

Investigation of Antioxidant Capacity, Some Phytochemicals and Antimicrobial Effects of *Thuja occidentalis* (AFS-Thuja) Plant

Thuja occidentalis (AFS-Thuja) Bitkisinin Antioksidan Kapasitesi, Bazı Fitokimyasalları ve Antimikrobiyal Etkilerinin İncelenmesi

Mustafa Yunus EMRE^{1*} , Muhammed GÜNGÖREN² , Ümmügülsüm TÜKENMEZ EMRE² 

¹ Mardin Artuklu University, Faculty of Health Sciences, Department of Nursing, Mardin

² Mardin Artuklu University, Vocational School of Health Services, Mardin

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*Sorumlu yazar/Corresponding author

Mustafa Yunus EMRE

e-mail: myemre@artuklu.edu.tr

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Abstract

Plants have been utilized by humans since ancient times for the treatment of various diseases. Among these, *Thuja occidentalis* has been reported to be traditionally used by indigenous populations in Canada for the treatment of scurvy. This plant, known for its therapeutic properties against a range of diseases, is predominantly distributed in Northeastern America. Owing to these characteristics, it has attracted considerable scientific interest, and the major constituents of its dried parts have been extensively investigated. Accordingly, the present study aims to evaluate the antioxidant activity, as well as the volatile and phenolic compositions, and the antimicrobial properties of *Thuja occidentalis*. The antioxidant activity, as determined by the DPPH radical scavenging assay, yielded an IC₅₀ value of 1.83. Among the volatile compounds, the most abundant constituents were identified as *p*-Cumic aldehyde in aldehydes, Carvotanacetone in ketones, Hexanoic acid in acids, Nonanoic acid methyl ester in esters, and D-carvone in terpenes. Among the phenolic compounds, catechin (28.320 mg/10 g extract) was found to be the predominant component in both methanolic and acetone extracts. Furthermore, the antimicrobial activity of *T. occidentalis* was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* strains. The results indicated that the plant exhibited significant antimicrobial effects in both solvents, with inhibition zone diameters ranging from 14 mm to 31 mm in a concentration-dependent manner.

Öz

Birçok bitki, insanlar tarafından çok eski çağlardan beri çeşitli hastalıkların tedavisinde kullanılmaktadır. *Thuja occidentalis* de bu bitkilerden biridir ve ilk olarak Kanada'daki yerli halk tarafından iskorbüt hastalığının tedavisinde kullanılmıştır. Hastalıklara karşı iyileştirici etkileri bilinen bu bitki, genellikle Kuzeydoğu Amerika'da yayılış göstermektedir. Bu özelliği nedeniyle bilim insanlarının dikkatini çekmiş ve kurutulmuş kısımlarının ana bileşenleri incelenmiştir. Bu çalışmada, *Thuja occidentalis*'in antioksidan aktivitesi, uçucu bileşenleri, fenolik bileşenleri ve antimikrobiyal etkileri araştırılmıştır. Antioksidan aktivite kapsamında DPPH radikal süpürme aktivitesi IC₅₀ değeri 1,83 olarak belirlenmiştir. Uçucu bileşenler içerisinde en yüksek miktara sahip bileşikler; aldehytler grubunda *p*-Cumic aldehyde, ketonlar grubunda Carvotanacetone, asitler grubunda Hexanoic acid, esterler grubunda Nonanoic acid methyl ester ve terpenler arasında D-Carvone olarak tespit edilmiştir. Fenolik bileşenler içerisinde ise Kateşin (28,320 mg/10 g ekstrakt) metanol ve aseton ekstraktlarında en yüksek miktarda bulunan bileşik olmuştur. Ayrıca *T. occidentalis* bitkisinin *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 ve *Candida albicans* ATCC 10231 suşları üzerinde antimikrobiyal etkisi incelendiğinde, her iki çözücüde de önemli aktivite gösterdiği ve konsantrasyona bağlı olarak 14–31 mm arasında değişen inhibisyon zonları oluşturduğu belirlenmiştir.

INTRODUCTION

Thuja occidentalis is a coniferous species belonging to the Cupressaceae family, generally distributed in eastern North America and cultivated as an ornamental tree in Europe, which can reach a height of approximately 15-20 m (Chang et al., 2000; Naser et al., 2005). This species is commonly known as *Arbor vitae* (tree of life) or white cedar among the public (Chang et al., 2000).



