



## Bioactive Potential of *Pistacia terebinthus* L. From the Atlas of Morrocco: Antioxidant and Antimicrobial Properties

Mohammed Bassouya<sup>1\*</sup>, Mohamed Chedadi<sup>1</sup>, Jawhari Fatima Zahra<sup>1</sup>, Khalid Chebbac<sup>2</sup>, Abdelfattah El Moussaoui<sup>3</sup>, Amina Bari<sup>1</sup>

<sup>1</sup>Laboratory of Biotechnology, Environment Agrifood and Health, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco

<sup>2</sup>Laboratory of Biotechnology Conservation and Valorisation of Natural Resources, Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, Fez 30000, Morocco

<sup>3</sup>Plant Biotechnology Team, Faculty of Sciences, Abdelmalek Essaadi University, Tetouan 93002, Morocco

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### ABSTRACT

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*Pistacia terebinthus* L., a tree native to the Middle Atlas of Morocco, is traditionally used in folk medicine. This study investigated the phytochemical profile, antioxidant, and antimicrobial properties of leaf essential oils (EOs) from three bioclimatic zones. EOs were extracted by hydrodistillation, analyzed by GC-MS, and tested for antioxidant activity using FRAP and DPPH assays. Molecular docking was performed to assess the interaction of major compounds with antioxidant targets, while antimicrobial activity was evaluated against bacterial and fungal strains by disk diffusion and microdilution. The chemical composition varied markedly among regions, with 61–98 compounds identified. Monoterpenes, particularly  $\alpha$ -pinene, were abundant in arid samples, whereas terpinen-4-ol (21.96%) appeared exclusively in subhumid oils. Other relevant constituents included  $\tau$ -muurolol (8.84%) and D-limonene (7.85%). Antioxidant activity also differed by origin, with IC<sub>50</sub> values ranging from 1.40 to 4.17 mg/mL and EC<sub>50</sub> from 2.86 to 9.47 mg/mL. Subhumid oils exhibited the highest antimicrobial activity, while arid samples were most effective against *Staphylococcus aureus*. Docking analysis revealed interactions of key compounds with NADPH oxidase (Ala11, Asp282, Ala300), suggesting a potential mechanism for their antioxidant action. Overall, results highlight that bioclimatic conditions strongly influence the chemical composition and bioactivity of *P. terebinthus* essential oils, supporting their potential for therapeutic and pharmaceutical applications.

**Keywords:** Terebinth, Chemotype, Antimicrobial, Monoterpenes, Atlas.

### Introduction

The genus *Pistacia*, belonging to the Anacardiaceae family, encompasses over 70 genera and approximately 600 species, predominantly distributed across Mediterranean regions, the Middle East, the southern United States and Mexico.<sup>1</sup> This species, comprising both deciduous and evergreen trees and shrubs, is characterized by resin production and an outstanding capacity to adapt to arid environments, with typical heights ranging from 8 to 10 meters.<sup>2</sup> Prominent species of the genus, including *P. vera* L., *P. lentiscus* L., *P. atlantica* subsp. *atlantica*, *P. terebinthus* L., and *P. khinjak* Stocks, are distributed across regions from the Mediterranean basin to Central Asia. In Morocco, three species, *P. lentiscus* L., *P. atlantica* subsp. *atlantica*, and *P. terebinthus* L. occur naturally. Of these, only *P. vera* is cultivated commercially, while the others primarily serve as rootstocks.

\*Corresponding author. E mail: [mohammed.bassouya@usmba.ac.ma](mailto:mohammed.bassouya@usmba.ac.ma)  
Tel: +212673557594

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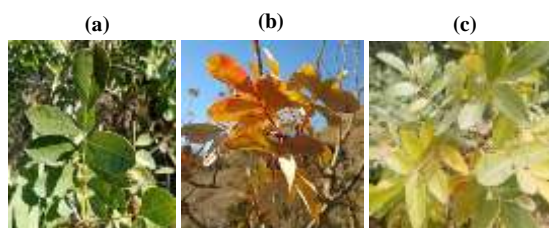
This diversity highlights the ecological relevance and economic importance of the genus. These species are abundant in bioactive compounds, such as phenolic compounds, flavonoids, terpenoids, saponins, alkaloids, sterols, fatty acids, and fibers.<sup>3</sup>  $\beta$ -sitosterol represents the principal sterol component of the fruits of *P. vera*, *P. atlantica*, *P. lentiscus*, and *P. terebinthus*, followed in descending order by campesterol,  $\Delta^5$ -avenasterol, stigmasterol, brassicasterol, and cholesterol.<sup>4–7</sup> The *Pistacia* genus is gaining increasing attention in the pharmaceutical sector.<sup>8,9</sup> Traditionally, these plants have been employed in the treatment of various ailments such as rheumatism, asthma, diabetes, hypertension, hemorrhoids and diarrhea.<sup>10–12</sup> They also exhibit antimicrobial, antioxidant, anti-inflammatory, antiviral, antitumor, antidiabetic, and hepatoprotective properties.<sup>13–16</sup> Various organs of *Pistacia* species have been examined for their biological activities, and their essential oils are widely utilized in the pharmaceutical, cosmetic, and food industries.<sup>17–19</sup> To further explore their therapeutic potential, a comparative analysis was conducted to assess the medicinal properties of three essential oils sourced from distinct bioclimatic regions of Morocco. Notably, *Pistacia terebinthus* L. (PT) is currently classified as Least Concern on the International Union for Conservation of Nature (IUCN) Red List.<sup>20,21</sup> This study aimed to compare previously reported data on the Atlas pistachio with new findings on the terebinth pistachio, with the objective of characterizing and differentiating these two *Pistacia* taxa in Morocco, while also evaluating and comparing the antioxidant activity of their essential oils. Despite existing morphological differences, distinguishing between these taxa and their subspecies remains challenging for non-specialist botanists. Therefore, the outcomes of this investigation may serve as a complementary approach for differentiating morphologically similar species, particularly for the

authentication of dried or processed plant material when conventional morphological criteria are insufficient.<sup>22</sup> The originality of this research resides in applying complementary methods, spanning chromatographic techniques and molecular modeling, to elucidate the mechanisms of the detected compounds. This integrated strategy enables a thorough examination and generates fresh perspectives on a species still little studied within Morocco.

