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# Investigation of Antimicrobial, Antioxidant and Enzyme Inhibitory Activity of *Pistacia terebinthus* Gall

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## Abstract

This study evaluates the antioxidant, antimicrobial, and enzyme inhibitory activities of *Pistacia terebinthus* L. gall induced by *Slavum* aff. *mordvilkoi*. The galls were extracted using petroleum ether, acetone, ethanol, and water. Antioxidant activity was assessed via DPPH, ABTS, and CUPRAC assays, along with total phenolic and flavonoid content determination. Antimicrobial activity was tested against Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria, as well as yeast (*Candida albicans*). Enzyme inhibition was evaluated for acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, and urease. The results indicate that acetone and ethanol extracts exhibited the highest antioxidant activity, while the water extract also demonstrated significant activity in specific assays. Antimicrobial testing revealed that Gram-positive bacteria and *C. albicans* were the most susceptible, whereas *E. coli* exhibited resistance. For enzyme inhibition, acetone and ethanol extracts showed strong inhibitory effects on AChE, BChE, and tyrosinase, while the water extract exhibited the highest urease inhibition. These findings suggest that *P. terebinthus* gall extracts could serve as natural antioxidants, antimicrobials, and enzyme inhibitors. Future studies should focus on isolating bioactive compounds and exploring their potential applications in pharmaceuticals, food preservation, and cosmetics.

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***Keywords:*** *Pistacia terebinthus*; Gall; Antimicrobial activity; Antioxidant activity; Enzyme inhibition.

## *Pistacia terebinthus* Galinin Antimikrobiyal, Antioksidan ve Enzim İnhibitör Aktivitesinin Araştırılması

**Öz**

Bu çalışma, oluşumu *Slavum* aff. *mordvilkoi* tarafından indüklenen *Pistacia terebinthus* galinin antioksidan, antimikrobiyal ve enzim inhibisyonu aktivitelerini değerlendirmektedir. Gal ekstreleri petrol eteri, aseton, etanol ve su kullanılarak elde edilmiştir. Antioksidan aktivite, DPPH, ABTS ve CUPRAC yöntemleri ile belirlenmiş, toplam fenolik ve flavonoid içerikleri ölçülmüştür. Antimikrobiyal etkinlik, Gram-pozitif (*Staphylococcus aureus*, *Streptococcus pyogenes*), Gram-negatif (*Escherichia coli, Pseudomonas aeruginosa*) bakterilere ve mayaya (*Candida albicans*) karşı test edilmiştir. Enzim inhibisyonu, asetilkolinesteraz (AChE), bütirilkolinesteraz (BChE), tirozinaz ve üreaz enzimleri üzerinde değerlendirilmiştir. Sonuçlar, aseton ve etanol ekstrelerinin en yüksek antioksidan aktiviteye sahip olduğunu, ancak su ekstraktının da belirli testlerde yüksek aktivite gösterdiğini ortaya koymuştur. Antimikrobiyal testlerde, Gram-pozitif bakteriler ve *C. albicans* en duyarlı mikroorganizmalar olurken, *E. coli* dirençli bulunmuştur. Enzim inhibisyonu açısından, aseton ve etanol ekstreleri AChE, BChE ve tirozinaz üzerinde güçlü etki gösterirken, su ekstraktı üreaz enzim inhibisyonunda en yüksek aktiviteye sahip olmuştur. Bu bulgular, *P. terebinthus* gal ekstrelerinin doğal antioksidan, antimikrobiyal ve enzim inhibitörü olarak değerlendirilebileceğini göstermektedir. Gelecekte yapılacak çalışmalar, biyoaktif bileşiklerin izolasyonu ve farmasötik, gıda koruma ve kozmetik alanlarında potansiyel uygulamalarına odaklanmalıdır**.**

***Anahtar Kelimeler:*** *Pistacia terebinthus*; Gal; Antimikrobiyal aktivite; Antioksidan aktivite; Enzim inhibisyonu.

### 1. Introduction

Medicinal plants have been an essential part of traditional medicine for centuries, and their therapeutic properties have been widely documented. The search for bioactive compounds from plants has gained significant momentum in modern science, leading to numerous studies on plant-derived secondary metabolites with pharmacological applications. Many plant species produce bioactive compounds, including flavonoids, phenolic acids, tannins, and terpenoids, which contribute to their antimicrobial, antioxidant, and anti-inflammatory properties [1]. These compounds play a crucial role in drug discovery, offering alternatives to synthetic drugs that often present side effects and risks of resistance [2].