Figure 1. Images of the plant and fruits of the species *Thuja occidentalis* (American Arborvitae- White Cedar, 2026)

Many plants have been traditionally used by humans for medicinal purposes since ancient times. It is well documented that the first widespread use of plants for therapeutic applications occurred in China, India, Egypt, and Greece (Agbor et al., 2005). The species *T. occidentalis* was first used by indigenous peoples in Canada for the treatment of scurvy (Chang et al., 2000). Traditionally, *T. occidentalis* has been used to treat various conditions, including rheumatism, amenorrhea, cystitis, warts, psoriasis, bronchitis, uterine cancer, and diarrhea (Dubey et al., 2009a; Patil et al., 2018). Its anti-inflammatory effects have also been demonstrated in recent studies (Bharti et al., 2022; El-Sayed, 2023). In addition to its beneficial effects on human health, it has also been reported that *T. occidentalis* exhibits insecticidal and anthelmintic activity (Ahmad et al., 2013; Song et al., 2022). Products derived from extracts of the *Thuja* genus have been used therapeutically in a wide range of conditions, including intestinal parasitic infections, fungal diseases, and cancer (Sunila and Kuttan, 2006; Biswas et al., 2011). When *T. occidentalis* is used in combination with *B. tinctoria*, *E. pallida*, and *E. purpurea*, it has been reported to be effective against angina, acute bronchitis, and pharyngitis (Bakht et al., 2020).

Many medicinal plants also contain antioxidants, which help protect cells from the harmful effects of reactive oxygen species (Brewer, 2011). Medicinal plants with antioxidant effects contain phenolic compounds that protect the body against damage caused by free radicals (Kahkonen et al., 1999). These phenolic compounds in medicinal plants can function in living organisms as radical scavengers, oxygen scavengers, hydrogen donors, and metal chelators (Proestos et al., 2006). Reactive oxygen species and free radicals are constantly formed in the human body. While these structures can be beneficial for physiological activities, their presence in excessive amounts in the human body can be quite dangerous and harmful. These free radicals accumulating in the body generally negatively affect DNA, proteins, carbohydrates, and lipids (Sies, 1991). Any increase in free radicals in the human body causes stress and harmful effects. Therefore, there should be a balance between free radicals and antioxidants in the body (Jain et al., 2013).

The importance of medicinal plants is increasing day by day because their phytochemicals contain significant microbial and viral inhibitors. Due to these effects, many researchers find it vital to investigate the phytochemical properties of medicinal plants in order to treat diseases occurring in humans (Jasuja et al., 2012). Tannins, saponins, terpenoids, flavonoids, and alkaloids, which are among the bioactive components of plants, show significant antimicrobial activity (Sah et al., 2017). The importance of medicinal plants in the treatment of drug-resistant microorganisms that cause infectious diseases, which is a significant treatment problem, is quite great today (Valgas et al., 2007). Therefore, the extracts of medicinal plants are becoming increasingly important as they are important sources of viral inhibitors and microbial inhibitors (Jasuja et al., 2012).

Studies have indicated that *T. occidentalis* has a therapeutic effect against diseases (Dubey & Batra, 2009b). Due to this property, pharmacologists have focused on examining the main components of the dried parts of this plant (Witte et al., 1983; Tsiri et al., 2009). Although there are some differences in the actual components of species belonging to the *Thuja* genus, in most species of *T. occidentalis*, essential oils are found as the main component in proportions ranging from 1.4% to 4%. In addition, other components include coumarins (*p*-coumaric acid, umbelliferone), flavonoids (catechin, gallic acid, etc.), tannic acid, polysaccharides, and proteins (Naser et al., 2005).

Studies have shown that *T. occidentalis* is quite effective against diseases; therefore, to reveal the difference caused by changes in climate, soil structure, etc. in its growing environment, we investigated the antioxidant and antimicrobial effects of the seeds of this species grown in Mardin province by extracting them separately in two different solvents (methanol and acetone). In addition to these effects, we determined its volatile components using gas chromatography (GC/MS) and its phenolic content using HPLC, thus identifying some important phytochemical components. This study is of great importance in terms of revealing the differences in *T. occidentalis* plants grown in Mardin province, which has different climatic conditions, as a result of different environments, showing the differences between extracts obtained with different solvents, and identifying its phytochemical components.

MATERIALS AND METHODS

Plant Material

In our study, the fruit parts of the *T. occidentalis* plant have been used. The fruit of this plant were collected from Artuklu district of Mardin province in 2025, dried in a dark environment at room temperature, and stored in the refrigerator until the study was conducted.



Figure 2. Seeds of *T. occidentalis* plant used in our study

Study Chemicals

To ensure the accuracy of the analyses included in our study and their repeatability in case of a possible problem, our chemicals with a high degree of purity have been selected from Merck Millipore (Germany), Merck EMSURE (Germany), ISOLAB (Germany) and J.T. Baker (USA) companies.