## Materials and Methods

### Plant collection and identification

The leaves of *Pistacia terebinthus* L. were collected in August 2023 from three distinct locations in the Middle Atlas region of Morocco: Amghas (Site 1: 33°68'63.9" N, 5°37'01.1" W), Lac Afourgagh (Site 2: 33°36'45.9" N, 4°53'02.6" W), and Ait Naamane (Site 3: 33°41'56.1" N, 5°21'23.2" W). The plant materials were identified by a Taxonomist Prof. Amina Bari at the Laboratory of Biotechnology, Environment Agrifood and Health, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco where a herbarium specimen with voucher number PT251022BM was deposited.



**Figure 1:** Samples of *Pistacia terebinthus* L. leaves (a: area 1, b: area 2, c: area 3)

### Preparation of extracts and essential oils

The leaves of *Pistacia terebinthus* L. (Figure 1) were air dried for one week, finely ground, and stored under controlled laboratory conditions. Hydro-distillation of 250 g of powdered leaves was performed in a Clevenger-type device using 2 L of distilled water for 4 hours to obtain the essential oil. The oil was then dehydrated with anhydrous sodium sulfate and kept at 4°C prior to analysis. All extractions were carried out in triplicate.<sup>23</sup> This procedure resulted in three essential oil samples of *Pistacia terebinthus* L. collected from different regions; EOPT1 Essential oil of *Pistacia terebinthus* collected from site 1 (Amghas), EOPT2 - Essential oil of *Pistacia terebinthus* collected from site 2 (Lac Afourgagh), and EOPT3 - Essential oil of *Pistacia terebinthus* collected from site 3 (Ait Naamane). The essential oils were diluted with hexane. The extraction yield was determined using the following formula:

$$R = \frac{M'}{M} \times 100 \dots\dots\dots (1)$$

Where;

R is the essential oil yield expressed as a percentage, M' is the mass (in grams) of the extracted oil, and M is the initial mass (in grams) of the plant material. Concurrently, hydroethanol extracts were prepared from leaves that had been air dried for two weeks, pulverized with an electric grinder, and preserved until extraction. For each extraction, 50 g of the powdered material was macerated in 500 mL of 70% ethanol (ratio 1:10, w/v). A 70% ethanol solution was selected as the extraction solvent owing to its high efficacy in extracting a wide range of bioactive compounds, its rapid evaporation rate, and its relatively low toxicity to both humans and the environment.<sup>24</sup>

### Phytochemical screening

The leaves of *Pistacia terebinthus* L. were subjected to preliminary phytochemical evaluation in order to reveal the major groups of active compounds.<sup>25</sup>

**Tannins:** The formation of a greenish or blackish coloration upon the addition of 1 mL of extract to 1 mL of 1% FeCl<sub>3</sub> solution.<sup>26</sup>

**Catechins:** Formation of a red, amyl alcohol-soluble precipitate after boiling 5 mL of infusion with 1 mL of concentrated HCl for 15 minutes.<sup>26</sup>

**Gallotannins:** Precipitate appears after heating 30 mL infusion with 15 mL of Stiasny reagent, saturating with sodium acetate, and adding FeCl<sub>3</sub>.<sup>27</sup>

**Flavonoids:** A distinct coloration (purple-pink, pink-orange, or reddish) appears when infusion is mixed with alcoholic HCl, magnesium, and isoamyl alcohol.<sup>27</sup>

**Saponins:** Persistent foaming after vigorous shaking of the extract with distilled water confirms their presence.<sup>27</sup>

**Sterols:** A reddish-brown ring forms during the Salkowski test after adding H<sub>2</sub>SO<sub>4</sub> to the extract.<sup>28</sup>

**Alkaloids:** The formation of a pale-yellow precipitate upon combining 1 mL of the extract with 1 mL of Mayer's reagent confirmed the presence of alkaloids.

**Cardiac glycosides:** A reddish-brown coloration appears when 2 mL chloroform and 1 mL extract are mixed, followed by the addition of H<sub>2</sub>SO<sub>4</sub>.<sup>29</sup>

**Monosaccharides and polysaccharides:** A red color appears after adding 2 - 3 drops of concentrated H<sub>2</sub>SO<sub>4</sub> to 1 mL extract, followed by 3 - 4 drops of thymol-saturated alcohol.<sup>30</sup>

**Mucilage:** A granular precipitate forms when 1 mL extract is mixed with 5 mL absolute alcohol.<sup>30</sup>

### Gas chromatography - mass spectrometry (GC-MS)

Gas chromatography mass spectrometry (GC-MS) analysis was conducted using a non-polar silica capillary column (HP-5MS column) under optimized parameters. The oven was programmed to start at 40°C for 2 minutes, then ramped at 2°C/min up to 260°C, which was held constant for 10 minutes. The injection was performed in split mode, with helium as the carrier gas flowing at 1 mL/min. Ionization occurred at 70 eV, with the ion source maintained at 200°C. Mass spectra were recorded within an m/z range of 40–650 Da. A 1 µL aliquot of the sample, diluted to 10% (v/v) in hexane, was injected into the system. Compound identification was performed by comparing retention indices with reference values reported in the Adams database.<sup>31</sup>

### Mineral analysis

Mineral analysis was carried out on leaf samples of *Pistacia terebinthus* L. collected from three sites in the Middle Atlas of Morocco: Ait Naamane (El Hajeb), Afourgagh (Sefrou), and Amghas (Ifrane). For each assay, 2 g of dried leaf material were calcined at 500°C for 5 h to obtain ash. From this residue, 0.15 g was taken and subjected to mineral digestion using the aqua regia method. The sample was treated with a nitric acid–hydrochloric acid mixture, evaporated to near dryness, and reconstituted in 2 M HCl. Elemental composition was then determined using inductively coupled plasma atomic emission spectrometry (ICP-AES), with argon employed as the plasma source.<sup>32</sup>

### Assessment of antioxidant activity

The antioxidant properties of *Pistacia terebinthus* L. essential oils originating from three bioclimatic zones (EOPT1, EOPT2, and EOPT3) were determined through DPPH radical scavenging and FRAP assays.