*Pistacia terebinthus* L. (Anacardiaceae) is a widely distributed plant species in the Mediterranean and Middle Eastern regions. It has been traditionally used for its medicinal properties, including its applications in treating respiratory diseases, gastrointestinal disorders, and skin ailments. Various parts of *P. terebinthus*, such as leaves, fruits, and resin, contain bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant activities [3].

Galls are abnormal growths on plants induced by various organisms such as insects, fungi, bacteria, and mites. These structures serve as a protective environment for the inducing organism while also causing significant chemical changes in the host plant [4]. Galls induced by insects, particularly those from the family Aphididae, are known to be rich in secondary metabolites such as tannins, flavonoids, and phenolic acids, which have been associated with numerous biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects [5].

The galls of *Pistacia* species, including *P. terebinthus*, have been traditionally used in folk medicine for their therapeutic properties. Studies suggest that these galls contain higher concentrations of bioactive compounds than other parts of the plant due to their unique chemical transformations during gall formation [6]. The galls of *P. terebinthus*, which develop as a result of interactions with gall-inducing insects, are particularly rich in tannins and phenolic compounds, contributing to their high biological activity. Despite their potential, limited studies have been conducted on the bioactive properties of *P. terebinthus* galls, necessitating further investigation [7].

Reactive oxygen species (ROS), such as superoxide ($O_2 \cdot^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals ($OH\cdot$), are continuously produced under aerobic conditions. These molecules play an essential role in cell signaling and homeostasis; however, their excessive accumulation can result in oxidative stress, leading to DNA damage, protein oxidation, lipid peroxidation, and ultimately cellular dysfunction which are key factors in the pathogenesis of many chronic diseases, including cancer, cardiovascular diseases, neurodegenerative disorders, and diabetes [8]. The interest in antioxidants has increased due to their potential to prevent and delay these diseases by scavenging ROS and reducing oxidative damage; moreover, the growing concern over the side effects of synthetic antioxidants has further intensified the interest in naturally derived antioxidants as safer and more effective alternatives [9].

The increasing antimicrobial resistance crisis has fueled interest in natural antimicrobial agents as potential alternatives to conventional antibiotics. The overuse and misuse of antibiotics have led to the emergence of multidrug-resistant pathogens, posing significant challenges in clinical treatment. Plant-derived antimicrobial agents, such as phenolics and flavonoids, exhibit broad-spectrum activity against bacteria, fungi, and viruses by disrupting microbial cell walls, inhibiting enzymes, and interfering with nucleic acid synthesis [10].

Several studies have demonstrated that *P. terebinthus* extracts exhibit strong antimicrobial activity against pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. However, the antimicrobial potential of its galls remains underexplored, warranting further investigation into their bioactive components and mechanisms of action [11].

Alzheimer's Disease (AD) is the most common type of dementia, characterized by cognitive impairments and severe behavioral disorders. It is a progressive neurodegenerative brain disease that ultimately leads to an individual's death [12]. Cholinesterase inhibitors (ChEIs) and Memantine (MEM) are the currently approved drugs that temporarily improve AD symptoms. However, an effective treatment for preventing or curing AD remains elusive. Given the increasing prevalence of AD, there is an urgent need for the discovery of new disease-modifying agents.

Tyrosinase is a copper-containing enzyme that plays a key role in the biosynthesis of melanin, which determines skin and hair color. The inhibition of tyrosinase is clinically used in the treatment of skin diseases associated with melanin hyperpigmentation. Additionally, it is important in cosmetics due to its skin-lightening effect. Although several synthetic inhibitors, such as kojic acid, have been developed to inhibit tyrosinase activity, their long-term toxic effects have accelerated research into identifying natural alternatives [13].

Urea is the most commonly used fertilizer in agricultural applications. The use of urease inhibitors in agriculture has long been studied as one of the strategies to ensure sufficient food supply. In addition to the agricultural industry, urease activity is also a significant viral marker in medicine. Ureases contribute to the formation of kidney stones and infections and can also cause hepatic coma [14]. Research is ongoing on urease inhibitors that can inhibit the urease enzyme for the treatment of such diseases [15]. Studies on urease inhibitors are of great importance for the development of drugs to treat diseases caused by urease-containing pathogens in humans and animals [16]. In recent years, various plant extracts have been investigated for their inhibitory effects on the urease enzyme for this purpose [17].