Preparation of Plant Extract

In our study, we ground the seeds of the *T. occidentalis* plant until they became a powder in a porcelain mortar. Then, we took 10 grams of this powdered seed mixture, added 10 times the weight of the plant in our solvents (methanol/acetone),

and let it incubate for 24 hours at room temperature in an orbital shaker at 100 rpm. After 24 hours, the solvent-plant mixture was filtered through Whatman No:1 filter paper and transferred to ground glass Erlenmeyer flasks. The evaporation (Hei-VAP Expert/Heidolph, Germany) process was carried out using an evaporator until the solvents were completely removed. At the end of this process, the plant extracts that collected as a thin layer on the glass surface were carefully collected with a spatula, weighed, and stored in tubes at +4°C until the experiments were conducted (Tawaha et al., 2007).

Determination of Antioxidant Properties

To determine the total antioxidant (TAC) property of *T. Occidentalis* plant, methods for determining the 1, 1-diphenyl-2-picryl hydrazyl (DPPH Sigma-Aldrich, Germany) free radical scavenging capacity and total phenolic content were used, and the analyses were performed using spectrophotometric analysis. This method was first used by M. S. Blois in 1958 and later improved by Sánchez-Moreno et al. (1998). This analysis is based on measuring the capacity to reduce the stable DPPH free radical as a result of its reaction with antioxidants.

0.3 mL of each extract was taken from plant concentrates prepared at different intensities, halved, and 2.7 mL of DPPH was added. The extracts were then incubated at room temperature in the dark for 30 minutes. After the incubation period, absorbance values were measured at 517 nm using a UV-VIS spectrophotometer (Biochrom Libra S70, Cambridge, England). Trolox was used as a positive control, and each group was tested in three parallels. IC₅₀ values were calculated using the obtained calibration curves, and the DPPH removal activity of the extracts was given as Trolox equivalent (TE).

The total phenolic content (TPC) of our plant extracts was determined using the Folin–Ciocalteu method, in accordance with the literature (Capanoglu & Beekwilder, 2013). For TPC analysis of our plant extracts, 100 µL of extract was taken from each sample, 900 µL of distilled water was added, and then 5 mL of 0.2 N Folin–Ciocalteu reagent was added. This solution was left in the dark for approximately 8 minutes, then 5 mL of sodium carbonate (Na₂CO₃) was added and vortexed. The mixtures were incubated at room temperature for 2 hours, and at the end of this period, absorbance values were measured using a UV–VIS spectrophotometer (Biochrom Libra S70, Cambridge, England) at a wavelength of 765 nm. The TPC levels of our plant were determined by creating a calibration curve with a gallic acid standard solution.

Volatile Component Analysis

Volatile components of *T. occidentalis* plant were determined using the Head Space-Solid Phase Microextraction (HS-SPME) method with a Shimadzu QP-2020 GC-MS (Kyoto, Japan) instrument (Genovese et al. 2015). To determine the volatile components of the plant, 2 grams of the plant were weighed into an SPME vial. This vial was kept at 40 °C for 30 minutes, and then an SPME fiber (Supelco, 2 cm) was placed inside the vial and allowed to adsorb at the same temperature for 30 minutes. After adsorption, the fiber was left to desorb at 250 °C for 5 minutes in the GC-MS injection port. Helium gas with a flow rate of 1.05 mL/min was used as the carrier gas, and chromatographic separation was performed using splitless injection mode. The oven temperature program of the device, started at 40 °C, was increased by 4 °C per minute up to 240 °C and held at this temperature for 6 minutes (Korkmaz, 2023). Isobutyl acetate was used as the internal standard and the concentration of volatiles was calculated relatively.

Analysis of Phenolic Compounds

High-Performance Liquid Chromatography (HPLC) system was used to determine phenolic compounds (Veneziani et al. 2018). Before starting the analysis, the prepared extracts were passed through a 0.45 µm PVDF membrane filter to prevent particle accumulation from affecting the column, and 20 µL from each sample was taken and injected into the Waters

Alliance E2695 HPLC system (USA). Separation was performed using a reverse-phase C18 column (5 µm, 4.6 × 250 mm; GL Sciences, Tokyo, Japan). *p*-coumaric, caffeic, vanillic, rosmarinic, gallic acids, and Catechin solutions were used as standards in the analysis of phenolic components. Chromatographic data were evaluated using Empower 3 software after measurements were taken at a wavelength of 280 nm with a Photodiode Array (PDA) detector (Waters 2996, Milford, MA, USA). The amounts of phenolic compounds we obtained were calculated in mg/10g.