### DPPH radical scavenging assay

For each oil sample, ten test tubes were prepared, each containing 750 µL of DPPH solution (4 mg/100 mL methanol) and 250 µL of various oil dilutions. After a 30-minute incubation period in the dark, the absorbance was recorded at 517 nm, the spectrophotometric readings were obtained using a UV2005 device (J.P. SELECTA S.A., Spain), with BHT and ascorbic acid employed as benchmarks. The negative control consisted of DPPH solution mixed with methanol only (without essential oil). The percentage of inhibition was determined using the following formula:

$$\% \text{ Inhibition} = [(A_0 - AE_0) / A_0] \times 100 \dots\dots\dots (2)$$

Where AE<sub>0</sub> is the absorbance of the sample and A<sub>0</sub> is the absorbance of the negative control.<sup>33</sup>

### Ferric reducing antioxidant power (FRAP) assay

The ferric reducing ability of the essential oils was evaluated using a modified protocol adapted from previously described methods.<sup>34,35</sup>

Briefly, 150  $\mu$ L of the methanol-diluted sample was mixed with 750  $\mu$ L of phosphate buffer (0.2 M, pH 6.6) and an equal volume (750  $\mu$ L) of potassium ferricyanide solution (1% w/v). The reaction mixture was incubated at 50 °C for 20 min, after which 750  $\mu$ L of trichloroacetic acid (10%) was added. The tubes were then centrifuged at 3000 rpm for 10 min. From the resulting supernatant, 2.5 mL was collected, mixed with 2.5 mL of distilled water, and followed by the addition of 0.5 mL of ferric chloride solution (0.1% w/v). The absorbance was immediately recorded at 700 nm. Butylated hydroxytoluene (BHT) and ascorbic acid were used as standards.

#### Molecular docking

Molecular docking was conducted to investigate the potential antioxidant mechanism of the major essential oil components;  $\alpha$ -pinene, terpinen-4-ol, and  $\tau$ -muurolol targeting NADPH oxidase (PDB ID: 2CDU). Protein preparation included water and ligand removal and Gasteiger charge addition. Ligand docking was performed using AutoDock, and binding interactions were detected using Discovery Studio 2021 in both 2D and 3D formats.<sup>36</sup>

#### Assessment of antimicrobial activity

##### Disk diffusion method

Pure cultures of bacterial and fungal strains, namely *Candida albicans* (ATCC 10231), *Bacillus subtilis* (DSM 6333), *Escherichia coli* (K12), *Pseudomonas aeruginosa* (CIP 82.114), *Klebsiella pneumoniae* (CIP A22), and *Staphylococcus aureus* (ATCC 6633), were sourced from the Biotechnology Laboratory of the Faculty of Sciences, Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, Fez, Morocco. The antimicrobial efficacy of the essential oils was evaluated against these test organisms using the disk diffusion method as previously described.<sup>37</sup> Sterile discs (5 mm in diameter) were saturated with 5  $\mu$ L of essential oils and placed on Mueller-Hinton agar for bacterial cultures and malt extract agar for fungal isolates previously inoculated with microbial suspensions ranging from 10<sup>6</sup> to 10<sup>8</sup> CFU/mL. Following an incubation at 37°C for 48 h (bacterial strain), and 72 h (fungal strain), the diameters of the inhibition zones were recorded.

##### Microdilution method

The minimum inhibitory concentration (MIC) was assessed using the microdilution technique in 96-well microplates. Serial dilutions of the essential oils were prepared in 10% dimethyl sulfoxide (DMSO), then inoculated with standardized microbial suspensions. After 24 hours of incubation at 37°C, 20  $\mu$ L of a 0.1% resazurin solution was added to each well. A change in color signified microbial viability, whereas the absence of color change indicated effective inhibition.<sup>38,39</sup>

#### Statistical analysis

The experimental data was analyzed based on six replicates for each measurement. Results were presented as maximum, minimum, and mean  $\pm$  standard deviation (SD), providing a clear view of the variability within the dataset. This descriptive statistical approach was chosen to summarize the central tendency and dispersion of the results. Graphical representations and data processing were carried out using GraphPad Prism version 8.0.1.

## Results and Discussion

### Phytochemical constituents of *Pistacia terebinthus* leaf extracts

Phytochemical analysis of *Pistacia terebinthus* L. leaf extracts identified a range of bioactive secondary metabolites, including terpenes, flavonoids, sterols, gallotannins, cardiac glycosides and saponins (Table 1). These compounds are widely recognized for their antioxidant, antimicrobial, and cardioprotective activities.

**Table 1:** Phytochemical screening of hydroethanol extracts from *Pistacia terebinthus* leaves.

Secondary metabolites	Results
Flavonoids	+
Tannins	+
Tannins	+
Alkaloids	-
Sterols and terpenes	+
Saponosides	+
Oses and holosides	+
Cardiac glycosides	+
Mucilage	-

+ indicates a positive reaction, - indicates a negative reaction.

### Compounds identified from GC-MS analysis of *P. terebinthus* essential oils

The chemical profiles of *P. terebinthus* L. essential oils differed markedly among the three sampling sites (Table 2). EOPT1 comprised 19 identified constituents, with  $\alpha$ -pinene (50.81%) and methyl commate A (17.24%) as the predominant components. In EOPT2, 26 compounds were detected, among which terpinen-4-ol (21.96%), and D-limonene (10.23 %) were the most abundant. The oil from Area 3 (EOPT3) exhibited the greatest compositional diversity, with 61 compounds identified;  $\tau$ -muurolol (8.84%) and D-limonene (7.85%) dominated this profile.