Given the promising bioactive potential of *P. terebinthus*, this study aims to evaluate the antioxidant, antimicrobial, and enzyme inhibitory activities of the gall induced by *Slavum* aff. *mordvilkoi* on this plant. Understanding these properties may contribute to the development of novel natural therapeutic agents, offering alternatives to synthetic compounds with potential applications in medicine and agriculture.

## 2. Materials and Methods

### 2.1. Plant Material and Extraction

*Pistacia terebinthus* galls were collected from Şirvan, Batman, Türkiye in September 2019. The galls were identified and confirmed by PhD Erdem SEVEN (Fig. 1). The samples were washed, dried at room temperature in a dark environment to prevent degradation of bioactive compounds, and then ground into a fine powder.

Extraction was performed sequentially using solvents with increasing polarity: petroleum ether, acetone, ethanol, and water. Each extraction was conducted for 48 hours under constant agitation. After filtration through filter paper, the solvents were evaporated using a rotary evaporator under reduced pressure. The obtained extracts were weighed and stored at +4°C until further analysis.



**Figure 1:** *P. terebinthus* gall induced by *Slavum* aff. *mordvilkoi*

### 2.2. Antioxidant Activity Assay

The antioxidant activity of the extracts was evaluated using three widely accepted methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay [18], ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay [19] and CUPRAC (cupric ion reducing antioxidant capacity) assay [20].

The total phenolic content was measured using the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE), while the total flavonoid content was determined using aluminum nitrate colorimetric methods and expressed as quercetin equivalents (QE).

### 2.3. Antimicrobial Activity Assay

The antimicrobial activity of the extracts was tested against Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615), and a yeast (*Candida albicans* ATCC 10231) using the disk diffusion and broth dilution methods [21-22]. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined using the broth microdilution method.

### 2.4. Enzyme Inhibitory Activity Assay

The enzyme inhibitory activities of the extracts were determined for acetylcholinesterase, butyrylcholinesterase, tyrosinase, and urease using well-established methods from the literature. Acetylcholinesterase and butyrylcholinesterase inhibitory activities were assessed using the spectrophotometric Ellman method [23]. Tyrosinase inhibitory activity was measured using the Hearing method [24]. Urease inhibitory activity was determined according to Mobley's method [25].

## 3. Results and Discussion

### 3.1. Extraction Yield

The extraction yields varied among the solvents. The highest extraction yield was obtained with acetone (14.37%), followed by ethanol (9.45%), petroleum ether (7.54%), and water (5.63%). The differences in yield are attributed to the varying solubility of bioactive compounds in solvents with different polarities. Acetone proved to be the most effective solvent in extracting phenolic compounds, which are known for their biological activities [7].

### 3.2. Antioxidant Activity Assay

The Table 1 presents the antioxidant activity (DPPH, ABTS, and CUPRAC assays) and total phenolic and flavonoid content of different solvent extracts. The results indicate significant variations in antioxidant capacity depending on the solvent used for extraction.

Among the extracts, ethanol exhibited the highest DPPH scavenging activity (lowest $IC_{50}$ value: 3.98 µg/ml), followed closely by acetone (4.51 µg/ml). The water extract showed a slightly weaker activity (5.79 µg/ml). In contrast, BHT demonstrated significantly lower antioxidant activity (49.06 µg/ml), while ascorbic acid, used as a positive control, had a comparable $IC_{50}$ value (4.32 µg/ml).

The water extract showed the highest ABTS scavenging activity ($IC_{50}$ = 1.87 µg/ml), followed by ascorbic acid (1.72 µg/ml) and acetone (2.17 µg/ml). Ethanol also demonstrated a notable activity (2.28 µg/ml), while BHT had relatively moderate antioxidant power (2.23 µg/ml).

The water extract exhibited the highest reducing power ($A_{0.5}$ = 10.84), indicating strong antioxidant potential. Ethanol followed with a value of 3.45, while acetone (1.36) and BHT (2.71) had significantly lower values.