Determination of Antimicrobial Effects

The antimicrobial effects of *T. occidentalis* plant were determined using one Gram-negative (*Escherichia coli* ATCC 25922) bacterial strain, one Gram-positive (*Staphylococcus aureus* ATCC 25923) bacterial strain, and one standard yeast (*Candida albicans* ATCC 10231) strain. Bacterial and yeast samples were cultured in sterile Nutrient Broth (Merck), Nutrient Agar (Merck), Sabouraud Dextrose Broth (Merck), and Sabouraud Dextrose Agar (Merck) media, respectively. As positive controls, discs containing Erythromycin 15 µg/disk (Bioanalyse®), Ciprofloxacin 1 µg/disk (Bioanalyse®), and Nystatin 100 µg/disk (Bioanalyse®) were selected, while DMSO (20%), used to adjust the concentration of the plant extract, was selected as a negative control. Using the disc diffusion method (NCCLS, 1997), 10 µL of different concentrations (50, 100 mg/mL) of acetone and methanol extracts were applied to sterile blank paper discs with a diameter of 6 mm (Bioanalyse, Ankara, Türkiye). For incubation, bacterial strains were kept in an oven at 37 °C for 24 hours, and yeast strains at 30 °C for 48 hours, and the diameters of the resulting inhibition zones were measured.

RESULTS AND DISCUSSION

Antioxidant Capacity

To determine the total antioxidant capacity (TAC) of *T. occidentalis* plant, DPPH radical scavenging activity (RAS) and total phenolic content (TPC) methods were used. The results obtained are given in Table 1.

Table 1. Antioxidant capacity of *T. occidentalis* plant

Samples	TPC (mg/g)	RAS (µg/mL)	IC ₅₀ (µg/mL)
<i>T. occidentalis</i> M	357.43 ± 8.11	1690.89 ± 3.36	1.83
<i>T. occidentalis</i> Ac	332.85 ± 4.60	1694.76 ± 6.71	6.25

M: Methanol, Ac: Acetone

The total phenolic acid content was calculated according to the gallic acid reference and the R²=0.993, while the DPPH scavenging capacity was calculated according to the trolox reference and the R²=0.998 (Table 2).

Table 2. Trolox's % radical scavenging activity and absorbance measurements of gallic acid standard used in TPC calculations

Trolox (mg/mL)	Trolox (% RAS)	Gallic Acid (µg/mL)	Gallic Acid Abs.
100.00	52.77	1000.00	1.058
50.00	27.80	500.00	0.582
25.00	12.15	125.00	0.083
12.50	6.56	62.50	0.069

When we examined the RAS and TPC values of *T. occidentalis*, we observed that it exhibited high antioxidant properties. Looking at previous studies, the TPC value in methanolic extracts of *T. occidentalis* leaves was determined as 135.32 mg GAE/g (Nazir et al., 2016). When we examined fruit samples of this plant grown in Mardin, the TPC value was measured as 357.43 mg/g. In another study, the DPPH radical scavenging activity of *T. occidentalis* leaves extracted in methanol was

investigated. The IC₅₀ value for DPPH was calculated as approximately 470 µg/mL (Nazir et al., 2016). In our study, the DPPH radical scavenging activity of the fruit of this plant was investigated, and the IC₅₀ value was calculated as 1.83 µg/mL. These results show that the fruit has much higher RAS and TPC values compared to the leaves.

In a study conducted by Yogesh and Ali in 2012, the antioxidant capacity of the fruit of *T. occidentalis* was determined by water extraction, and the total phenolic content was calculated as 7.80 ± 0.04 mg/gr (Yogesh & Ali, 2014). However, in our study, due to the difference in solvent, it showed much better antioxidant properties, as seen in Table 1.

When we compare the data obtained from the literature review of *T. occidentalis* with the results we obtained from our study, it is seen that, in addition to the environment in which this plant grows, the solvent from which this plant is extracted also creates significant differences in terms of RAS, TPC and antimicrobial properties.

Volatile Component Analysis

The volatile components of *T. occidentalis* plant were measured chromatographically, and the chromatogram was obtained as shown below (Figure 3).