**Table 2:** Chemical composition of essential oils of *Pistacia terebinthus* L.

No.	Compound	T (min)	Percentage composition (%)		
			EOPT1	EOPT2	EOPT3
1	$\alpha$ -Pinene	7.923	50.81	2.17	1.49
2	Camphene	8.394	1.24	-	-
3	Pin-2-ene	8.998	0.6	-	-
4	$\beta$ -Pinene	9.205	0.72	-	-
5	3-Carene	10.125	1.56	-	-
6	1-Isopropyl-2-methylbenzene	10.581	1.42	-	-
7	D-Limonene	10.735	2.37	10.23	7.85
8	Eucalyptol	10.835	0.44	-	-
9	Trans-Verbenol	13.152	1.47	-	-
10	Pinocarveol	14.105	0.89	-	-
11	2-Pinen-4-one	16.033	0.77	-	-
12	$\beta$ -Bourbonene	20.975	0.78	-	-
13	B-Elemene	21.087	0.71	-	-

14	$\beta$ -Selinene	23.657	0.59	-	-
15	Trielaidin	38.944	0.46	-	-
16	24-Norursa-3,9(11),12-triene	44.697	3.67	-	-
17	$\beta$ -Amyrenyl acetate	45.408	8.6	-	-
18	Methyl commate A	47.094	17.24	-	-
19	Methyl commate B	47.276	5.64	-	-
20	4-Isopropylidene-1-cyclohexene	12.391	-	3.38	-
21	p-(1-Propenyl)-toluene	12.510	-	1.51	-
22	Cyclooctanone	13.501	-	3.19	-
23	dimethylhydrocoumarin	14.085	-	6.26	-
24	1-Terpinen-4-ol	15.204	-	0.93	-
25	p-Isopropylbenzyl alcohol	15.351	-	8.76	3.26
26	Muurolol	18.470	-	1.55	-
27	Myrcene acetal	18.632	-	1.58	-
28	$\beta$ -Caryophyllene alcohol	18.950	-	7.72	0.78
29	Myrcene acetal	19.131	-	3.38	-
30	trans-Carveol diol	19.743	-	0.97	-
31	terpinen-4-ol	21.223	-	21.96	-
32	Carvone	22.724	-	4.83	-
33	2-Methylisoborneol	22.985	-	2.54	-
34	2-Bornanol, 2-methyl-	23.45	-	2.39	-
35	Mesityl oxide	23.859	-	0.99	3.07
36	Artedouglasia oxide A	25.424	-	1.12	-
37	(-)-Spathulenol	25.870	-	1.31	3.64
38	Cryptomeridiol	28.122	-	6.83	-
39	Benzyl Benzoate	30.971	-	0.89	0.81
40	Aromadendrane-4,10-diol	33.279	-	1.21	-
41	Pregnenolone	37.972	-	1.23	-
42	Phytol	38.316	-	2.09	-
43	Solanesol	49.625	-	0.98	-
44	<i>o</i> -Cymene	10.536	-	-	1.01
45	4-Carvomenthenol	14.078	-	-	0.61
46	$\alpha$ -Terpineol	15.205	-	-	1.66
47	p-Methylacetophenone	15.293	-	-	1.01
48	$\alpha$ -Bisabolol	15.613	-	-	1.63
49	Bornyl acetate	18.143	-	-	0.65
50	Caryophyllene	21.824	-	-	1.49
51	4-(Tetradecyloxy)phenol	22.194	-	-	0.67
52	$\alpha$ -Humulene	22.726	-	-	1.6
53	$\gamma$ -Cadinene	23.185	-	-	3.99
54	$\alpha$ -Amorphene	23.29	-	-	0.85
55	$\gamma$ -Muurolene	23.623	-	-	0.84
56	$\alpha$ -Muurolene	23.763	-	-	1.89
57	(R)-p-Cymene	24.024	-	-	0.55
58	$\gamma$ -Muurolene	24.131	-	-	3.05
59	$\delta$ -Cadinene	24.244	-	-	1.63
60	Tetrahydronaphthalene	24.317	-	-	1.26
61	$\alpha$ -Cadinene	24.728	-	-	0.85
62	$\alpha$ -Calacorene	24.841	-	-	1.2
63	Linalool	25.002	-	-	0.87
64	$\gamma$ -Muurolol	25.207	-	-	1.04
65	$\alpha$ -Calacorene	25.423	-	-	0.99
66	Caryophyllene oxide	26.06	-	-	4.39
67	(-) -Globulol	26.170	-	-	0.6
68	(-)-Myrtenol	26.870	-	-	1.58

69	Cubebol	26.997	-	-	1.84
70	Junenol	27.240	-	-	1.14
71	$\gamma$ -Himachalene	27.353	-	-	0.96
72	$\gamma$ -Eudesmol	27.481	-	-	2.07
73	$\tau$ -Cadinol	27.745	-	-	0.97
74	$\tau$ -Muurolol	27.799	-	-	1.86
75	$\alpha$ -Cadinol	27.867	-	-	1
76	$\tau$ -Muurolol	28.132	-	-	8.84
77	$\alpha$ -Cedrol	28.230	-	-	0.8
78	$\delta$ -Cadinene	28.363	-	-	0.77
79	$\alpha$ -Bisabolol	29.074	-	-	1.2
80	Tetradecanoic acid	30.775	-	-	0.67
81	$\gamma$ -Cadinene	31.761	-	-	1
82	$\alpha$ -Cedrol	31.835	-	-	0.91
83	$\beta$ -Caryophyllene	32.048	-	-	0.57
84	3 $\alpha$ ,7 $\beta$ -Dihydroxy-5 $\beta$ ,6 $\beta$ -epoxycholestane	32.145	-	-	0.66
85	Dihydroboldenone	32.440	-	-	1.35
86	Neophytadiene	32.653	-	-	0.63
87	Hexahydrofarnesylacetone	32.746	-	-	1.99
88	Globulol	33.286	-	-	1.93
89	Labdane dialdehyde	34.197	-	-	0.58
90	Mintoxide	34.378	-	-	0.61
91	Ascorbyl dipalmitate	35.339	-	-	0.68
92	Aromadendrene oxide-(2)	35.528	-	-	0.71
93	Phytol	38.318	-	-	4.86
94	Palmitaldehyde, diallyl acetal	38.88	-	-	1.8
95	Octacosanol	41.549	-	-	0.58
96	1-Hexacosanol	44.896	-	-	0.89
97	Tetrapentacontane	45.039	-	-	0.72
98	Hexacontane	48.042	-	-	0.6

