A STUDY ON PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF QUERCUS MACRANTHERA SUBSP. SYSPIRENSIS (K. KOCH) MENITSKY BRANCH AND LEAF EXTRACTS

QUERCUS MACRANTHERA SUBSP. SYSPIRENSIS (K. KOCH) MENITSKY’İN DAL VE YAPRAK EKSTRELERİNİN FİTOKİMYASAL ANALİZİ VE ANTİBAKTERİYEL AKTİVİTESİ ÜZERİNE BİR ÇALIŞMA

Merve Eylul KIYMACI 1*, Kenan Can TOK 2*, Muhammed Mesud HÜRKUL 3

1University of Health Sciences Turkey, Gülhane Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara, Turkey
2Ankara University, Institute of Forensic Sciences, Department of Forensic Toxicology, Ankara, Turkey
3Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Turkey

ABSTRACT

Objective: Oak species are medicinal plants with traditional use around the world. These species, which are very rich in tannins, have potential as antibacterial agents in terms of the polyphenolic compounds content. In this study, the antibacterial potential and phytochemical content of the branches and leaves of Quercus macranthera subsp. syspirensis, which is endemic to Turkey, were investigated.

Material and Method: Plant materials were collected from Araç (Kastamonu/Turkey) in 2020. Methanol extracts were prepared from dried and powdered branches and leaves. The antibacterial activity test was evaluated by broth microdilution method as a minimal inhibition concentration (MIC) against Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis ATCC 35984, Enterococcus faecalis ATCC 29212, Klebsiella pneumoniae ATCC 13883, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922, Acinetobacter baumannii ATCC 19606 and Bacillus subtilis ATCC 6633. The GC-MS analysis of extracts were performed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973N quadrupole mass spectrometer (Agilent, USA). The compounds were identified by comparing the mass spectrum ratio of the sample with the data available in NIST 2014 Mass Spectral Library.

* Corresponding Author / Sorumlu Yazar: Merve Eylul Kiymaci
e-mail / e-posta: mekiymaci@gmail.com, Phone / Tel.: +90 312 304 6073

Submitted / Gönderilme: 09.12.2021
Accepted / Kabul: 21.12.2021
**Result and Discussion:** As a result, it was found that the branch extracts were more effective than the leaf extracts and both branch and leaf extracts showed the highest activity against Bacillus subtilis ATCC 6633 strain (48.8 µg/ml, 97.6 µg/ml, respectively). The extracts also showed antibacterial activity at varying concentrations on other test strains.

**Keywords:** Antibacterial, branch, GC-MS, leaf, Quercus macranthera subsp. syssiphenis

**INTRODUCTION**

In the search for a solution to antimicrobial resistance that has emerged in recent years, active substances obtained from plants come to the fore. Although 25-50% of existing pharmaceuticals are obtained from herbal raw materials. Plants contain various secondary metabolites with antimicrobial activity such as tannins, terpenoids, alkaloids and flavonoids that are one of the go-to reservoirs to alleviate this problem [1].

The distribution areas of the genus *Quercus* L. are in the Northern Hemisphere and these plants, called oaks, have 461 accepted species worldwide [2, 3]. Oak species are rich in tannins, they are also known to contain gallic acid, caffeic acid, ferulic acid, ellagic acid, (-)-epicatechin, (-)-epigallocatechin, (+)-catechin and (+)-gallocatechin [4-10]. It is widely used as traditionally in the treatment of diabetes, wounds, respiratory diseases, diarrhea, obesity, fungus, ulcers, toothache, hemorrhoids, abscesses, dermatitis and burns [11-24]. It has been proven that the medically important *Quercus* species have antibacterial, anticancer, gastroprotective, antiviral, cardioprotective and hepatoprotective activities [25-34]. *Quercus macranthera* subsp. syssiphenis (K. Koch) Menitsky is endemic to Turkey, also called "ispir meşesi", the plant is a small deciduous tree, the leaves are obovate with 6-10 primary veins and the stipules are filiform [35-36].
In this study, the antibacterial activity of the branch (BM) and leaf (LM) methanol extracts of *Q. macranthera* subsp. *syspirensis* were investigated and the phytochemical analysis of the extracts were carried out with Gas Chromatography-Mass Spectrometry (GC-MS).