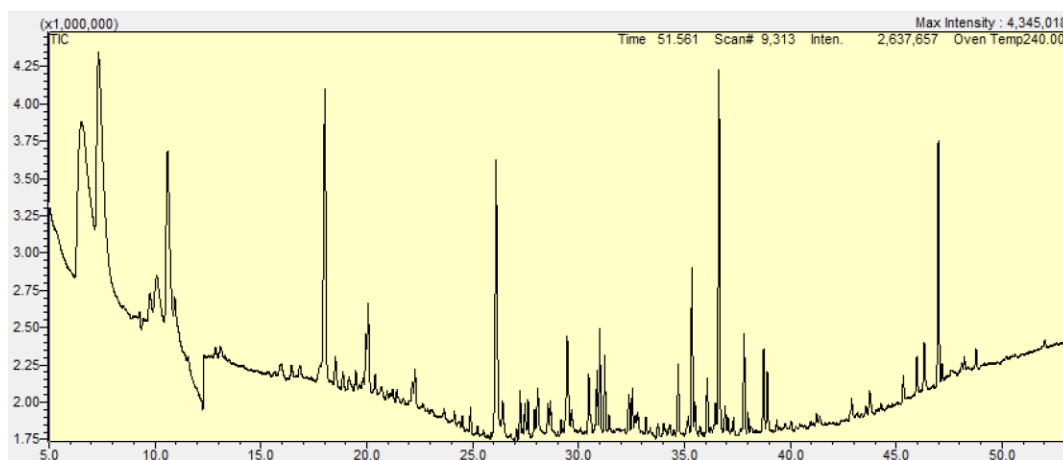


Figure 3. Chromatogram of *T. occidentalis* plant obtained from a GC-MS device

When the volatile components of the fruit of the *T. occidentalis* plant were analyzed using a GC-MS device, a total of 70 compounds were detected, of which 9 were alcohols, 7 were aldehydes, 7 were ketones, 4 were acids, 7 were esters, 12 were terpenes, and 24 were various other components (Table 3).

Among alcohols, methanol had the highest amount (10.7406 mg/kg), while among aldehydes, *p*-cuminaldehyde (0.9465 mg/kg) and 2-nonenal, (E)- (0.5781 mg/kg) were found in the highest amounts. Among ketones, the compounds with the highest amounts were carvotanacetone (2.0588 mg/kg), while among acids, hexanoic acid (CAS) had the highest amount (0.5225 mg/kg). Among esters, nonanoic acid, methyl ester had the highest amount (1.2706 mg/kg), while among terpenes, D-carvone (3.3646 mg/kg) and α-guaiene (1.4868 mg/kg) were the compounds with the highest amounts. In addition to these, lots of other components were found, the ones present in the highest amounts being Dodecane (3.8980 mg/kg), Tetradecane (3.8459 mg/kg), and Dodecane, 4,6-dimethyl- (2.2989 mg/kg), respectively (Table 3).

When the effects of the compounds we identified with the GC-MS device were examined, carvones, which are ketone compounds and were the most abundant among ketones in our study, have antibacterial, antifungal, anti-inflammatory, antioxidant, and anticancer effects found in most essential oils (Pina et al., 2022). Besides, Caryophyllene, measured in significant amounts, is the main sesquiterpene found in essential oils and is known to have antioxidant, anticancer, antimicrobial and anti-inflammatory properties, as well as protective and strengthening effects on many systems in the human body (Machado et al., 2018).

Table 3. List of volatile components of *T. occidentalis* plant detected by GC-MS device

Compound Name	RT	RI	Amount
ALCOHOLS			
Methanol (CAS)	7.330	945	10.7406
1-Hexanol, 2-ethyl- (CAS)	29.179	1463	0.1206
7-Octen-2-ol, 2,6-dimethyl-	28.566	1445	0.2514
Linalool, formate	31.006	1519	0.7896
1-Octanol	31.241	1527	0.6098
trans-2-Dodecen-1-ol	35.741	1683	0.1061
1-Dodecene (CAS)	34.009	1621	0.1483
1-Nonanol	34.110	1624	0.0753
Phenol, 2-methoxy-4-(2-propenyl)- (CAS)	46.331	2130	0.5145
ALDEHYDES			
Hexanal (CAS)	13.098	1101	0.1564
Octanal	22.159	1281	0.2841
2-Nonenal, (E)-	30.877	1515	0.5781
p-Cumic aldehyde	37.823	1764	0.9465
2-Furancarboxaldehyde (CAS)	28.675	1448	0.2681
p-Anisic aldehyde	43.570	2008	0.1166
Decanal	29.672	1478	0.2539
KETONES			
5-Hepten-2-one, 6-methyl-	24.140	1327	0.0866
2-propanoate Propionic acid	26.419	1385	0.5776
(-)-Alcanfor	30.488	1502	0.6774
3-Cyclohexen-1-one, 2-isopropyl-5-methyl-	36.469	1711	0.4006
Carvotanacetone	46.993	2159	2.0588
Acetone, geranyl-	39.366	1826	0.0755
cis-Dihydrocarvone	33.190	1592	0.1464
ACIDS			
Acetic acid (CAS)	28.080	1431	0.4975
Hexanoic acid (CAS)	38.918	1807	0.5225
Octanoic acid	43.752	2016	0.3134
Nonanoic acid (CAS)	45.977	2114	0.3338
ESTERS			
Nonanoic acid, methyl ester	29.471	1472	1.2706
Decanoic acid, methyl ester	32.546	1570	0.3876
Dodecanoic acid, methyl ester	38.007	1771	0.1474
Acetic acid, isobutyl ester	39.750	1842	0.0712
Oleic acid, methyl ester	52.022	2381	0.0677
Dipropyl sulphite	19.969	1235	0.5654
Hexadecanoic acid, methyl ester	47.176	2167	0.1476
TERPENES			
Caryophyllene	32.708	1576	0.1859
cis-Dihydrocarvone	33.190	1592	0.1464
γ-Murolene	35.179	1663	0.2497
α-Guaiene	35.359	1670	1.4868
α-Murolene	36.082	1696	0.5821