The distribution of major chemical classes in the essential oils differed markedly among the three sampling sites (Table 3). The oil from Area 1 (EOPT1) was dominated by monoterpene hydrocarbons, which accounted for 57.48% of its total composition. In contrast, EOPT2 displayed a substantially higher content of oxygenated monoterpenes,

representing 37.45%. EOPT3 was distinguished by its high proportion of hydrocarbon sesquiterpenes, comprising 31.23%. Other compound families were most abundant in EOPT1 together they made up 35.61% of the oil while the levels of oxygenated sesquiterpenes remained comparable across all three essential oil samples (Table 3).

**Table 3:** Subclasses of terpenes in the essential oils

Subclasses of terpenes	Percentage composition (%)		
	EOPT1%	EOPT2%	EOPT3%
Hydrocarbon monoterpenes	57.48	13.91	12.16
Oxygenated monoterpenes	4.81	37.45	7.98
Hydrocarbon sesquiterpenes	2.08	1.55	31.23
Oxygenated sesquiterpenes	0	18.19	25.80
Other	35.61	28.90	22.83

EOPT1: Essential oil of *Pistacia terebinthus* collected from site 1 (Amghas), EOPT2: Essential oil of *Pistacia terebinthus* collected from site 2 (Lac Afouragh), EOPT3: Essential oil of *Pistacia terebinthus* collected from site 3 (Aït Naamane).

The GC-MS spectrum (Figure 2) provides a detailed chemical profile of the essential oils, illustrating the presence and intensity of various volatile compounds based on their retention times and peak areas. Each peak corresponds to a specific compound identified in the oil sample. The accompanying figure highlights the major constituents detected,

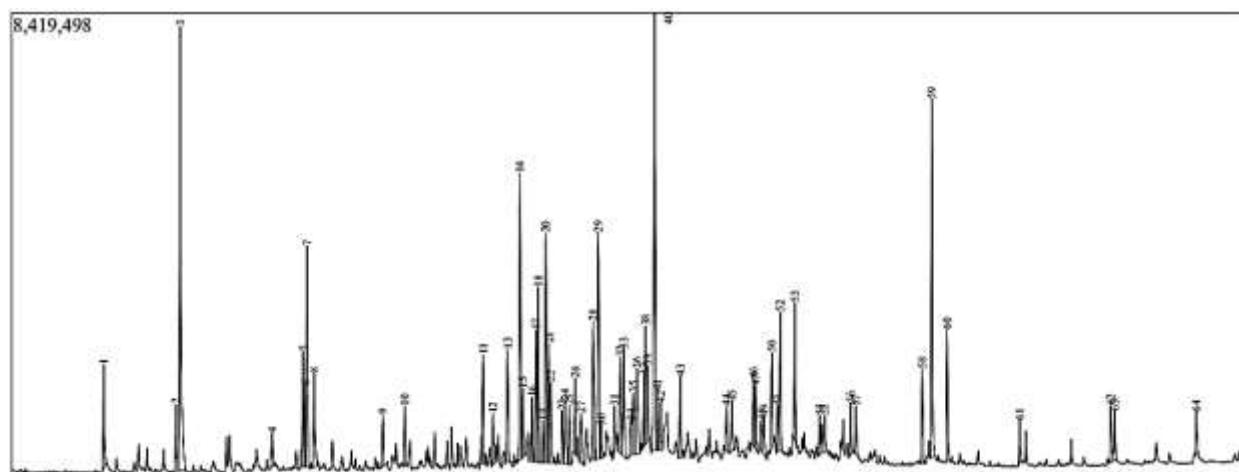
showing the predominant compounds with the highest relative abundance (Figure 3).

The essential oil extracted from the leaves of *Pistacia terebinthus* L. collected in Turkey was primarily composed of  $\alpha$ -terpineol (5.00%),  $\delta$ -cadinene (5.10%), phytol (5.40%), bornyl acetate (4.40%), and

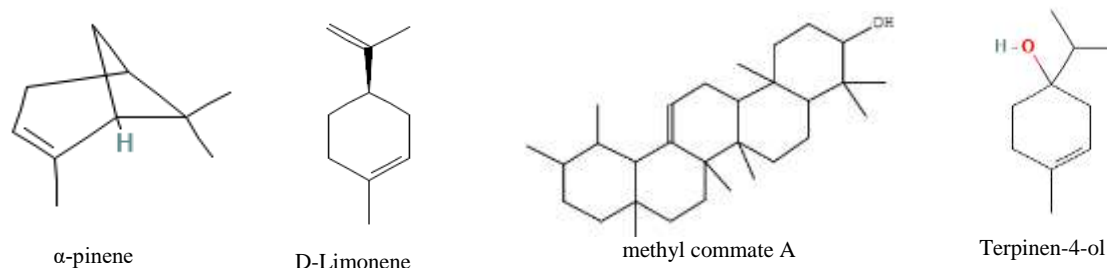
$\alpha$ -cadinol (6.90%). In comparison, the steam-distilled oil from twigs, in which 61 compounds were identified, was largely dominated by cubeol (5.40%),  $\alpha$ -cubebene (5.90%),  $\beta$ -pinene (7.50%), bornyl acetate (6.0%), and germacrene D (10.0%)<sup>41</sup>. In the corresponding fixed (fatty) oils, oleic acid was the principal fatty acid, whereas  $\alpha$ -pinene (26.31%) predominated in the volatile fraction. A survey of Kosovar populations revealed 33 volatile constituents, with  $\alpha$ -pinene ranging from 12.58% to 66.29%, D-limonene from 13.95% to 46.29%,  $\beta$ -ocimene from 0.03% to 40.49%,  $\beta$ -pinene from 2.63% to 20.47%, sabinene up to