**MATERIAL AND METHOD**

**Plant materials and preparation of extracts**

Plant materials were collected from Araç (Kastamonu/Turkey) in 2020. A voucher specimen was deposited in the Ankara University Faculty of Pharmacy Herbarium (AEF). The collected plant parts (branches and leaves) were dried in the shade. The plant parts were extracted by using the maceration method with methanol.

**Antibacterial activity**

Antibacterial activity of the branch and leaf extracts of *Q. macranthera* subsp. *syspirensis* was tested against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 35984, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606 and *Bacillus subtilis* ATCC 6633. Antibacterial activity test was evaluated by broth microdilution method as a minimal inhibition concentration (MIC) according to European Committee on Antimicrobial Susceptibility Testing standarts [37].

**GC/MS analysis**

For GC-MS analysis of plant extracts, a two-step derivatization method including methoximation (methoxyamine derivatization) and silylation was used [38]. Methoxyamine reacts with the carbonyl groups of sugars to form oxime derivatives, thus preventing ring formation that causes multiple chromatographic peaks [39]. It also helps to protect α-keto acids from decarboxylation. Before the methoxyamine derivatization, methoxyamine hydrochloride (MeOX) (Germany, Sigma-Aldrich) solution freshly prepared in pyridine (25 mg/ml). 30 µl MeOX solution added to the dried extracts and waited 90 min at 30 °C for oximation of sugars. In the second step of derivatization, silylation was performed using 30 µl of BSTFA-1%TMCS (Germany, Sigma-Aldrich).

The analysis was performed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973N quadrupole mass spectrometer detector (Santa Clara, USA). All samples were analyzed using the RTX-5MS Low-Bleed fused silica gas chromatography capillary column (30m × 0.25mm i.d. × 0.25µm film thickness) (Restek, USA). Ultrapure helium was preferred as the carrier gas and a constant flow rate of 1.5 ml/min was used. The injection port was maintained at 280 °C. The ion source, quadrupole and transfer line temperatures were adjusted at 230 °C, 150 °C and 280 °C, respectively. The GC oven program was held at 50 °C for 2 min, and then increased to 280 °C at 4 °C/min and held
for 10 min. Total analysis time was 70 min. The mass range was 40–550 m/z and the scan rate was 0.45 scan per second in full scan mode. Electron ionization was carried out using 70 eV ionization energy. Compounds were identified using MS Search software and the NIST 2014 Mass Spectral Library.

RESULT AND DISCUSSION

The MIC results of tested extracts were shown in Table 1. It was determined that the branch extracts (BM) were more effective than the leaf extracts (LM) and both extracts showed the highest antibacterial activity against *Bacillus subtilis* ATCC 6633 strain. The extracts also showed activity at varying concentrations on other test strains.

**Table 1.** Antibacterial activity results for tested extracts as MIC.

| Extracts | Minimal inhibition concentrations (µg/ml) |
|----------|------------------------------------------|
|          | *S. aureus* ATCC 29213 | *S. epidermidis* ATCC 35984 | *E. faecalis* ATCC 29212 | *E. coli* ATCC 25922 | *P. aeruginosa* ATCC 27853 | *A. baumannii* ATCC 19606 | *K. pneumoniae* ATCC 13883 | *B. subtilis* ATCC 6633 |
| BM       | 781.25 | 1562.5 | 6250 | 3125 | 1562 | 781.25 | 781.25 | 48.8 |
| LM       | 3125 | 1562.5 | 6250 | 6250 | 6250 | 781.25 | 781.25 | 97.6 |

MIC results of *E.coli* for ciprofloxacin was found 0.078 µg/ml.

