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Some Biological Activities of Ethanol Extract of *Marrubium globosum*

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ABSTRACT

Plants have been used for centuries to treat various diseases. In this study, *Marrubium globosum* Montbret and Aucher ex Benth. plant was used as a material. The extract of the plant was extracted with the help of soxhlet device using ethanol, which is a good polar solvent, and Rel Assay kits were used to determine the oxidant and antioxidant levels in the plant extract. Antifungal and antibacterial activities of *M. globosum* were tested against standard bacteria and fungus strains by agar dilution method. As a result of the analysis, TAS value of plant extract was determined as 7.677±0.231, TOS value as 12.387±0.083 and OSI value as 0.162±0.004. In this context, it has been observed that the plant has an important antioxidant potential. In addition, the plant extract was found to be effective against test microorganisms at 50-200 µg/mL extract concentrations. As a result, it has been determined that *M. globosum* can be a natural antioxidant and antimicrobial source.

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Introduction

Breathing, which is an indispensable action for the continuation of life, actually brings some dangers with it. Although undoubtedly the element is essential for life, most of the biochemical reactions it participates in generate free radicals that contain oxygen as a by-product. These highly reactive chemical wholes can damage or even kill cells when they reach high levels (Marx, 1987; Loix et al., 2017). Free radicals have an important effect on tissue damage as well as cellular damage. Oxidative stress is an increase in oxidant level and/or a decrease in antioxidant level, especially in conditions that are pathological for the body, shifting the balance in oxidative metabolism to the oxidative direction (Sevindik, 2020). Oxidants often have a damaging effect on cells, mitochondria, nuclei and membranes. Free radicals that affect DNA in particular cause irreversible damage to the organism (Dadheech et al., 2008). The antioxidant system especially ensures that the effect of reactive oxygen species (ROS) formation is minimized and neutralized (Tunçel et al., 2015). In cases where the antioxidant system is insufficient, taking supplemental antioxidants plays an important role in reducing oxidative damage. In this context, it is very important to investigate the antioxidant effects of natural products (Tewari et al., 2021). Many materials obtained from natural ecosystems have been used by humans in the treatment of diseases. Plants in particular have been used

by humans for different purposes such as the treatment of diseases, nutrition, shelter, defense and warming, and they are still being used. In recent years, many researchers have reported that different plant species have different biological activities such as antioxidant, antimicrobial, anticancer, antiproliferative, anti-inflammatory, DNA preservative, anti-aging, antidepressant, antiallergic, hypoglycemic (Hu et al., 2017; Ayoub et al., 2018; Khan et al., 2018; Lichota and Gwozdzinski, 2018; Miastkowska and Sikora, 2018; Ribeiro et al., 2018; Salehi et al., 2019; Guo et al., 2020; Salehi et al., 2020; Mohammed et al., 2021).

In this study, antioxidant and antimicrobial activities of *M. globosum* were determined. The genus *Marrubium* (Lamiaceae) includes about 40 species growing mainly throughout the Mediterranean and in the temperate regions of the Eurasian continent. Most of the species are annual or perennial rhizomatous plants, usually containing a separate indumentum of very complex hairs (Mabberley, 1997). It has been reported that *M. globosum* ssp. *libanoticum* is used in the treatment of inflammatory diseases, asthma, cough and other lung and urinary problems (Rigano et al., 2009). Flavonoids and phenylethanoids have also been reported as chemical constituents of *Marrubium* spp., but it is also well known that the genus contains a wide variety of diterpenoids (Hatam et al., 1995; Karioti et al., 2005).

Materials and Methods

M. globosum examples of Turkey (Gaziantep) were collected from. Plant identification was made using Flora of Turkey volume 7 (Davis, 1982; 173). The aerial parts of the samples were separated and dried in a shaded and ventilated environment. Then 30 g of the samples were weighed. It was then extracted at 50 0C with 200 mL of EtOH for about 6 hours (Gerhardt EV 14). The solvents of the extracts obtained were removed in a concentrator. (Heidolph Laborota 4000 Rotary Evaporator).