5.61% and (Z)- $\beta$ -ocimene up to 44.85%.<sup>42</sup> The essential oil obtained from seeds of *Pistacia terebinthus* grown in Tunisia was found to be mainly composed of  $\beta$ -pinene, accounting for 38.28%<sup>43</sup>. In addition, monoterpene hydrocarbons represent the dominant chemical class, constituting 84.66% of total volatile compounds

Oxygenated monoterpenes and sesquiterpenes accounted for 6.58% and 3.29%, respectively. The variation in the chemical composition of essential oils depends mainly on the plant organs used for extraction and the geographical origin of *Pistacia terebinthus* L.<sup>44</sup>



**Figure 2:** GC Chromatogram of essential oils of *Pistacia terebinthus* collected from Afourgagh (Area 2). The peaks indicate absolute abundances, while the x-axis shows retention times in minutes



**Figure 3:** Chemical structures of major bioactive compounds identified in the essential oils of *Pistacia terebinthus* L.

#### Mineral composition of *Pistacia terebinthus* L. leaves

Table 4 illustrates the variation in the mineral element content of *P. terebinthus* L. leaves from three bioclimatic zones. Potassium (> 2 250 mg·kg<sup>-1</sup>) was found to be the most abundant cation across all sites, underscoring its role in drought resilience. Magnesium concentrations were highest in EOPT3 (973.53 mg·kg<sup>-1</sup>), potentially enhancing photosynthetic and metabolic functions. Sodium concentrations were highest in EOPT1 (382.13 mg·kg<sup>-1</sup>), indicating possible adaptation to saline or arid soils. Iron content varied significantly, with the highest level in EOPT2 (1 501.77 mg·kg<sup>-1</sup>) and the lowest in EOPT3 (973.53 mg·kg<sup>-1</sup>), reflecting localized soil and environmental influences. Minerals are indispensable for metabolic functions, particularly in supporting cellular growth and differentiation.<sup>45</sup> The analysis of *Pistacia terebinthus* L. foliage revealed notably high levels of both macronutrients, including calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P) and micronutrient such as iron (Fe). Previous studies have reported different mineral profiles in two pistachio cultivars, the mineral elements were ranked in the following order K<sup>+</sup> > Fe<sup>2+</sup> > Ca<sup>2+</sup> > Na<sup>+</sup> > Zn<sup>2+</sup> > Mn<sup>2+</sup> > Cr<sup>3+</sup>.<sup>46,47</sup> Such disparities likely reflect the strong influence of soil composition, irrigation water quality, and broader geographic factors on mineral uptake and accumulation in pistachio tissues.

#### Antioxidant activity of essential oil of *Pistacia terebinthus*

Three essential oils were subjected to antioxidant testing through FRAP and DPPH methods, employing BHT and ascorbic acid as reference standards. The half-maximal inhibitory concentrations (IC<sub>50</sub>), which reflect the dose necessary to achieve 50% inhibition of radical activity, differed markedly among the three collection sites. Essential oil of *Pistacia terebinthus* leaves from Area 1 (EOPT1) showed the lowest IC<sub>50</sub> value, ranging from 1.40 to 1.51 mg/mL, with mean value of 1.45 ± 0.03 mg/mL. In comparison, EOPT2 exhibited higher IC<sub>50</sub> value, ranging from 2.34 to 2.48 mg/mL, with mean value of 2.42 ± 0.04 mg/mL, while EOPT3 yielded the highest IC<sub>50</sub> value, ranging from 4.14 to 4.17 mg/mL (mean = 4.15 ± 0.01 mg/mL).

Similarly, the ferric reducing antioxidant power (FRAP) assay revealed significant differences in reducing capacity (Table 5). The strongest activity was observed in EOPT1, which showed an average IC<sub>50</sub> of 3.20 mg/mL, in contrast to EOPT2 and EOPT3, whose reducing power was considerably weaker, with IC<sub>50</sub> values of 8.36 mg/mL and 8.64 mg/mL. Together, these findings indicate that essential oil of *Pistacia terebinthus* leaves from Area 1 exerts the most potent antioxidant effects, followed by those from Area 2 and Area 3. Such geographical variation in bioactivity likely arises from local environmental factors including temperature fluctuations, precipitation patterns, wind exposure, solar intensity, UV radiation, and humidity that influence secondary metabolite production.<sup>48</sup>



In mountainous regions, for example, lower mean temperatures, greater diurnal temperature range, and intensified light exposure impose stress conditions that can alter plant physiology, morphology, and phytochemical synthesis as adaptive responses.<sup>48,49</sup> A study from India demonstrated that species of the *Pistacia* genus exhibit remarkable antioxidant effect, with crude extracts and flavonoids from *Pistacia integerrima* showing strong antioxidant activity. This activity is mainly due to direct free radical scavenging, where flavonoids are oxidized by free radicals, leading to the formation of more stable and less reactive radicals.<sup>50</sup>

The antioxidant efficacy of *P. terebinthus* L. essential oils differed significantly among the three bioclimatic zones studied. Such discrepancies may be attributed to variations in the qualitative and quantitative profiles of phenolic and flavonoid constituents, their applied doses, and the environmental and climatic parameters prevailing at each collection site. Extant research on *Pistacia spp.* consistently highlights their value as natural antioxidant reservoirs, owing primarily to their high levels of phenolics (e.g., quercetin) and tocopherols ( $\alpha$ -tocopherol), which serve as reference antioxidants. In comparative assays, methanol extracts of *P. terebinthus* leaves outperformed acetone extracts across multiple test systems. Notably, at