Since *Q. macranthera* subsp. *sypirensis* is an endemic plant, there is no literature data other than a study conducted in 2007 reported [40] that *Q. macranthera* subsp. *sypirensis* extracts prepared with different solvents (petroleum ether, ethyl acetate, *n*-butanol fractions and lyophilized water phase of methanol extract) showed the antibacterial activity at different concentrations (512≥1024 µl) against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853. Therefore, the current study is important in terms of bringing data to the literature. Previous studies have shown that different *Quercus* species have antibacterial activity against various Gram positive and Gram negative bacteria. Ahmed et al. (2021) [41] determined that *Quercus floribunda* Lindl. ex A. Camus acorn extract showed antibacterial activity against *B. subtilis, E. coli, K. pneumoniae* and *S. aureus*. Aleebrahim-Dehkordy et al. (2019) [42] showed that *Quercus brantii* Lindl. acorn ethanol (70%) extracts had inhibitory activity against *S. aureus* and *E. faecalis*. In the study of Elansary et al. (2019) [43], the antibacterial activities of the bark methanolic extracts of three *Quercus* species (*Q. robur, Q. macrocarpa* and *Q. acutissima*) exhibited antibacterial activities against most species of microorganism studied. The highest antibacterial activities were found against *S. aureus* ATCC 6538 (MIC 0.23 mg/ml), *P. aeruginosa* ATCC 27853 (MIC 0.05 mg/ml), *Bacillus cereus* ATCC 14579 (MIC 0.11 mg/ml), *Listeria monocytogenes* (clinical isolate) (MIC 0.25 mg/ml), *E. coli* ATCC 35210 (MIC 0.10 mg/ml) for the extracts of *Q. robur*, compared to streptomycin. The methanol extracts of *Quercus alba* L. barks were tested for growth inhibition of *S. aureus* (ICso 64 µg/ml), *K. pneumoniae* (ICso 32 µg/ml), and *A. baumannii* (ICso 32 µg/ml), and evaluated for biofilm
inhibition (IC\textsubscript{50} 1 μg/ml) against S. aureus by Dettweiler et al. (2019) [44]. Sánchez-Burgosa et al. (2013) [45] investigated the antibacterial activity of leaf aqueous extracts of Q. resinosa against E. coli ATCC 35218 (MIC 1.895 mg/ml), S. epidermidis ATCC 12228 (MIC 0.348 mg/ml), K. pneumoniae ATCC 13883 (MIC 0.547 mg/ml), P. mirabilis ATCC 12453 (MIC 0.708 mg/ml) and P. vulgaris ATCC 49132 (MIC 0.265 mg/ml).

Figure 1 and Figure 2 show the major compounds identified in branch and leaf extract by GC-MS. The analyzes show the presence of 17 and 19 compounds (Table 2-3), respectively in branch and leaf samples. Q. macranthera subsp. sypirensis branch extract contains 1.49% Carbonitrile, 1.50% Flavanoid, 1.63% Terpenoid, 2.28% Acid, 2.3% Carboxylic Acid, 2.58% Sugar Alcohol, 2.95% Steroids, 5.9% Cylopentapyrazoles, 5.94% Sulfonamide, 22.95% Phenols, 50.48% Sugars. However, Q. macranthera subsp. sypirensis leaf contains 0.59% Carbonitrile, 1.45% Steroids, 2.07% Sulfonamide, 2.32% Cylopentapyrazoles, 2.58% Sugar Alcohol, 5.41% Acids, 23.95% Phenols, 24.81% Carboxylic Acids, 36.82% Sugars.

**Figure 1.** Compounds identified by GC-MS in Q. macranthera subsp. sypirensis branch extract.

**Figure 2.** Compounds identified by GC-MS in Q. macranthera subsp. sypirensis leaf extract.
Table 2. Compounds identified by GC-MS in *Q. macranthera* subsp. *sypirensis* branch extract.