Antimicrobial Activity Studies

The extract concentrations of the plant's EtOH extract against bacteria and fungi were determined using the agar dilution method (CLSI, 2012; EUCAST, 2014; EUCAST, 2015). Different concentrations of the plant extract were adjusted with distilled water. Bacterial strains (*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 cultured in Muller Hinton Broth medium and Amikacin, Ampicillin and Ciprofloxacin were used as reference drugs. Fungus strains (*Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030) were cultured in RPMI 1640 Broth medium and Fluconazole and Amphotericin B were used as reference drugs (Bauer et al., 1966; Hindler et al., 1992; Matuschek et al., 2014).

Antioxidant and Oxidant Tests

The total antioxidant and oxidant levels of the plant extract were measured according to the protocol specified in the Rel Assay kits. The antioxidant status was determined using TAS kit, oxidant status was determined using TOS kit (Erel, 2004; Erel, 2005). Trolox was used as the calibrator in the antioxidant status test and hydrogen peroxide as the calibrator in the oxidant status test. The oxidative stress index (OSI: Arbitrary unit (AU)) was determined according to the formula below (Sevindik, 2019).

$$\text{OSI (AU)} = \frac{\text{TOS, } \mu\text{mol } H_2O_2 \text{ equiv./L}}{\text{TAS, mmol Trolox equiv./L} \times 10}$$

Results and Discussion

Antimicrobial Potential

Plants have many biological activities thanks to the secondary metabolites they produce. In recent years, the number of microbial diseases has been increasing (Danish et al., 2020). Chemically synthesized antibiotics are used extensively in the fight against these diseases (Zazharskyi et al., 2019). However, due to the possible side effects of chemical antibiotics and resistant microorganisms, the trend towards natural products has increased (Mostafa et al., 2018). In this context, the use of plants with high biological activities is increasing. In our study, the potential of EtOH extract of *M. globosum* against bacteria and fungi was investigated. The findings obtained are shown in Table 1.

Antimicrobial potentials of different species belonging to the genus *Marrubium* have been reported in previous studies. From these studies, it has been reported that *Marrubium peregrinum*, acetone, ethyl acetate and methanol extracts are effective against *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella enterica, Proteus mirabilis, Enterococcus faecalis, Staphylococcus aureus, Sarcina lutea, Bacillus subtilis, Bacillus cereus* and *Bacillus pumilus* in different concentrations (Radojević et al., 2013). Methanol extract of *Marrubium globosum* ssp. *libanoticum* has been reported to be effective against *Staphylococcus epidermidis, S. aureus, Enterococcus faecalis, Bacillus subtilis, Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Enterobacter aerogenes, E. cloacae, Klebsiella pneumoniae* and *Escherichia coli* (Rigano et al., 2007). It has been reported that the essential oil of *Marrubium astracanicum* ssp. *astracanicum* is effective against *Brevibacillus brevis, B. megaterium, B. subtilis, B. cereus, Staphylococcus aureus* and *Listeria monocytogenes* (Kilic and Özdemir, 2017). In our study, it was determined that the EtOH extract of *Marrubium globosum* was effective against *E. faecalis, E. coli,* and *A. baumannii* at 50 µg/mL, *P. aeruginosa* and *C. glabrata* at 100 µg/mL, *S. aureus, S. aureus* MRSA, *C. albicans* and *C. krusei* at 100 µg/mL. In this context, it has been determined that *M. globosum* has antimicrobial activity. As a result, it has been determined that it can be an antimicrobial natural source.

Antioxidant Status

Aerobic organisms reduce the effects of oxidant compounds with their antioxidant defense system, which includes enzymatic and non-enzymatic mechanisms. The use of supplemental antioxidants is very important in situations where endogenous antioxidants are insufficient (Korkmaz et al., 2018). Due to the effects of herbal antioxidants, interest in these natural materials is increasing.