Ar-tumerone	48.223	2213	0.1032
D-Carvone	36.634	1717	3.3646
Farnesan	21.421	1266	0.1502
α -Curcumene	37.326	1744	0.1312
(-)-Terpinen-4-ol	32.801	1579	0.1952
(+)- δ -Cadinene; δ -Cadinene	36.925	1729	0.3042
Trans-Anethole	38.739	1800	0.6945
MISCELLANEOUS			
Dodecane, 4, 6-dimethyl-	10.939	1051	2.2989
Nonane, 5-(2-methylpropyl)-	12.851	1095	0.1150
Pentadecane (CAS)	15.961	1155	0.2880
Undecane, 3-methyl-	16.458	1165	0.1574
Dodecane	18.026	1195	3.8980
Dodecane, 4-methyl-	18.521	1205	0.3049
Tridecane	19.168	1218	0.1898
Dodecane, 2-methyl-	20.403	1244	0.1917
Benzene, (2, 3-dimethyldecyl)-	20.673	1250	0.1294
m-Cymol	21.236	1262	0.1157
2-Methyloctadecane	24.514	1337	0.1073
Hexadecane (CAS)	24.899	1347	0.2003
Heptadecane	25.237	1355	0.0609
Tetradecane	26.115	1377	3.8459
Heneicosane	26.806	1394	0.0773
Benzene, 1,3-bis(1,1-dimethylethyl)-	27.247	1406	0.3994
Decyl ether	27.468	1413	0.3724
Octadecane (CAS)	27.619	1417	0.3984
Tridecane, 1-iodo-	27.918	1426	0.2228
2-Pentadecanone, 6,10,14-trimethyl-	45.342	2086	0.2502
Methyleugenol	42.903	1978	0.2137
Phenol, 2,4-bis-(1,1-dimethylethyl), TMS	48.779	2238	0.1651
2-pentadecyl-4,4,5,5-tetradeutero-1,3-dioxolane	33.749	1611	0.1651
Benzene, 1-methoxy-4-(2-propenyl)- (CAS)	34.710	1646	0.6200

RT: Retention Time (min), **RI:** Retention Index, **Amount:** mg/kg **dry** plant

Aldehydes, which are aroma components, are important compounds in terms of biological activity and food. Cumic aldehydes, which are among the aldehydes, play a role in the manifestation of many pharmacological properties such as immunomodulatory, hypotensive, hepatoprotective, analgesic, antimicrobial, antioxidant, anti-inflammatory, wound healing, antidiabetic, hypolipidemic, anti-obesity, anti-osteoporosis, antispasmodic, anti-asthmatic, antipyretic, and anticancer (Singh et al., 2025). In our study, *p*-Cumic aldehyde, which belongs to this group, was the type of aldehyde found in the highest amount, too. In their Salem et al. (2014) study, investigated fatty acid methyl esters obtained from air-dried wood, bark, and leaves of *Brachychiton diversifolius* R. Br. and determined their antibacterial, antifungal, and antioxidant activities (Salem et al., 2014). In our study, a total of 8 ester compounds were identified, 5 of which were methyl ester compounds.

Phenolic Compounds

When we examined the phenolic compounds we tested from the plant *T. occidentalis*, Catechin (28.320 mg/10g) was the compound with the highest amount. When we examined the results of the plant extracted in acetone, catechin was not detected, while vanillic acid (6.775 mg/10g) and rosmarinic acid (6.268 mg/10g) were the compounds detected in the highest amounts. When we examined the methanol extracts, catechin (28.320 mg/10g) was detected in the highest amount (Table 4).

Table 4. Amounts of phenolic compounds in *T. occidentalis* plant (mg/10g ext.)

Plant Extracts	Catechin	Caffeic Acid	Vanillic Acid	<i>p</i> -Coumaric Acid	Gallic acid	Rosmarinic Acid
<i>T. occidentalis</i> Ac	-	1.359	6.775	0.612	0.858	6.268
<i>T. occidentalis</i> M	28.320	1.612	6.929	0.647	1.300	6.561

M: Methanol, Ac: Acetone

While the amount of catechin was not detected in the plant extracted in acetone, it was found in the highest amount in the plant extracted in methanol. Catechin is known to have many health effects such as anti-obesity, anti-diabetic, anti-tumor, anti-inflammatory, antioxidant, and antimicrobial properties (Beltz et al., 2006; Yang et al., 2009; Singh et al., 2011). Vanillic acid, which was detected in similar amounts in both extracted plant samples, has been shown in studies to have significant antioxidant activity (Kähkönen et al. 1999). In addition, vanillic acid is also known to have pharmacological antifilarial, antimicrobial, anti-inflammatory, antioxidant, and snake venom inhibitory effects (Amin et al., 2017). Similarly, studies have shown that rosmarinic acid, detected in similar amounts, exhibits significant anticancer and antioxidant properties (Kowalczyk et al., 2024). Caffeic acid, which we detected in both of our extract samples, is known to be abundant in fruits, vegetables, and beverages, and is considered important for health because it reaches significant concentrations in the colon (up to 126 µM) and exhibits anti-inflammatory effects (Zielińska et al., 2021).