| #  | RT (min) | Identified compounds                                      | %   | Classification     |
|----|---------|----------------------------------------------------------|-----|--------------------|
| 1  | 9.671   | Boric acid                                               | 2.28| Acid               |
| 2  | 11.712  | *N*-2-Hydroxy-1-phenylethyl)benzenesulfonamide           | 5.95| Sulfonamide        |
| 3  | 18.233  | Benzo[e][1,2,5]-thiadiazole, 4,5,6,7-tetramethyl-       | 5.90| Cylopentapyrazoles |
| 4  | 18.919  | Glycerol                                                 | 2.58| Sugar alcohol      |
| 5  | 19.536  | 3-Amino-2,6,6,7-tetramethyl-1-thioxo-1,2,5,6,7,8-hexahydro-[2,7]naphthyridine-4-carbonitrile | 1.49| Carbonitrile       |
| 6  | 33.778  | Androst-5,7-dien-3-ol-17-one, acetate                   | 1.27| Steroid            |
| 7  | 34.453  | Myo-Inositol                                             | 15.6| Phenol             |
| 8  | 34.550  | Scylio-Inositol                                          | 7.35| Phenol             |
| 9  | 35.002  | Androst-5-en-3-ol17-one, 16,16-trimethylenedithio-      | 1.58| Steroid            |
| 10 | 36.156  | Quinic acid                                              | 2.30| Carboxylic acid    |
| 11 | 36.545  | D(-)-Fructose                                            | 14.1| Sugar              |
| 12 | 36.825  | D(-)-Fructose                                            | 8.64| Sugar              |
| 13 | 37.162  | D(+)-Talose                                              | 15.4| Sugar              |
| 14 | 37.648  | D-Allose                                                 | 3.02| Sugar              |
| 15 | 53.079  | Sucrose                                                  | 9.32| Sugar              |
| 16 | 56.908  | Catechine                                                | 1.50| Flavanoid          |
| 17 | 65.904  | Lupeol                                                   | 1.63| Terpenoid          |

Table 3. Compounds identified by GC-MS in *Q. macranthera* subsp. *sypirensis* leaf extract.

| #  | RT (min) | Identified compounds                                      | %   | Classification     |
|----|---------|----------------------------------------------------------|-----|--------------------|
| 1  | 9.671   | Boric acid                                               | 0.76| Acid               |
| 2  | 11.712  | *N*-2-Hydroxy-1-phenylethyl)benzenesulfonamide           | 2.07| Sulfonamide        |
| 3  | 12.603  | Methylphosphonic acid                                    | 0.45| Acid               |
| 4  | 14.421  | Benzo[h][1,2,5]-thiadiazole, 4,5,6,7-tetramethyl-       | 4.20| Acid               |
| 5  | 18.233  | Glycerol                                                 | 2.58| Sugar alcohol      |
| 6  | 19.536  | 3-Amino-2,6,6,7-tetramethyl-1-thioxo-1,2,5,6,7,8-hexahydro-[2,7]naphthyridine-4-carbonitrile | 0.59| Carbonitrile       |
| 7  | 33.778  | β-D-Glucopyranosidronic acid                             | 1.13| Sugar              |
| 8  | 33.887  | Pregnane-3,17,20,21-tetrol, (3a,5f,17a,20a)-             | 1.45| Steroid            |
| 9  | 34.470  | Myo-Inositol                                             | 16.3| Phenol             |
| 10 | 34.550  | Scylio-Inositol                                          | 7.65| Phenol             |
| 11 | 34.750  | Shikimic acid                                            | 2.01| Carboxylic acid    |
| 12 | 36.156  | Quinic acid                                              | 22.8| Carboxylic acid    |
| 13 | 36.545  | D(-)-Fructose                                            | 6.51| Sugar              |
| 14 | 36.825  | D(-)-Fructose                                            | 2.73| Sugar              |
| 15 | 37.162  | D(+)-Talose                                              | 6.50| Sugar              |
| 16 | 37.648  | D-Allose                                                 | 1.22| Sugar              |
| 17 | 38.722  | D-Allofuranose                                           | 1.63| Sugar              |
| 18 | 53.079  | Sucrose                                                  | 17.1| Sugar              |

