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Antioxidant and antimicrobial activities of ethanol extract of *Helianthemum salicifolium* (Cistaceae)

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Plants have been used by humans to treat diseases. Different plant species have been very successful in treating different diseases. *Helianthemum salicifolium* (L.) Mill. was used as a material in our study. The plant was extracted with ethanol (EtOH) in a Soxhlet apparatus. Then, the antioxidant (TAS) and oxidant status (TOS) of the plant extract were determined using Rel Assay kits. Their antimicrobial activities were tested against standard bacteria and fungus strains by the agar dilution method. As a result of the analysis, the TAS value of plant extract was determined as 9.490±0.195, TOS value as 14.839±0.253, and OSI value as 0.157±0.005. In this context, it was seen that the plant has important antioxidant potential. In addition, the plant extract was found to be effective against test microorganisms at 25-100 µg/mL extract concentrations. Also, the extract was found to be more effective against fungus strains (*C. albicans*, *C. Glabrata*, and *C. krusei*). As a result, it was determined that *H. salicifolium* could be a natural antioxidant and antimicrobial source.

Keywords: Antimicrobial, Antioxidant, *Helianthemum*, Medicinal plant, Oxidant.
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Introductıon

Medicinal plants have been used in the treatment of diseases in many civilizations. Many plants from past to present have been very important in curing and preventing different diseases[1]. Plants produce phenolic compounds, which are not nutritious but have medicinal properties[2]. Phenolic compounds are quite common in the plant kingdom as complex substances (gallic acid, caffeic acid, stilbenes, flavonoids, and polymers, etc.)[2]. In recent years, many researchers reported that different plant species have different biological activities such as antioxidant, antimicrobial, anticancer, antiproliferative, anti-inflammatory, DNA preservative, anti-ageing, antidepressant, antiallergic, hypoglycemic[3-10]. In this study, the biological activity of *Helianthemum salicifolium* (L.) Mill. was determined.

Helianthemum (Cistaceae) genus is known as rockrose, sunrose, rushrose or frostweed. The Cistaceae family includes about 110 species of flowering plants[11]. It has a widespread distribution in the Northern Hemisphere and especially in the Mediterranean. Usually, shrubs or sub-shrubs and some are herbaceous annuals or perennials[11]. *Helianthemum* members form mycorrhiza with fungi. It is particularly associated with Terfeziaceae members. Plants and fungi can prevent soil erosion and desertification, especially with the mycorrhizal association. Among *Helianthemum* members, this mycorrhizal association between *Helianthemum salicifolium* and truffles is striking[12]. Besides mycorrhizal association, *Helianthemum* members are food plants for the larvae of Lepidoptera species. *Bucculatrix helianthemi* and *B. regaella* feed exclusively on *Helianthemum sessiliflorum* like *Coleophora eupreta*[13,14]. In this study, the antioxidant, oxidant, and antimicrobial potentials of *H. salicifolium* were determined. In this context, the potential of the plant to be a natural drug was evaluated.

Materials and Methods

Plant identification and extract preparation

H. salicifolium samples were collected from Turkey (Gaziantep/Şahinbey). Plant specimens are stored in a personal (Dr. Falah Saleh Mohammed) herbarium. Plant identification was done by one of the authors, Falah Saleh Mohammed using Flora Turkey vol 1[15]. Aerial parts of the plant specimens were dried in a shady and airy environment. Exactly 30 g of the dry samples were weighed and pulverized. Then, extraction

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was performed at 50 °C with 200 mL EtOH for 6 hours (Gerhardt EV 14). The solvents of the extracts obtained were removed in a concentrator (Heidolph Laborota 4000 Rotary Evaporator). EtOH was purchased from Merck (Darmstadt, Germany).

Antioxidant and oxidant activity

The antioxidant and oxidant status of the EtOH extract of the plant were determined using Rel Assay TAS and TOS kits[16,17]. Trolox was used as a calibrator in the TAS kit. Hydrogen peroxide was used as a calibrator in the TOS kit. The oxidative stress index (OSI) was determined by proportioning TOS values to TAS values by equalizing units and the following formula was used[18].

$$\text{OSI (AU)} = \frac{\text{TOS (}\mu\text{moL } H_2O_2 \text{ equiv./L)}}{\text{TAS(mmoL Trolox equiv./L)x10}}$$

Antimicrobial activity

The antimicrobial activity of the plant sample was determined using the agar dilution method. The plant extract was adjusted at 6.25-800 µg/mL concentrations. Concentrations were adjusted with distilled water. The lowest concentrations of the plant extract that inhibit the growth of bacterial and fungal strains were detected[19-21]. *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606 were used as bacterial strains. *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135, *C. glabrata* ATCC 90030 were used as fungus strains. Bacterial strains were pre-cultured in Muller Hinton Broth medium. Fungus strains were pre-cultured in RPMI 1640 Broth medium. Fluconazole and amphotericin B were used as reference drugs for fungi. Amikacin, ampicillin and ciprofloxacin were used as reference drugs for bacteria[22-24].

Results and Discussion

Antimicrobial potential

Plants interact with many living things in their natural ecosystems. Thanks to these interactions, they produce compounds with antimicrobial effects. These compounds have varying levels of antimicrobial activity[25,26]. In this study, the effects of *H. salicifolium* against the bacteria and fungus strains were investigated. The results obtained are shown in Table 1.