Antimicrobial Tests

E. coli, *S. aureus*, and *C. albicans* strains were used to determine the antimicrobial properties of *T. occidentalis* plant, and the results obtained are given in Table 5.

Table 5. Antimicrobial test results of *T. occidentalis* plant

Strains	Ciprofloxacin (1 µg/disk)	Nystatin (100µg/disk)	Erythromycin (15 µg/disk)	Negative Control	1M	2M	1Ac	2Ac
<i>E. coli</i>	30±0.57	TE	TE	0	31±0.57	29±0.57	31±0	29±1
<i>S. aureus</i>	TE	TE	28±0.57	0	21±0.57	18±0.57	21±0.57	18±1
<i>C. albicans</i>	TE	27±0.47	TE	0	17±1	14±0	16±0.57	16±0.57

1M: 100 mg/ml concentration of *T. occidentalis* plant extracted in methanol, 2M: 50 mg/ml concentration of *T. occidentalis* plant extracted in methanol, 1A: 100 mg/ml concentration of *T. occidentalis* plant extracted in acetone, 2A: 50 mg/ml concentration of *T. occidentalis* plant extracted in acetone, TE: not tested, 0: no effect

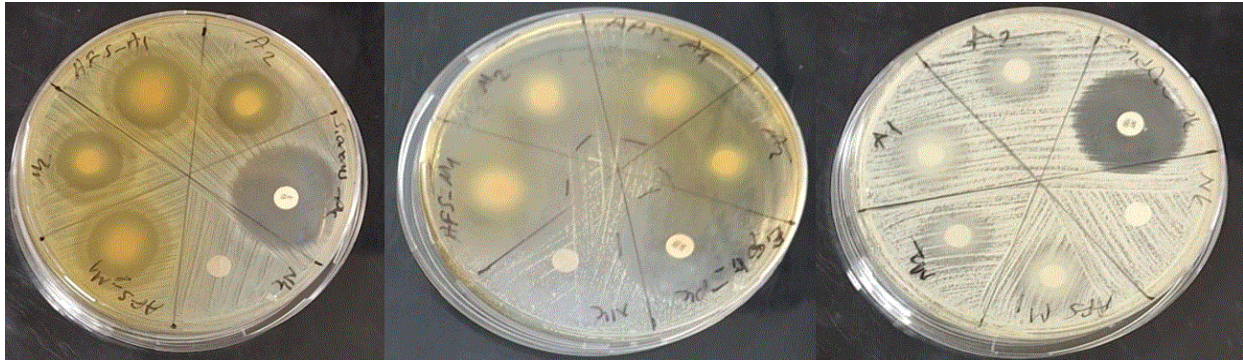


Figure 4. Antimicrobial effect of *T. occidentalis* plant on *E. coli*, *S. aureus*, and *C. albicans* strains

When the effects of *T. occidentalis* plant on the Gram-negative strain *E. coli* were examined, it was observed that the positive control formed a 30 mm inhibition zone, while the sample extracted in metonal formed a 31 mm inhibition zone at concentration 1 and a 29 mm inhibition zone at concentration 2. When the plant sample extracted in asteon was examined, the positive control formed a 31 mm inhibition zone, with concentration 1 forming a 31 mm zone and concentration 2 forming a 29 mm zone. No inhibition zone was observed in any of the negative controls.

When the effects of *T. occidentalis* plant on *S. aureus* bacteria, a Gram-positive strain, were examined, it was observed that the positive control formed an inhibition zone of 28 mm, while the sample extracted in methonal formed an inhibition zone of 21 mm at concentration 1 and 18 mm at concentration 2. When the plant sample extracted in acetone was examined, it was observed that the positive control formed an inhibition zone of 28 mm, the first concentration formed a 21 mm inhibition zone, and the second concentration formed an 18 mm inhibition zone. No inhibition zone was observed in any of the negative controls.

When the effects of *T. occidentalis* plant on the *C. albicans* yeast strain were examined, it was observed that the positive control formed an inhibition zone of 27 mm, while the sample extracted in methanol formed an inhibition zone of 17 mm at concentration 1 and 14 mm at concentration 2. When the plant sample extracted in asteon was examined, the positive control formed an inhibition zone of 27 mm, while the inhibition zone was 16 mm at concentration 1 and 16 mm at concentration 2. No inhibition zone was observed in any of the negative controls.

