Some Biological Activities of Ethanol Extract of *Marrubium globosum*

Mustafa Pehlivan$^{1,a,*}$, Falah Saleh Mohammed$^{2,b}$, Ali Erdem Şabik$^{3,f}$, Eylem Kına$^{4,d}$, Muhittin Dogan$^{4,e}$, Önder Yumrutaş$^{4,f}$, Mustafa Sevindik$^{6,g}$

$^1$Department of Medical and Aromatic Plants, Nurdagi Vocational Higher School, Gaziantep University, 27310 Gaziantep, Turkey
$^2$Department of Biology, Faculty of Science, Zakho University, Duhok, Iraq
$^3$Department of Chemistry and Chemical Processing Technologies, Bahçe Vocational School, Osmaniye Korkut Ata University, 80010 Osmaniye, Turkey
$^4$Department of Biology, Faculty of Science and Literature, Gaziantep University, 27310 Gaziantep, Turkey
$^5$Department of Medical Biology, Faculty of Medicine, Adıyaman University, 02100 Adıyaman, Turkey
$^6$Department of Food Processing, Bahçe Vocational School, Osmaniye Korkut Ata University, 80010 Osmaniye, Turkey

Corresponding author

Research Article

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.

Keywords: Antimicrobial, Antioxidant, Medicinal Plants, *Marrubium globosum*, Oxidant

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 (Dadhicheh 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, anti-allergic, hypoglycemic (Hu et al., 2017; Ayoub et al., 2018; Khan et al., 2018; Lichota and Gwozdziński, 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* ssp., 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 ºC 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).

\[
\text{OSI (AU)} = \frac{\text{TOS, } \mu\text{mol H}_2\text{O}_2 \text{ equiv./L}}{\text{TAS, mmol Trolox equiv./L} \times 10}
\]

**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 (Zazharsky 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* (Kılıç 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.
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 24.199 μmol/L and OSI value as 0.473 (Mohammed et al., 2020a). The TAS value of Gundelia 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 TAS 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 TAS 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 TAS value, the more harmful the level of oxidant compounds in the plant. In this context, it is seen that the TAS 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.

Table 1. Antimicrobial Activity of Marrubium globosum

| Material       | S. aureus | E. coli | P. aeruginosa | C. albicans | C. glabrata | K. pneumonia | MRSA | C. glabrata | C. albicans | C. jeikeium | C. tropicalis |
|----------------|-----------|---------|---------------|-------------|-------------|--------------|------|-------------|-------------|-------------|---------------|
| EtOH           | A         | B       | C             | D           | E           | F            | G    | H           | I           | J           |               |

Table 2. TAS, TOS and OSI values of Marrubium globosum

| Material       | TAS       | TOS      | OSI          |
|----------------|-----------|----------|--------------|
| M. globosum    | 7.677±0.231| 12.387±0.083| 0.162±0.004  |

Values are presented as mean±SD

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.

References

Akgül H, Korkmaz N, Dayangaz A, Sevindik M. 2020. Antioxidant Potential of Endemic Salvia absconditiflora. Turkish Journal of Agriculture-Food Science and Technology, 8(10): 2222-2224.

Ayoub IM, Korinek M, Hwang TL, Chen BH, Chang FR, El-Shazy M, Singab ANB. 2018. Probing the antiallergic and anti-inflammatory activity of biflavonoids and dihydroflavonols from Dietses bicolor. Journal of natural products, 81(2): 243-253.

Bauer AW, Kirby WM, Sherris JC, Tuck M. 1966. Antibiotic susceptibility testing by a standardized single disk method, Am J Clin Pathol, 45: 493-96.

CLSI, 2012. (The Clinical and Laboratory Standards Institute) Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Eighth Edition (M11-A8).

Dadheech G, Mishra S, Gautam S, Sharma P. 2008. Evaluation of antioxidant deficit in schizophrenia. Indian Journal of Psychiatry, 50(1): 16-20.

Danish P, Ali Q, Hafeez MM, Malik A. 2020. Antifungal and antibacterial activity of aloe vera plant extract. Biol Clin Sci Res J, 1-8.

Daştan SD, Durukan H, Demirbaş A, Dönmez E. 2019. Bioactivity and Therapeutic Properties of Evelik (Rumex crispus), A Naturally Growing and Edible Plant in Sivas Province. Turkish Journal of Agriculture-Food Science and Technology, 7(sp2): 67-71.

Erel O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical biochemistry, 37(4): 277-285.

Erel O. 2005. A new automated colorimetric method for measuring total oxidant status. Clinical biochemistry, 38(12): 1103-1111.

EUCAST, 2014. (European Committee on Antimicrobial Susceptibility Testing) Breakpoint tables Fungal isolate for interpretation of MICs. Version 7.0.

EUCAST, 2015. (European Committee on Antimicrobial Susceptibility Testing) Breakpoint tables for Bacteria interpretation of MICs and zone diameters, Version 5.0.

Guo Y, Zhang H, Zhao J, Zhang H, Chen S. 2020. Enzyme-assisted extraction of a cup plant (Silphium perfoliatum L.) polysaccharide and its antioxidant and hypoglycemic activities. Process Biochemistry, 92: 17-28.