Table 1. Antimicrobial Activity of *Marrubium globosum*

Extract	A	B	C	D	E	F	G	H	J
EtOH	200	200	50	50	100	50	100	200	200

*(A) *S. aureus*, (B) *S. aureus* MRSA, (C) *E. faecalis*, (D) *E. coli*, (E) *P. aeruginosa*, (F) *A. baumannii*, (G) *C. glabrata*, (H) *C. albicans*, (J) *C. Krusei*, *200, 100, 50 µg/mL extract concentrations

Table 2. TAS, TOS and OSI values of *Marrubium globossum*

Material	TAS	TOS	OSI
M. globossum	7.677±0.231	12.387±0.083	0.162±0.004

Values are presented as mean±SD

The use of herbal products as supplementary antioxidants as supplements to endogenous antioxidants may delay oxidative damage (Kim et al., 2017). In this study, antioxidant (TAS) and oxidant (TOS) levels of *M. globosum* were determined. The findings obtained are shown in Table 2.

TAS, TOS and OSI values of *M. globosum* were not previously reported, and were determined for the first time in this study. In studies on different plant species, TAS value of *Allium calocephalum* has been reported as 5.853 mmol/L, TOS value as 16.288 µmol/L and OSI value as 0.278 (Mohammed et al., 2019). The TAS value of *Mentha longifolia* subsp. *longifolia* has been reported as 3.628 mmol/L, TOS value as 4.046 µmol/L and OSI value as 0.112 (Sevindik et al., 2017). The TAS value of *Rhus coriaria* var. *zebaria* has been reported as 7.342 mmol/L, TOS value as 5.170 µmol/L and OSI value as 0.071 (Mohammed et al., 2018). TAS value of *Scorzonera papposa* has been reported as 5.314 mmol/L, TOS value as 24.199 µmol/L and OSI value as 0.473 (Mohammed et al., 2020a). The TAS value of *Gundellia tournefortii* has been reported as 6.831 mmol/L, TOS value as 3.712 µmol/L and OSI value as 0.054 (Saraç et al., 2019). The TAS value of *Ferulago platycarpa* has been reported as 5.688 mmol/L, TOS value as 15.552 µmol/L and OSI value as 0.273 (Mohammed et al., 2020b). The TAS value of *Rumex crispus* has been reported as 6.758 mmol/L, TOS value as 5.802 µmol/L and OSI value as 0.086 (Daştan et al., 2019). When compared with these studies, the TAS value of *M. globosum* 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 the antioxidant active compounds in the plant (Akgül et al., 2020). Plants with high TAS values are very important in terms of antioxidant compound. According to the data of the study we conducted in this context, it was seen that the TAS value of *M. globosum* was high, and as a result, it was determined that the plant could be an important natural antioxidant source.

The TOS value shows the whole of the oxidant compounds produced by the plant as a result of environmental and structural effects and metabolic activities (Akgül et al., 2020). In this context, the TOS value of *M. globosum* was determined higher than *R. crispus*, *R. coriaria* var. *zebaria*, *G. tournefortii* and *M. longifolia* subsp. *longifolia*, and lower than *F. platycarpa*, *S. papposa* and *A. calocephalum*. 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 *M. globosum* used in our study is at normal levels. In addition, the OSI value indicates the balance of the oxidant compounds in the plant's body and the antioxidant defense system. As the OSI value increases, it indicates that the antioxidant defense system is insufficient to suppress oxidant compounds (Akgül et al., 2020). In this context, it was seen that the OSI value of *M. globosum* was lower than *F. platycarpa*, *S. papposa* and *A. calocephalum*, and higher than *R. coriaria* var. *zebaria*, *R. crispus*, *G. tournefortii* and *M. longifolia subsp. longifolia.* As a result, it is seen that *M. globosum* is successful in suppressing oxidant compounds. In this context, it is thought that the plant can be used as a natural antioxidant agent.

Conclusion

In this study, the antioxidant, antibacterial and antifungal potential of *M. globosum* was determined. The study has shown that the plant has an important antioxidant potential. In this context, it is thought that it can be used as a natural antioxidant agent. In addition, it was determined that the plant has antimicrobial activity against standard bacteria and fungi. As a result, it is thought that *M. globosum* can be used as a natural pharmacological agent.

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