**AUTHOR CONTRIBUTIONS**

Conception: M.E.K., K.C.T., M.M.H.; Design: M.E.K., K.C.T., M.M.H.; Supervision: M.E.K., K.C.T., M.M.H.; Resources: M.E.K., K.C.T., M.M.H.; Materials: M.E.K., K.C.T., M.M.H.; Data collection and/or processing: M.E.K., K.C.T., M.M.H.; Analysis and/or interpretation: M.E.K., K.C.T., M.M.H.; Literature search: M.E.K., K.C.T., M.M.H.; Writing manuscript: M.E.K.; Critical review: M.E.K., K.C.T., M.M.H.; Other: -
CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Mahizan, N. A., Yang, S. K., Moo, C. L., Song, A. A., Chong, C. M., Chong, C. W., Abushelaibi, A., Lim, S. E., Lai, K. S. (2019). Terpene Derivatives as a Potential Agent against Antimicrobial Resistance (AMR) Pathogens. *Molecules*, 24(14), 2631. [CrossRef]

2. Morales, D. (2021). Oak trees (*Quercus* spp.) as a source of extracts with biological activities: A narrative review. *Trends in Food Science & Technology*, 109, 116-125. [CrossRef]

3. POWO. (2021). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org Accessed: 21.11.2021.

4. Buche, G., Colas, C., Fougère, L., Giordanengo, T., Destandau, E. (2020). Untargeted UHPLC-Q-TOF-HRMS based determination of discriminating compounds for oak species *Quercus robur* L. and *Quercus petraea* Liebl. identification. *Phytochemical Analysis*, 32(5), 660-671. [CrossRef]

5. Evans, W. (2002). Trease and Evans pharmacognosy (15th ed.). WB Saunders.

6. Marinov, M. G., Dimitrova, E. D., Puech, J. L. (1997). Kinetics of ellagitannin extraction from oak wood using white wine. *Journal of Wine Research*, 8(1), 29-40. [CrossRef]

7. Perez, A. J., Pecio, Ł., Kowalczyk, M., Kontek, R., Gajek, G., Stopinsek, L., Mirt, I., Oleszek, W., Stochmal, A. (2017). Triterpenoid components from oak heartwood (*Quercus robur*) and their potential health benefits. *Journal of Agricultural and Food Chemistry*, 65(23), 4611-4623. [CrossRef]

8. Ricci, A., Parpinello, G. P., Palma, A. S., Teslić, N., Brilli, C., Pizzi, A., Versari, A. (2017). Analytical profiling of food-grade extracts from grape (*Vitis vinifera* sp.) seeds and skins, green tea (*Camellia sinensis*) leaves and Limousin oak (*Quercus robur*) heartwood using MALDI-TOF-MS, ICP-MS and spectrophotometric methods. *Journal of Food Composition and Analysis*, 59, 95-104. [CrossRef]

9. Şöhretoğlu, D., Sakar, M. K. (2004). Polyphenolic constituents and biological activities of *Quercus* species. *Journal of Faculty of Pharmacy of Ankara University*, 33(3), 183-215. [CrossRef]

10. Vivas, N., Nonier, M. F., de Gaulejac, N. V., de Boissel, I. P. (2004). Occurrence and partial characterization of polymeric ellagitannins in *Quercus petraea* Liebl. and *Q. robur* L. wood. *Comptes Rendus Chimie*, 7(8-9), 945-954. [CrossRef]
11. Bulut, G., Haznedaroğlu, M. Z., Doğan, A., Koyu, H., Tuzlacı, E. (2017). An ethnobotanical study of medicinal plants in Acipayam (Denizli-Turkey). *Journal of Herbal Medicine*, 10, 64-81. [CrossRef]

12. Caklicioğlu, U., Türkoglu, I. (2010). An ethnobotanical survey of medicinal plants in Sivrice (Elazığ-Turkey). *Journal of Ethnopharmacology*, 132(1), 165-175. [CrossRef]

13. Senkardes, I., Tuzlacı, E. (2014). Some Ethnobotanical Notes from Gundogmus District (Antalya/Turkey). *Clinical and Experimental Health Sciences*, 4(2), 63.