In 2017, Ayoub and Hamid extracted the seeds of *T. occidentalis* plant in various solvents (petroleum ether, chloroform, ethyl acetate, methanol) and studied their antimicrobial activity against several strains, including *E. coli*, *S. aureus*, and *C. albicans*, which were also included in our study. The inhibition zones obtained from this study varied between 20 ± 0.57 mm and 12 ± 1.25 mm in different extracts (2017).

Ololade et al. (2014) investigated the antimicrobial effects of essential oils from the fruit of the *T. occidentalis* plant on several strains. Two of these strains are the bacteria also included in our study. When comparing our results with that study, it is seen that our sample has a more effective antimicrobial effect on *E. coli* and *S. aureus*, although there are differences depending on the concentration. When our study results are examined, the antimicrobial activity of the seeds extracted with methanol as a common solvent was also determined with acetone-based solvents. It is seen that the results obtained with both solvents are more effective than those in this study.

When the essential oil of *T. occidentalis* fruit is compared with other species in the Cupressaceae family, similar results are observed. In our study, the zones we obtained were between 21 mm and 17 mm for *S. aureus*, while they were between 3.2 mm and 28 mm for *E. coli*. *T. plicata* has an inhibition diameter of 30 mm on *S. aureus* and 11 mm on *E. coli* (Pham-Huy et al., 2008). When the results of *T. plicata* are examined, it is more effective on *S. aureus* than *E. coli*, but in

our study, the fruit extract of *T. occidentalis* was more effective on *E. coli*. In *Thuja koraiensis* extract, the zone was 17 mm on *S. aureus* and 15 mm on *E. coli*. This plant was also more effective against *E. Coli* (Pham-Huy et al., 2008).

The essential oil of *T. orientalis* fruit yielded comparable results to other related species in the Cupressaceae family, such as *T. occidentalis*: *S. aureus* (17 mm), *E. faecalis* (11 mm), *E. coli* (11 mm), *P. vulgaris* (-), *P. aeruginosa* (9 mm), *Salmonella* sp. (11 mm), *K. pneumoniae* (13 mm). Furthermore, *Thuja plicata* showed the following activity against the following organisms: *S. aureus* (30 mm), *E. faecalis* (10 mm), *E. coli* (11 mm), *P. vulgaris* (7 mm), *P. aeruginosa* (10 mm), *Salmonella* sp. *Thuja koraiensis* extract showed the following inhibition zones against *S. aureus* (17 mm), *B. subtilis* (13 mm), *E. coli* (15 mm) and *S. typhimurium* (12 mm) and contributed to disease prevention (Pham-Huy et al., 2008).

In a study conducted by Kamona, Z.K. in 2011, the antimicrobial activity of hot water and alcohol extracts of *Thuja orientalis* fruit at different concentrations was tested against various pathogenic microorganisms. The results showed that both the water and alcohol extracts had antimicrobial activity with varying effects, but the alcohol extract showed significantly higher activity than the water extract. In this study, the highest antimicrobial activity was observed against *S. aureus* and *C. albicans*, with zones reaching up to 16 mm (Kamona, 2011). In our study, similar results were obtained against *C. albicans*; acetone extracts formed zones of 15-16 mm, while methanol extracts formed zones between 14 mm and 18 mm, depending on the concentration of the plant extract.

CONCLUSION

When we extracted the fruit of the *T. occidentalis* plant, grown in Mardin, in different solvents (methanol, acetone) and examined the results, we observed some significant differences which we believe to be due to the difference in solvents and growing environment. In this study, the antioxidant capacity, volatile components, phenolic compound contents, and antimicrobial activity of this plant were determined. In *T. occidentalis*, a plant with very high antioxidant capacity, the volatile components detected in the highest amounts were methanol (10.7406 mg/kg), D-carvone (3.3646 mg/kg), hexanoic acid (CAS) (0.5225 mg/kg), nonanoic acid, methyl ester (1.2706 mg/kg), and D-carvone (3.3646 mg/kg). The amounts of phenolic compounds p-coumaric acid, caffeic acid, vanillic acid, catechin, gallic acid, and rosmarinic acid were investigated. Except for catechin, all components were detected in both solutions; however, no significant differences were observed in the obtained amounts due to the difference in solvents. The antimicrobial effects of *T. occidentalis* were investigated on 3 strains, and it was found to be lethal against all of them, with the greatest lethal effect against *E. coli* bacteria. Specifically, the methanol extract was found to be more effective than the acetone extract in terms of antimicrobial activity.

When these determined properties of *T. occupationalis* are compared with similar studies in the literature, although its antioxidant properties and volatile components yield similar results, it has been found to be more effective in terms of antimicrobial properties, and the methanol extract is significantly more effective depending on the solvent used. Because of these properties we have identified, we believe that this species may have significant health implications. Therefore, we believe that research on the effects of this plant on cancer research and on experimental animals will make a significant contribution to the literature.

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