The acetone and ethanol extracts contained the highest amounts of total phenolics (204.28 µg GAEs/mg and 202.59 µg GAEs/mg, respectively). Water had a lower phenolic content (118.61 µg GAEs/mg). Flavonoid content was highest in ethanol (21.21 µg QEs/mg), followed by water (12.40 µg

QEs/mg) and acetone (10.91 µg QEs/mg). The petroleum ether extract had the lowest phenolic and flavonoid content.

**Table 1:** Results of antioxidant activity and total phenol-phenolic content of the extracts

| Samples | DPPH ($IC_{50}$ µg/ml) | ABTS ($IC_{50}$ µg/ml) | CUPRAC ($A_{0.5}$) | Phenolic content (µgGAEs/mg extract) | Flavonoid content (µgQEs/mg extract) |
|---|---|---|---|---|---|
| **Petroleum ether** | NA | NA | NA | 27.32 ± 0.00 | 6.2 ± 0.6 |
| **Acetone** | 4.51±0.01 | 2.17±0.06 | 1.36±0.05 | 204.28 ± 0.04 | 10.91 ± 0.47 |
| **Ethanol** | 3.98±0.04 | 2.28±0.02 | 3.45±0.07 | 202.59 ± 0.02 | 21.21 ± 0.14 |
| **Water** | 5.79±0.06 | 1.87±0.09 | 10.84±0.08 | 118.61 ± 0.02 | 12.40 ± 0.07 |
| **Ascorbic acid** | 4.32±0.08 | 1.72±0.02 | 11.3±0.05 | | |
| **BHT** | 49.06±0.01 | 2.23±0.05 | 2.71±0.07 | | |

Values are means of three parallel measurement ± Standard deviation, n = 3. Ascorbic acid and BHT were used as positive control.

These findings suggest that ethanol and acetone are the most effective solvents for extracting phenolic compounds with high antioxidant potential, while water also proves to be a good solvent, particularly in specific antioxidant assays such as ABTS.

It is well known that there is a correlation between the phenolic/flavonoid content of plants and their antioxidant activity. Numerous studies have demonstrated the antioxidant potential of phenolic compounds.

In this study, the acetone, ethanol, and water extracts of gall were found to be rich in total phenolic content. In line with their phenolic composition, all tested antioxidant activity methods yielded high activity levels. The extracts were ranked in terms of their phenolic compound richness as acetone > ethanol > water, and the activity values obtained from the DPPH, ABTS, and CUPRAC assays followed the same order. This finding supports the correlation between phenolic compound richness and antioxidant activity.

However, variations in antioxidant activity among different assays suggest that factors other than total phenolic content may also contribute to the observed effects. Particularly, the high CUPRAC activity of the water extract indicates that additional antioxidant compounds or mechanisms may be involved.

### 3.3. Antimicrobial Activity Assay

The antimicrobial activity assessment revealed that the effectiveness of the extracts varied depending on the type of microorganism and the solvent used for extraction (Table 2). Among the Gram-negative bacteria tested, *E. coli* displayed complete resistance to all extracts, whereas *P. aeruginosa* exhibited moderate susceptibility to acetone, ethanol, and water extracts, with inhibition zones of 13 mm, 13 mm, and 11 mm, respectively. The minimum inhibitory concentration (MIC) values for *P. aeruginosa* were 200

µg/ml for these extracts, while the minimum bactericidal concentration (MBC) values were significantly higher, indicating a bacteriostatic effect rather than bactericidal.

In contrast, Gram-positive bacteria showed greater sensitivity to the extracts. The acetone extract demonstrated the strongest inhibition against *S. aureus* (14 mm inhibition zone, MIC = 100 µg/ml, MBC = 500 µg/ml), followed by ethanol and water extracts, which exhibited moderate antimicrobial activity. Similarly, *S. pyogenes* was effectively inhibited by the acetone extract, with ethanol and water extracts showing weaker effects. The reference antibiotic (ampicillin) exhibited considerably higher inhibition, indicating that while the extracts possessed antimicrobial properties, their efficacy was lower than conventional antibiotics.

In terms of antifungal activity, the acetone extract was the most potent against *C. albicans*, with an inhibition zone of 13 mm, an MIC of 50 µg/ml, and an MBC of 700 µg/ml. Ethanol and water extracts also exhibited antifungal properties but required higher concentrations to achieve inhibitory effects. Petroleum ether extract displayed only weak antifungal activity. When compared with fluconazole, the positive control, all extracts demonstrated lower inhibition, confirming their relatively weaker antifungal potential.