50  $\mu\text{g/mL}$  in the DPPH scavenging assay, the methanol fraction was comparable to the inhibitory activity of standard antioxidants. Its ferric reducing capacity also exceeded that of  $\alpha$ -tocopherol, whereas the acetone fraction exhibited reducing power on par with  $\alpha$ -tocopherol.<sup>51</sup> Another investigation reported that the leaf extract of *P. terebinthus* L. possessed an antioxidant potency roughly twelve-fold greater than that of both butylated hydroxyanisole (BHA) and ascorbic acid.<sup>52</sup> Moreover, fruit extracts displayed remarkable metal chelating ability surpassing EDTA and radical scavenging activity comparable to commercial standards; thermal roasting further enhanced this antioxidant performance.<sup>53</sup> In a Turkish study, foliar extracts of *P. terebinthus* achieved over 85  $\mu\text{mol}$  Trolox equivalent antioxidant capacity (TEAC), suggesting a potential role in mitigating oxidative damage linked to carcinogenesis.<sup>52</sup> Further Turkish studies quantified the antioxidant metrics of the methanol extract as follows: ABTS, 3.29 mmol TE/g; DPPH, 2.06 mmol TE/g; CUPRAC, 2.67 mmol TE/g; FRAP, 1.62 mmol TE/g; and metal chelation activity of 65.65 mg EDTA/g.<sup>54</sup> In a Jordanian study, *P. palaestina* leaf extracts exhibited strong ABTS<sup>+</sup> and DPPH radical scavenging activity, with IC<sub>50</sub> values of 6.86  $\mu\text{g/mL}$  and 8.31  $\mu\text{g/mL}$ , respectively, which were comparable to those of ascorbic acid (6.09  $\mu\text{g/mL}$  and 6.96  $\mu\text{g/mL}$ ).<sup>55</sup>

**Table 4:** Mineral content of *Pistacia terebinthus* leaves

Sample	Mineral content (mg/kg)											
	Ca	Cu	Pb	Mg	K	Mg	Mn	Na	K	Fe	Se	Zn
LPT1	>2250.00	0.81	<0.02	743.65	1153.35	743.65	1.49	382.13	1153.35	13.17	<0.02	1.05
LPT2	>2250.00	0.60	0.02	769.18	1501.77	769.18	0.98	209.29	1501.77	27.86	<0.02	1.06
LPT3	>2250.00	0.77	<0.02	973.53	1098.76	973.53	1.26	228.09	1098.76	26.68	<0.02	1.89

LPT1 = Leaves of *Pistacia terebinthus* collected from site 1, LPT2 = Leaves of *Pistacia terebinthus* collected from site 2, LPT3 = Leaves of *Pistacia terebinthus* collected from site 3.

**Table 5:** Antioxidant activity of *Pistacia terebinthus* leaf oils and synthetic antioxidants (DPPH and FRAP assays)

	DPPH IC <sub>50</sub> (mg/mL)			FRAP EC <sub>50</sub> (mg/mL)		
	Max	Min	Mean $\pm$ SD	Max	Min	Mean $\pm$ SD
BHT	0.009	0.009	0.009 $\pm$ 0.000	1.051	1.279	1.165 $\pm$ 0.114
Ascorbic acid	0.001	0.001	0.001 $\pm$ 0.001	0.005	0.001	0.003 $\pm$ 0.001
EOPT 1	1.51	1.40	1.45 $\pm$ 0.03	3.52	2.86	3.20 $\pm$ 0.22
EOPT 2	2.48	2.34	2.42 $\pm$ 0.04	8.89	7.82	8.36 $\pm$ 0.36
EOPT 3	4.17	4.14	4.15 $\pm$ 0.01	9.47	7.82	8.64 $\pm$ 0.55

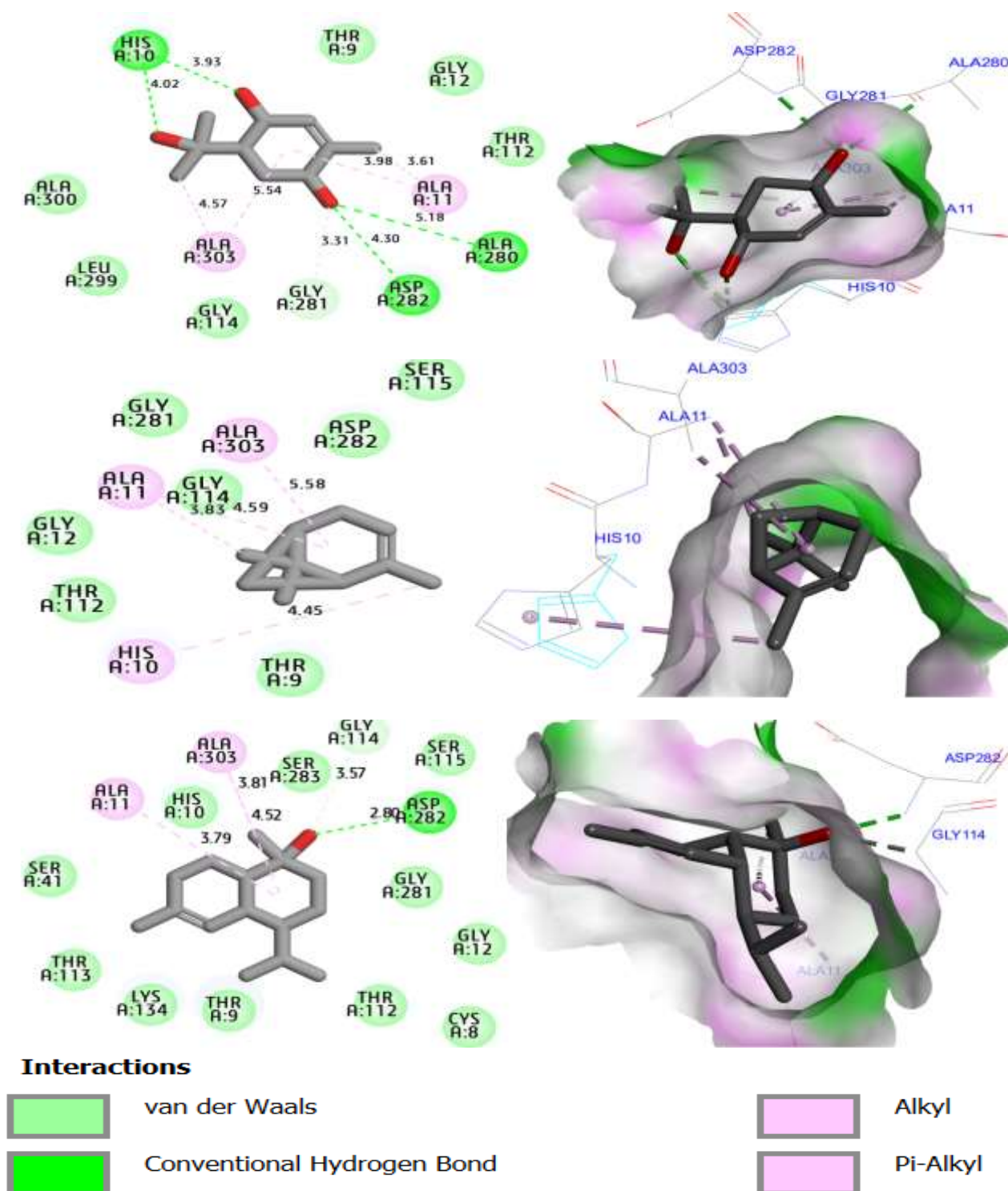
Data are expressed as Mean  $\pm$  SD (n = 3). EOPT1: essential oil of *Pistacia terebinthus* from site 1 (Amghas); EOPT2: from site 2 (Lac Afourgagh); EOPT3: from site 3 (Aït Naamane); BHT: butylated hydroxytoluene.

