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ORIGINAL ARTICLE / ÖZGÜN MAKALE

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

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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.*

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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. syspirensis*

ÖZ

Amaç: *Meşe türleri dünya genelinde geleneksel kullanımı olan tıbbi bitkilerdendir. Tanen bakımından oldukça zengin olan bu türlerin içerdikleri polifenolik bileşikler açısından antibakteriyel ajan olarak potansiyelleri vardır. Bu çalışmada Türkiye için endemik olan Quercus macranthera subsp. syspirensis'in dal ve yapraklarının antibakteriyel potansiyeli ve fitokimyasal içeriği araştırılmıştır.*

Gereç ve Yöntem: *Bitki materyalleri 2020 yılında Araç'tan (Kastamonu/Türkiye) toplanmıştır. Kurutulmuş ve toz haline getirilmiş dal ve yapraklardan metanol ekstreleri hazırlanmıştır. Antibakteriyel aktivite, minimum inhibisyon konsantrasyonu (MIC) olarak sıvı mikrodilüsyon yöntemiyle, 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 ve Bacillus subtilis ATCC 6633 mikroorganizmaları üzerine test edilmiştir. Ekstrelerin GC-MS analizi, Agilent 5973N dört kutuplu kütle spektrometresi (Agilent, ABD) ile donatılmış bir Agilent 6890 gaz kromatografı kullanılarak yapılmıştır. Bileşikler, numunenin kütle spektrum oranı NIST 2014 Kütle Spektral Kütüphanesinde bulunan verilerle karşılaştırılarak tanımlanmıştır.*

Sonuç ve Tartışma: *Sonuç olarak, dal ekstrelerinin yaprak ekstrelerinden daha etkili olduğu bulundu ve her iki ekstrenin de en yüksek antibakteriyel aktiviteyi Bacillus subtilis ATCC 6633 suşuna karşı gösterdiği belirlendi. Ekstreler ayrıca diğer test suşları üzerinde değişen konsantrasyonlarda aktivite gösterdi.*

Anahtar kelimeler: *Antibakteriyel, GC-MS, dal, yaprak, Quercus macranthera subsp. syspirensis*

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. *syspirensis* (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. pneumonie* 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* (IC_{50} 64 µg/ml), *K. pneumoniae* (IC_{50} 32 µg/ml), and *A. baumannii* (IC_{50} 32 µg/ml), and evaluated for biofilm

inhibition (IC_{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.

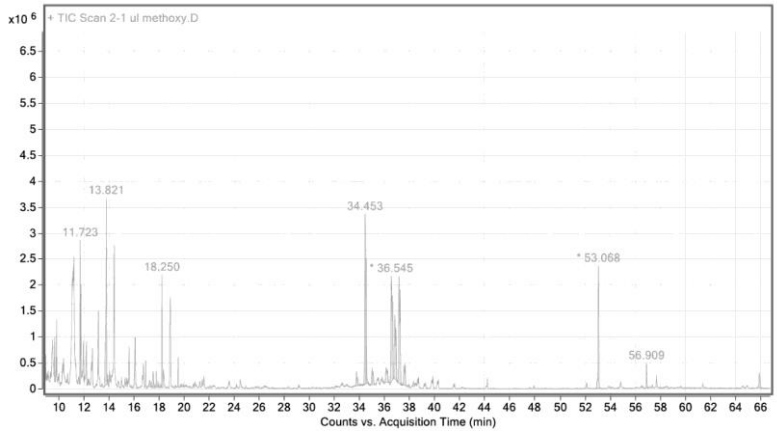

Figure 1. Compounds identified by GC-MS in *Q. macranthera* subsp. *sypirensis* branch extract.

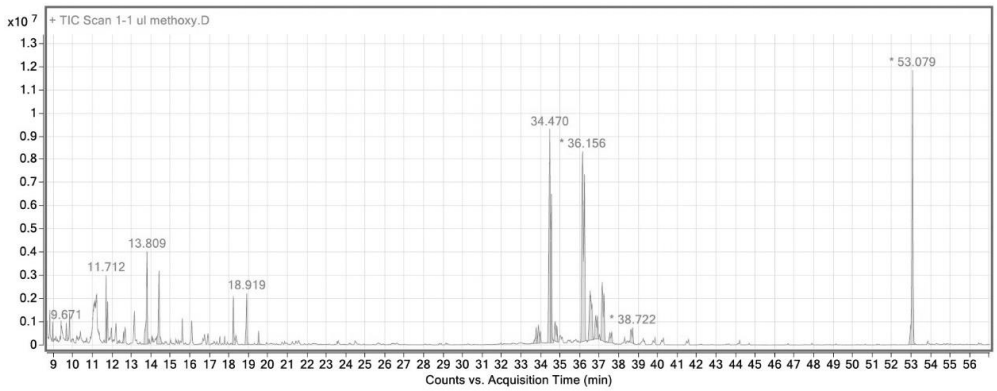

Figure 2. Compounds identified by GC-MS in *Q. macranthera* subsp. *sypirensis* leaf extract.

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[c][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	Cabonitrile
6	33.778	*Androst-5,7-dien-3-ol-17-one, acetate*	1.37	Steroid
7	34.453	*Myo-Inositol*	15.6	Phenol
8	34.550	*Scyllo-Inositol*	7.35	Phenol
9	35.002	*Androst-5-en-3-ol17-one, 16, 16-trimethylenedithio-*	1.58	Steroid
10	36.156	*Quininic 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	*Benzohydroxamic acid*	4.20	Acid
5	18.233	*Benzo[c][1,2,5]-thiadiazole, 4,5,6,7-tetramethyl-*	2.32	Cylopentapyrazoles
6	18.919	*Glycerol*	2.58	Sugar alcohol
7	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	Cabonitrile
8	33.778	*β-D-Glucopyranosiduronic acid*	1.13	Sugar
9	33.887	*Pregnane-3,17,20,21-tetrol, (3α,5β,17α,20α)-*	1.45	Steroid
10	34.470	*Myo-Inositol*	16.3	Phenol
11	34.550	*Scyllo-Inositol*	7.65	Phenol
12	34.750	*Shikimic acid*	2.01	Carboxylic acid
13	36.156	*Quininic acid*	22.8	Carboxylic acid
14	36.545	*D-(-)-Fructose*	6.51	Sugar
15	36.825	*D-(-)-Fructose*	2.73	Sugar
16	37.162	*D-(+)-Talose*	6.50	Sugar
17	37.648	*D-Allose*	1.22	Sugar
18	38.722	*D-Allofuranose*	1.63	Sugar
19	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.

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