Among the different solvents used, acetone was the most efficient in extracting antimicrobial compounds, followed by ethanol. While the water extract showed some antimicrobial activity, its MIC and MBC values were generally higher, suggesting lower effectiveness. The petroleum ether extract exhibited minimal activity, implying that the active antimicrobial compounds in the plant are more soluble in polar solvents. These findings highlight the selective antimicrobial potential of the extracts, with stronger activity against Gram-positive bacteria and yeast compared to Gram-negative bacteria.

**Table 2:** Results of antimicrobial activity of the extracts

| | | **Gram negative** | | **Gram positive** | | **Yeast** |
|---|---|---|---|---|---|---|
| **Extracts** | | ***E.coli*** | ***P.aeruginosa*** | ***S.aureus*** | ***S.pyogenes*** | ***C. albicans*** |
| **Petroleum ether** | **DD** | NA | NA | NA | 8±0 | 9±0 |
| | **MIC** | NA | NA | NA | 100±0 | 200±0.5 |
| | **MBC** | NA | NA | NA | 2000±0.3 | 900±0.2 |
| **Acetone** | **DD** | NA | 13 ±0 | 14 ±0.1 | 12 ±0.1 | 13 ±0.1 |
| | **MIC** | NA | 200±0.5 | 100±0.2 | 100±0.1 | 50±0.4 |
| | **MBC** | NA | 1000±0.3 | 500±0.1 | 500±0.1 | 700±0 |
| **Ethanol** | **DD** | NA | 13 ±0 | 13 ±0 | 11 ±0.2 | 11 ±0.1 |
| | **MIC** | NA | 200±0.5 | 300±0.7 | 200±0.4 | 300±0 |
| | **MBC** | NA | 700±0.4 | 750±0.5 | 1000±0.6 | 1000±0.4 |
| **Water** | **DD** | NA | 11 ±0.2 | 10 ±0 | 9 ±0.1 | 10 ±0.2 |
| | **MIC** | NA | 200±0 | 300±0.5 | 1100±0.5 | 1200±0.6 |
| | **MBC** | NA | 900±0 | 900±0.3 | 10000±0.5 | 5000±0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Positive control** | **DD** | 20±0.1 | NA | 35±0.2 | 19±0.2 | 30±0.3 |

Values are means of three parallel measurement ± Standard deviation, n = 3. DD: Results of the disc diffusion method in mm at a concentration of 30 µg/ml, MIC: Results of minimum inhibitory concentration in µg/ml, MBC: Results of minimum bactericidal concentration in µg/ml, NA: Not active, positive control ampicillin for bacteria, fluconazole for yeast.

The resistance of *E. coli* and the lower sensitivity of *P. aeruginosa* could be attributed to structural differences in their outer membranes, which may limit the penetration of bioactive compounds. Although the antimicrobial efficacy of these extracts is lower than that of standard antibiotics, the results suggest that they may serve as natural antimicrobial agents, particularly against Gram-positive bacterial and fungal infections. Further investigations are necessary to isolate and characterize the bioactive compounds responsible for these effects and to evaluate their potential applications in antimicrobial formulations.

### 3.4. Enzyme Inhibitory Activity Assay

The enzyme inhibitory activity results (Table 3) reveal that the effectiveness of the extracts varies depending on the enzyme tested and the solvent used for extraction. The inhibitory potential was evaluated for acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, and urease at a concentration of 200 µg/ml, with the standard inhibitors (galantamine, kojic acid, and thiourea) serving as positive controls.

Among the extracts, ethanol showed the highest inhibition across all enzymes, with 69.23% for AChE, 80.75% for BChE, 75.93% for tyrosinase, and 57.04% for urease. Acetone extract followed closely, exhibiting strong inhibitory effects, particularly against BChE (78.63%) and tyrosinase (63.08%). The water and petroleum ether extracts displayed relatively lower enzyme inhibition, except for the water extract, which exhibited the highest urease inhibition (64.73%) among the extracts. This suggests that different solvents extract bioactive compounds with varying inhibitory capacities.