14. Sargın, S. A. (2021). Plants used against obesity in Turkish folk medicine: A review. *Journal of Ethnopharmacology*, 113841. [CrossRef]

15. Sargin, S. A., Akçicek, E., Selvi, S. (2013). An ethnobotanical study of medicinal plants used by the local people of Alaşehir (Manisa) in Turkey. *Journal of Ethnopharmacology*, 150(3), 860-874. [CrossRef]

16. Sargin, S. A., Selvi, S., Büyükçengiz, M. (2015). Ethnomedicinal plants of Aydınçık district of Mersin, Turkey. *Journal of Ethnopharmacology*, 174, 200-216. [CrossRef]

17. Polat, R., Caklicioğlu, U., Satılı, F. (2013). Traditional uses of medicinal plants in Solhan (Bingöl-Turkey). *Journal of Ethnopharmacology*, 148(3), 951-963. [CrossRef]

18. Kültür, Ş. (2007). Medicinal plants used in Kırklareli province (Turkey). *Journal of Ethnopharmacology*, 111(2), 341-364. [CrossRef]

19. Sezik, E., Yeşilada, E., Honda, G., Takaishi, Y., Takeda, Y., Tanaka, T. (2001). Traditional medicine in Turkey X. Folk medicine in central Anatolia. *Journal of Ethnopharmacology*, 75(2-3), 95-115. [CrossRef]

20. Carrió, E., Vallès, J. (2012). Ethnobotany of medicinal plants used in eastern Mallorca (Balearic Islands, Mediterranean Sea). *Journal of Ethnopharmacology*, 141(3), 1021-1040. [CrossRef]

21. Gîlca, M., Tiplica, G. S., Salașvastru, C. M. (2018). Traditional and ethnobotanical dermatology practices in Romania and other Eastern European countries. *Clínics in Dermatology*, 36(3), 338-352. [CrossRef]

22. Leporatti, M. L., Ivancheva, S. (2003). Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy. *Journal of Ethnopharmacology*, 87(2-3), 123-142. [CrossRef]

23. Sõukand, R., Pieroni, A. (2016). The importance of a border: medical, veterinary, and wild food ethnobotany of the Hutsuls living on the Romanian and Ukrainian sides of Bukovina. *Journal of Ethnopharmacology*, 185, 17-40. [CrossRef]

24. Šarić-Kundalić, B., Dobeš, C., Klatté-Asselmeyer, V., Saukel, J. (2010). Ethnobotanical study on medicinal use of wild and cultivated plants in middle, south and west Bosnia and Herzegovina. *Journal of Ethnopharmacology*, 131(1), 33-55. [CrossRef]
25. Alkofahi, A., Atta, A. H. (1999). Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats. *Journal of Ethnopharmacology*, 67(3), 341-345. [CrossRef]

26. Andrešek, S., Simonovska, B., Vovk, I., Fyhrquist, P., Vuorela, H., Vuorela, P. (2004). Antimicrobial and antioxidative enrichment of oak (*Quercus robur*) bark by rotation planar extraction using ExtraChrom®. *International Journal of Food Microbiology*, 92(2), 181-187. [CrossRef]

27. Berahou, A., Auhmani, A., Fdil, N., Benharref, A., Jana, M., Gadhi, C. A. (2007). Antibacterial activity of *Quercus ilex* bark’s extracts. *Journal of Ethnopharmacology*, 112(3), 426-429. [CrossRef]

28. Deryabin, D. G., Tolmacheva, A. A. (2015). Antibacterial and anti-quorum sensing molecular composition derived from Quercus cortex (Oak bark) extract. *Molecules*, 20(9), 17093-17108. [CrossRef]

29. Frédérich, M., Marcowycz, A., Cieckiewicz, E., Mégalizzi, V., Angenot, L., Kiss, R. (2009). *In vitro* anticancer potential of tree extracts from the Walloon Region forest. *Planta medica*, 75(15), 1634-1637. [CrossRef]

