. spinosum* used in our study could be a natural antioxidant and antimicrobial source. In this context, it is thought that natural material may be used in pharmacological designs.

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Introduction

Plants contain many biologically active natural materials. Although these bioactive compounds are not nutritional, they have important medicinal properties (Diab et al., 2021; Ivanišová et al., 2021). Plants are used for many different purposes in different communities. Plants are important natural materials used in complementary medicine. Many studies by different researchers have reported that plants have many important biological activities such as antioxidant, antimicrobial, anti-aging, anticancer, antitumor, DNA protective, anti-allergic (Mani et al., 2018; Abd Rani et al., 2019; Nisa et al., 2019; Zazharskyi et al., 2019; Nguyen et al., 2020; Cocelli et al., 2021; Dias et al., 2021; Vaou et al., 2021; Akgül et al., 2022). In this context, revealing the biological activities of plants with new studies will reveal the importance of their

use in complementary medicine and modern medicine. In our study, *Lepidium spinosum* Ard. was used as the material. The genus *Lepidium* is in the Brassicaceae family and includes 175 species worldwide. *Lepidium spinosum* is popularly known as Muşurat. The aerial parts of the plant are consumed fresh, salad and cooked (Özdemir and Kültür, 2017). In this context, in this study, it was aimed to determine the antioxidant and antimicrobial potentials of the ethanol extract of the aerial parts of the plant.

Materials and Methods

Lepidium spinosum specimens were collected from Gaziantep (Turkey). The identification of the plant was made using Flora of Turkey volume 1 (Davis, 1965). The

aerial parts of the plant samples were dried in a shaded and ventilated environment. Then, 30 g of dry samples were weighed. It was then extracted using ethanol for about 6 hours in a soxhlet apparatus at 50°C (Gerhardt EV 14). The solvents of the solvent-containing extracts were concentrated with a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

Antimicrobial Activity Tests

The agar dilution method was used to determine the effects of the ethanol extract of the plant against bacteria and fungi (Hindler et al., 1992; EUCAST, 2014; Matuschek et al., 2014; EUCAST, 2015). Bacteria used as standard strains were pre-cultured in Muller Hinton Broth medium, and fungal strains were pre-cultured in RPMI 1640 Broth medium. Colony formation in the media was evaluated as the presence of reproduction, and the absence of colony was evaluated as inhibition. The microorganisms used are listed below:

Gram positives, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212,

Gram negatives, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606.

Fungi; *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135, *Candida glabrata* ATCC 90030

Antioxidant and Oxidant Tests

The antioxidant and oxidant potentials of EtOH extracts of the plant were determined using Rel Assay kits. The kits were used according to the protocols specified by the manufacturer. Trolox was used in the calibration of TAS tests in the kit. Hydrogen peroxide was used in the calibration of TOS tests (Erel, 2004; Erel, 2005). In determining the oxidative stress index, the units of TOS value and TAS value were equalized. Then, they were proportioned and their percentages were taken (Sevindik and Akata, 2019).

Results and Discussion

Antimicrobial Activity

Plants are faced with many abiotic and biotic factors in the ecosystem. During this struggle, they synthesize many biologically active compounds of different types and properties (Mostafa et al., 2018; Esmael et al., 2020). Thanks to these compounds, they form a defense against the microorganisms they encounter in the ecosystem (Mohammed et al., 2018; Kina et al., 2021). In this context,

many plants can be actively used in the fight against microorganisms. In this study, antimicrobial effects of *L. spinosum* against standard bacterial and fungal strains were investigated. The obtained results are shown in Table 1

In our study, the ethanol extract of *L. spinosum* was against *S. aureus*, *S. aureus* MRSA and *C. krusei* at 200 µg/mL, against *E. faecalis*, *P. aeruginosa* and *C. albicans*, and *E. coli* at 200 µg/mL, and against *A. baumannii* at 25 µg/mL concentrations. It is seen that the plant extract generally shows the highest effect against gram-negative bacteria. In previous studies on *Lepidium* species, it was reported that *Lepidium sativum* was effective against *Proteus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus mutans* using the ethanolic and aqueous extracts agar dilution method (Akrayi and Tawfeeq, 2012). It has been reported that petroleum ether extract of *Lepidium sativum* is effective at different levels against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* by using Microdilution method (Alqahtani et al., 2019). In our study, the ethanol extract of *Lepidium spinosum* was tested against *S. aureus*, *S. aureus* MRSA, *E. faecalis*, *E. coli*, *P. aeruginosa*, *A. baumannii*, *C. albicans*, *C. glabrata* and *C. krusei*. As a result, it was determined that there was an inhibitory effect on the growth of microorganisms at 25-200 µg/mL extract concentrations. In this context, it has been determined that *L. spinosum* may be a natural antimicrobial agent.

Antioxidant Activity

Antioxidants are involved in the defense system against harmful reactive oxygen species in living organisms (Selamoglu et al., 2020). The increase in the levels of oxidant compounds reveals negative results in living things. Antioxidant defense system is activated against oxidant compounds and suppresses/eliminates these effects. Oxidative stress occurs when the antioxidant defense system is insufficient (Chen et al., 2020; Bal et al., 2022). As a result of oxidative stress, very dangerous diseases such as cancer, cardiovascular diseases, Alzheimer's and Parkinson's may occur in humans (Sevindik et al., 2018; Uysal et al., 2021). In such cases, antioxidants to be supplemented may prevent the occurrence of these effects (Xiang et al., 2019; Sevindik et al., 2020). Plants are highly valuable sources as natural antioxidant agents. In this study, TAS, TOS and OSI values of *L. spinosum* were determined. The obtained results are shown in Table 2.