When compared to the positive controls, the extracts displayed moderate enzyme inhibition. Galantamine, used as a standard inhibitor for AChE and BChE, showed the highest activity (82.85% and 81.33%, respectively), outperforming all extracts. Similarly, kojic acid exhibited the strongest tyrosinase inhibition (95.05%), and thiourea demonstrated the highest urease inhibition (98.37%), highlighting the superior efficiency of commercial inhibitors. While the extracts exhibited promising inhibitory activity, their effects were lower than those of the synthetic standards.

**Table 3:** Results of enzyme inhibitory activity of the extracts

| Samples | AChE (%I) | BChE (%I) | Tyrosinase (%I) | Urease (%I) |
|---|---|---|---|---|
| **Petroleum ether** | 35.00±0.28 | 52.63±0.18 | 33.97±0.97 | NA |
| **Acetone** | 58.50±1.32 | 78.63±0.88 | 63.08±0.61 | 52.85±1.48 |

| | | | | |
|---|---|---|---|---|
| **Ethanol** | 69.23±1.07 | 80.75±0.66 | 75.93±1.07 | 57.04±0.47 |
| **Water** | 32.78±1.05 | 57.75±1.88 | 32.32±1.00 | 64.73±0.49 |
| **Galantamine** | 82.85 ± 0.21 | 81.33±1.04 | - | - |
| **Kojic acid** | - | - | 95.05±0.37 | - |
| **Thiourea** | - | - | - | 98.37±0.40 |

Values are means of three parallel measurement ± Standard deviation, n = 3. It was studied at a concentration of 200 µg/ml. Galantamine, kojic acid and thiourea were used as positive controls. NA: not active.

The variation in enzyme inhibition among the different extracts indicates that solvent selection plays a crucial role in extracting bioactive compounds with enzyme inhibitory potential. Ethanol and acetone extracts, in particular, demonstrated notable inhibition against cholinesterases and tyrosinase, suggesting their potential use in neuroprotective and skin-related applications. On the other hand, the water extract stood out for urease inhibition, which could be relevant for treatments targeting urease-related disorders. These findings emphasize the importance of selecting appropriate solvents to optimize enzyme inhibitory effects, warranting further studies to isolate and characterize the active compounds responsible for these bioactivities.

Numerous studies have investigated the biological and phytochemical properties of *Pistacia* species. However, research on *Pistacia* galls remains limited, and no prior studies have been found specifically on the gall examined in this study.

Several studies have explored the biological activities of *Pistacia terebinthus* and its various parts. In a recent study, Uysal et al. investigated the chemical composition, antioxidant activity, and enzyme inhibitory potential of *P. terebinthus* leaf extracts [26]. Their results indicated that the extracts exhibited strong antioxidant capacity and significant enzyme inhibitory effects, suggesting the presence of bioactive secondary metabolites. The total phenolic and flavonoid contents ranged between 65.51–210.54 mg GAE/g and 54.94–169.89 mg RE/g, respectively. While methanol extract contained the highest phenolic content, the ethyl acetate extract exhibited the highest flavonoid concentration. The antioxidant evaluations revealed that methanol extract displayed considerable antioxidant activity across various assays, with recorded values of 3.29 mmol TE/g (ABTS), 2.06 mmol TE/g (DPPH), 2.67 mmol TE/g (CUPRAC), 1.62 mmol TE/g (FRAP), and 65.65 mg EDTA/g (metal chelating activity). Additionally, the methanol extract demonstrated notable enzyme inhibitory potential. These findings align with our study on *P. terebinthus* gall extracts, further supporting the pharmacological potential of different plant parts. However, the observed variations in bioactivity between leaves and galls emphasize the significance of tissue-specific chemical composition in determining biological activity.

Fidan et al. examined the Vitamin C content, enzyme activity, phenolic composition, antioxidant capacity, and antimicrobial properties of ripe and unripe *Pistacia terebinthus* fruits [27]. Ripe fruits had significantly higher Vitamin C (63.2 mg/100g) and enzyme inhibition (0.136 mg/mL) compared to unripe fruits (15.4 mg/100g, 2.14 mg/mL). The main phenolic compounds identified were rutin, syringic acid, and gallic acid. Methanol extracts of ripe fruits exhibited stronger antioxidant activity (DPPH, ABTS, FRAP, TPC, TFC, and TAC) and showed antimicrobial effects against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, whereas unripe fruits displayed no antimicrobial activity. These findings align with our study, particularly regarding the selective antimicrobial and antioxidant potential of *P. terebinthus* extracts. Differences in bioactivity between fruit, leaf, and gall extracts underscore the influence of tissue type and maturation stage on phytochemical composition, necessitating further comparative research.