In this study, ethanol extract of *H. salicifolium* was used. As a result of the study, it was determined that the plant extract was effective against fungi (*C. albicans*, *C. glabrata* and *C. krusei*) at 25 µg/mL concentration. In addition, it was determined that it was effective against *E. faecalis*, *E. coli* and *A. baumannii* at 50 µg/mL concentration and *S. aureus*, *S. aureus* MRSA and *P. aeruginosa* at 100 µg/mL concentration.

In previous studies on *Helianthemum* species, it was reported that the methanol extract of *H. lippii* was effective against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans* at varying concentrations[27]. It was reported that ethylacetate and n-butanol extracts of *H. sessiliflorum* were effective against *Streptococcus faecalis*, *Salmonella typhimurium*, and *Aeromonas hydrophyla*[28]. In addition, methanol extracts of *H. kahiricum* and *H. lippi* were reported to be effective against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*[29]. On the other hand, methanol and water extracts of *H. glomeratum* were reported to be effective at different levels against *Escherichia coli*, *Vibrio cholera*, *Shigella*, *Salmonella* and *Vibrio parahaemolyticus* isolates[30].

In the present study, it was observed that the plant had high antimicrobial activity and hence, it may be an antimicrobial natural drug.

Antioxidant and oxidant status

Free radicals have a very important effect on tissue damage as well as cellular damage. Aerobic organisms reduce the effects of oxidant compounds with their antioxidant defence system, which includes enzymatic

Table 1 — Antimicrobial activity of *H. salicifolium*

	S. aureus	*S. aureus* MRSA	*E. faecalis*	*E. coli*	*P. aeruginosa*	*A. baumannii*	*C. albicans*	*C. glabrata*	*C. krusei*
EtOH	100	100	50	50	100	50	25	25	25
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloxacin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Fluconazole	-	-	-	-	-	-	3.12	3.12	-
Amphotericin B	-	-	-	-	-	-	3.12	3.12	3.12

*The MIC values are presented in units of µg/mL; - not worked

Table 2 — TAS, TOS and OSI values of *H. salicifolium*

	TAS	TOS	OSI
H. salicifolium	9.490±0.195	14.839±0.253	0.157±0.005

Values are presented as mean±SD

and non-enzymatic mechanisms. The use of supplemental antioxidants is very important in situations where endogenous antioxidants are insufficient[31,32]. Due to the effects of herbal antioxidants, interest in these natural materials is increasing. The use of herbal products as supplementary antioxidants to endogenous antioxidants may delay oxidative damage. In this study, the antioxidant (TAS) and oxidant (TOS) status of *H. salicifolium* were determined. The findings are given in Table 2.

In studies on different plant species, TAS value of *Mentha longifolia* subsp. *longifolia* was reported as 3.628, TOS value as 4.046 and OSI value as 0.112[(Ref. 8)]. The TAS value of *Allium calocephalum* has been reported as 5.853, the TOS value as 16.288, and the OSI value as 0.278[33]. The TAS value of *Gundellia tournefortii* was reported as 6.831, TOS value 3.712, and OSI value 0.054[34]. The TAS value of *Rhuszebaria* var. *zebaria* was reported as 7.342, TOS value as 5.170, and OSI value as 0.071[35]. The TAS value of *Rumexcrispus* was reported as 6.758, TOS value as 5.802, and OSI value as 0.086[36]. The TAS value of *Scorzonera papposa* was reported as 5.314, TOS value 24.199 and OSI value 0.473[37]. The TAS value of *Ferulago platycarpa* was reported as 5.688, the TOS value as 15.552 and the OSI value as 0.273[38]. Compared to these studies, the TAS value of *H. salicifolium* was determined to be higher than *M. longifolia* subsp. *longifolia*, *A. calocephalum*, *G. tournefortii*, *R. coriaria* var. *zebaria*, *R. crispus*, *S. papposa* and *F. platycarpa*. TAS value shows the whole of compounds with antioxidant capacity within the living organism[39]. The higher the TAS value, the higher the antioxidant potential of the plant. According to the results of the study, it was seen that the TAS value of *H. salicifolium* was high. In this context, it was determined that the plant could be a natural source of antioxidants.

When TOS values were examined, it was seen that *H. salicifolium* was higher than *Rumex crispus*, *R. coriaria* var. *zebaria*, *G. tournefortii*, and *M. longifolia* subsp. *longifolia*, and lower than *F. platycarpa*, *S. papposa*, and *A. calocephalum*. The TOS value shows the whole of oxidant compounds produced as a result of environmental factors and metabolic activities in living organisms[38]. It is seen that the higher the TOS value, the more harmful the level of oxidant compounds in the plant. In this context, it is seen that the TOS value of *H. salicifolium* used in our study is at normal levels. In addition, the OSI value shows how much it suppresses oxidant compounds produced within its body with endogenous antioxidant compounds[38]. Increasing OSI value indicates that the plant is more affected by oxidative stress. It was observed that the oxidant compounds of *H. salicifolium* used in our study were more suppressed with antioxidant compounds compared to *F. platycarpa*, *S. papposa* and *A. calocephalum*, and less compared to *R. crispus*, *G. tournefortii*, *R. coriaria* var. *zebaria* and *M. longifolia* subsp. *longifolia*. As a result, it was determined that *H. salicifolium* could be an important natural antioxidant source.

Conclusion

In our study, the antioxidant, oxidant, and antimicrobial potentials of *H. salicifolium* were determined. According to the results obtained, it was seen that the plant has high antioxidant activity. In addition, oxidant compound status was found to be at normal levels. Also, it was seen that plant extracts were more effective against fungal strains. As a result, it was seen that *H. salicifolium* can be a natural antioxidant and antimicrobial source.

Conflict of interest

The authors declare no conflict of interest.

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