30. Gharzouli, K., Khennouf, S., Amira, S., Gharzouli, A. (1999). Effects of aqueous extracts from *Quercus ilex* L. root bark, *Punica granatum* L. fruit peel and Artemisia herba-alba Asso leaves on ethanol-induced gastric damage in rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 13(1), 42-45. [CrossRef]

31. Güllüce, M., Adıgüzel, A., Öğütçü, H., Şengül, M., Karaman, I., Şahin, F. (2004). Antimicrobial effects of *Quercus ilex* L. extract. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 18(3), 208-211. [CrossRef]

32. Jassim, S. A. A., Naji, M. A. (2003). Novel antiviral agents: a medicinal plant perspective. *Journal of Applied Microbiology*, 95(3), 412-427. [CrossRef]

33. Khennouf, S., Benabdallah, H., Gharzouli, K., Amira, S., Ito, H., Kim, T. H., Yoshida, T., Gharzouli, A. (2003). Effect of tannins from *Quercus suber* and *Quercus coccifera* leaves on ethanol-induced gastric lesions in mice. *Journal of Agricultural and Food Chemistry*, 51(5), 1469-1473. [CrossRef]

34. Panchal, S. K., Brown, L. (2013). Cardioprotective and hepatoprotective effects of ellagitannins from European oak bark (*Quercus petraea* L.) extract in rats. *European Journal of Nutrition*, 52(1), 397-408. [CrossRef]

35. Davis, P. H. (1982). Flora of Turkey and the East Aegean Islands. Edinburgh, UK: Edinburgh University Press.

36. Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M. T. (2012). Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyigit Botanik Baçesi Yayınları, Flora Dizisi I.
37. EUCAST. (2021). European Committee on Antimicrobial Susceptibility Testing. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf Accessed: 19.11.2021.

38. Villas-Boas, S. G., Mas, S., Åkesson, M., Smedsgaard, J., Nielsen, J. (2005). Mass spectrometry in metabolome analysis. Mass spectrometry reviews, 24, 613-646. [CrossRef]

39. Blau, K., Halket, J. M. (1993). Handbook of derivatives for chromatography. Wiley.

40. Şöhretoğlu, D., Ekizoglu, M., Kılıç, E., Sakar, M. K. (2007). Antibacterial and antifungal activities of some Quercus species growing in Turkey. FABAD Journal of Pharmaceutical sciences, 32(3), 127.

41. Ahmed, M., Adil, M., Haq, I., Tipu, M. K., Qasim, M., Gul, B. (2021). RP-HPLC-based phytochemical analysis and diverse pharmacological evaluation of Quercus floribunda Lindl. ex A. camus nuts extracts. Natural Product Research, 35(13), 2257-2262. [CrossRef]

42. Aleebrahim-Dehkordy, E., Rafieian-kopaei, M., Amini-Khoei, H., Abbasi, S. (2019). In vitro evaluation of antioxidant activity and antibacterial effects and measurement of total phenolic and flavonoid contents of Quercus brantii L. fruit extract. Journal of Dietary Supplements, 16(4), 408-416. [CrossRef]

43. Elansary, H. O., Szopa, A., Kubica, P., Ekiert, H., Mattar, M. A., Al-Yafrasi, M. A., El-Ansary, D. O., Zin Elabadin, T. K., Yessoufou, K. (2019). Polyphenol profile and pharmaceutical potential of Quercus spp. bark extracts. Plants, 8(11), 486. [CrossRef]

44. Dettweiler, M., Lyles, J., Nelson, K., Dale, B., Reddinger, R., Zurawski, D., Quave, C. L. (2019). American civil war plant medicines inhibit growth, biofilm formation, and quorum sensing by multidrug-resistant bacteria. Scientific Reports, 9, 7692. [CrossRef]

45. Sánchez-Burgos, J. A., Ramirez-Mares, M. V., Larrosa, M. M., Gallegos-Infante, J. A., González-Laredo, R. F., Medina-Torres, L., Rocha-Guzmán, N. E. (2013). Antioxidant, antimicrobial, antitopoiseromerase and gastroprotective effect of herbal infusions from four Quercus species. Industrial Crops and Products, 42, 57-62. [CrossRef]