Table 1. MIC values of ethanol extract of *L. spinosum*

Sample	<i>S. aureus</i>	<i>S. aureus</i> MRSA	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>
EtOH	200	200	100	25	100	25	100	200	200

*The MIC values are presented in units of µg/mL.

Table 2. TAS, TOS and OSI values of *Lepidium spinosum*

Sample	TAS	TOS	OSI
<i>L. spinosum</i>	4.550±0.132	12.610±0.221	0.277±0.007

Values are presented as mean±SD

In previous studies, it has been reported that methanol and aqueous extracts of *Lepidium meyenii* have antioxidant activity (Sandoval et al., 2002; Rodríguez-Huamán et al., 2016). The oil extract of *Lepidium sativum* has been reported to have antioxidant potential (Alqahtani et al., 2019; Chatoui et al., 2020). In our study, it was determined that *L. spinosum* has antioxidant potential. Data on TAS, TOS and OSI values of *L. spinosum* were not found in the literature. In this context, it was determined for the first time in our study. In studies using Rel Asay kits on different plant species, the TAS values of *Mentha longifolia* subsp. *longifolia*, *Helianthemum salicifolium*, *Galium aparine*, *Allium calocephalum*, *Scorzonera papposa*, *Ferulago platycarpa*, *Rumex crispus* and *Gundellia tournefortii* were reported as 3.628, 9.490, 5.147, 5.853, 6.328, 5.688, 6.758 and 6.831, respectively (Sevindik et al., 2017; Mohammed et al., 2019; Durna Dastan et al., 2019; Saraç et al., 2019; Mohammed et al., 2020a; Mohammed et al., 2020b; Korkmaz et al., 2021; Mohammed et al., 2021). Compared to these studies, the TAS value of *L. spinosum* was higher than *M. longifolia* subsp. *longifolia* and lower than *H. salicifolium*, *G. aparine*, *A. calocephalum*, *S. papposa*, *F. platycarpa*, *R. crispus* and *G. tournefortii*. TAS value shows the whole of compounds with antioxidant properties produced in living organisms. High TAS value is equivalent to potentially high antioxidant properties (Korkmaz et al., 2018). In this context, it was determined that *L. spinosum* used in our study has antioxidant potential.

In studies using Rel Asay kits on different plant species, the TOS values of *M. longifolia* subsp. *longifolia*, *H. salicifolium*, *G. aparine*, *A. calocephalum*, *S. papposa*, *F. platycarpa*, *R. crispus* and *G. tournefortii* were reported as 4.046, 14,839, 18,679, 16,288, 11,525, 15,552, 5,802 and 3.712 mmol/L, respectively (Sevindik et al., 2017; Mohammed et al., 2019; Durna Dastan et al., 2019; Saraç et al., 2019; Mohammed et al., 2020a; Mohammed et al., 2020b; Korkmaz et al., 2021; Mohammed et al., 2021). Compared to these studies, the TOS value of *L. spinosum* was higher than *M. longifolia* subsp. *longifolia*, *S. papposa*, *R. crispus* and *G. tournefortii*, and lower than *H. salicifolium*, *G. aparine*, *A. calocephalum* and *F. platycarpa*. TOS value shows the whole of the oxidant compounds produced as a result of metabolic activities in living organisms under the influence of abiotic and biotic factors. The increase in TOS value increases the formation of oxidative stress in living organisms (Korkmaz et al., 2018). TOS values of *L. spinosum* used in our study were found to be at normal levels.

In studies using Rel Asay kits on different plant species, the OSI values of *M. longifolia* subsp. *longifolia*, *H. salicifolium*, *G. aparine*, *A. calocephalum*, *S. papposa*, *F. platycarpa*, *R. crispus* and *G. tournefortii* were reported as 0.112, 0.157, 0.346, 0.278, 0.182, 0.273, 0.086 and 0.054, respectively (Sevindik et al., 2017; Mohammed et al., 2019; Durna Dastan et al., 2019; Saraç et al., 2019; Mohammed et al., 2020a; Mohammed et al., 2020b; Korkmaz et al., 2021; Mohammed et al., 2021). Compared to these studies, the OSI value of *L. spinosum* was higher than *M. longifolia* subsp. *longifolia*, *H. salicifolium*, *S. papposa*, *F. platycarpa*, *R. crispus* and *G. tournefortii*, and lower than *G. aparine* and *A. calocephalum*. The OSI value is found by dividing the TOS values with the TAS value.

A high OSI value indicates that oxidant compounds are not sufficiently suppressed by antioxidant compounds (Korkmaz et al., 2018). In our study, it was determined that the suppression of oxidant compounds produced in *L. spinosum* was at normal levels.

Conclusion

In this study, antibacterial, antifungal activities and antioxidant and oxidant status of *L. spinosum* were determined. As a result of the studies carried out in this context, it was determined that the antioxidant and oxidant levels of *L. spinosum* were at normal levels. In this context, it has been determined that the plant can be used as a natural antioxidant agent. In addition, it has been observed that the plant has significant levels of antimicrobial activity against gram-negative bacteria. In this context, the plant is thought to be a natural antimicrobial agent. As a result, it is thought that *L. spinosum* may be an important antioxidant and antimicrobial agent that can be taken through diet.

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