Tilkat et al. investigated the antihypertensive, anticholinesterase, antiurease, antityrosinase, antielastase, and anticancer activities *of Pistacia khinjuk* Stocks using in vivo (male and female trees) and in vitro (root, stem, and leaf) samples [28]**.** The study revealed that all extracts exhibited apoptotic effects against MCF-7 (breast cancer) and HT-29 (colon cancer) cell lines**.** In enzyme inhibition assays, butyrylcholinesterase (BChE) activity was strong across all samples**,** while acetylcholinesterase (AChE) inhibition was absent**.** Female in vivo leaf extracts exhibited the highest BChE inhibition (75.20%)**,** while elastase inhibition in female root and leaf extracts (58.72% and 58.25%, respectively) surpassed that of the standard oleanolic acid (39.46%)**.** Overall, in vivo samples exhibited greater biological activity than in vitro samples**,** though in vitro stem extracts showed promise as potential antihypertensive, antielastase, and anticancer agents**.** These findings align with our study on *Pistacia terebinthus* galls, particularly regarding enzyme inhibition and bioactive potential, highlighting the need for further comparative research on different *Pistacia* species and plant tissues.

Mandrone et al. investigated the biological properties of 36 plant extracts from Sardinia, including *Pistacia lentiscus* and *Pistacia terebinthus* ssp. *terebinthus* [29]. Their study demonstrated that leaf extracts from both species exhibited strong antimicrobial effects, with *P. terebinthus* ssp. *terebinthus* showing higher flavonoid content and lower cytotoxicity than *P. lentiscus*. The minimum inhibitory concentration (MIC) values revealed that *P. lentiscus* fruit and leaf extracts inhibited *Staphylococcus aureus* ATCC 25293 at 144.0 µg/ml and 27.3 µg/ml, respectively. Meanwhile, the MIC of *P. terebinthus* leaf extract was 62.9 µg/ml against *S. aureus*. Interestingly, *P. lentiscus* extracts exhibited no antimicrobial activity against *Escherichia coli* ATCC 25922, whereas *P. terebinthus* leaf extract demonstrated an MIC of 156.3 µg/ml against this strain. These findings are consistent with our study, where no antimicrobial activity was observed against *E. coli*, reinforcing the selective efficacy of *P. terebinthus* extracts against Gram-positive

bacteria. Such variations in antimicrobial potency suggest that differences in plant tissue composition may influence bioactivity, warranting further comparative research.

Similar to the studies mentioned above, numerous investigations have focused on the antimicrobial, antioxidant, and phytochemical properties of *Pistacia* species; however, research specifically targeting *Pistacia* galls remains scarce, making direct comparisons challenging. In evaluating antimicrobial activity, our findings align with previous reports, particularly in the lack of activity against *E. coli* and strong inhibition of *S. aureus*.

Aromatic and medicinal plants containing secondary metabolites are widely recognized for their antioxidant, antifungal, and antibacterial properties, making them valuable in food preservation and pharmaceuticals [30-31]. Several mechanisms have been proposed to explain the antibacterial effects of phenolic compounds, including disrupting proton gradients, interfering with electron transport, and impairing cytoplasmic membrane function [32].

The correlation between phenolic/flavonoid content and antioxidant activity is well established, with numerous studies confirming the antioxidant potential of phenolic compounds. In this study, acetone, ethanol, and water extracts were found to be rich in phenolic compounds, which correlated with high antioxidant activity across all tested assays. The ranking of extracts in terms of phenolic content (acetone > ethanol > water) corresponded to their performance in DPPH, ABTS, and CUPRAC assays, reinforcing this correlation.

## 4. Conclusion

This study demonstrates that *P. terebinthus* galls possess antioxidant, antimicrobial, and enzyme inhibitory properties, with bioactivity varying based on solvent extraction. Ethanol and acetone extracts exhibited strong antioxidant and enzyme inhibitory activity, while antimicrobial effects were most pronounced against Gram-positive bacteria and yeast.

These findings support the pharmacological potential of *P. terebinthus* galls as a natural source of bioactive compounds. Future research should focus on compound isolation, mechanistic studies, and potential applications in medicine, food preservation, and cosmetics.

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