The present results on *Pistacia terebinthus* show notable similarities and differences compared to the study on *Pistacia lentiscus* from Algeria,<sup>56</sup> while the Kabylie study used a hydro-methanol extract rich in flavonoids and tannins, the present work focused on essential oils dominated by  $\alpha$ -pinene and other monoterpenes. Antioxidant activity was higher in the *P. lentiscus* extract (IC<sub>50</sub> = 0.024 mg/mL) compared to the essential oils (IC<sub>50</sub> = 1.40–4.17 mg/mL), likely due to the polar nature of the extract. Additionally, the present study included molecular docking analysis, providing mechanistic insights not addressed in the Algerian study. Moreover, studies on *Pistacia vera* have corroborated our findings on its antioxidant activity. *Pistacia vera* L. demonstrated strong antioxidant effects against DPPH radicals, with an IC<sub>50</sub> value of

72.60  $\mu\text{g/mL}$ , along with selective and dose-dependent cytotoxicity toward breast cancer cells.<sup>57</sup>

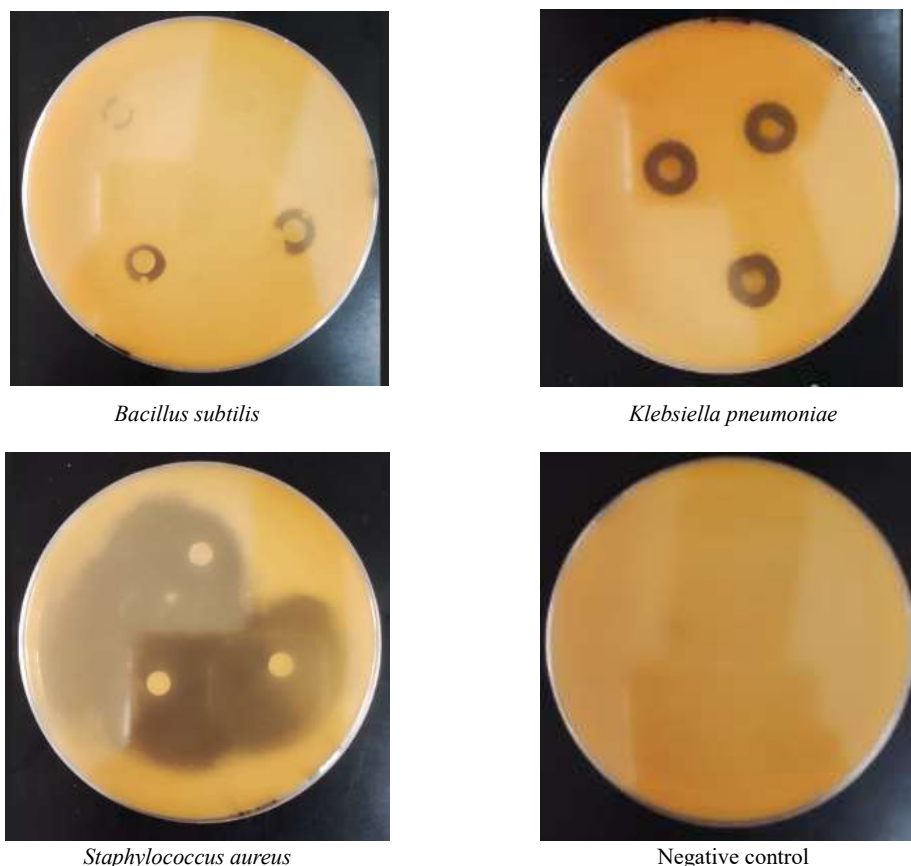
#### Molecular docking result

Chemical profiling of the essential oils from the three Moroccan Middle Atlas regions identified  $\alpha$ -pinene (50.81%), terpinen-4-ol (21.96%), and  $\tau$ -muurolol (8.84%) as the major constituents. Docking these ligands against the NADPH oxidase structure (PDB ID: 2CDU) produced the following lowest binding energies:  $\alpha$ -Pinene,  $-7.05$  kcal/mol; terpinen-4-ol,  $-6.97$  kcal/mol; and  $\tau$ -muurolol,  $-6.80$  kcal/mol.  $\alpha$ -Pinene formed three alkyl interactions with His10, Ala11, and Ala300 on chain A (Figure 4A).



**Figure 4:** 2D and 3D Visualization of the intermolecular interactions of terpinen-4-ol and  $\alpha$ -pinene with the NADPH oxidase protein (PDB ID: 2CDU)





**Figure 5:** Effect of *Pistacia terebinthus* leaves essential oil on bacterial growth

The zones of inhibition around the discs indicate the antimicrobial activity of *Pistacia terebinthus* essential oil against the tested bacterial strains. The negative control did not contain any essential oil.

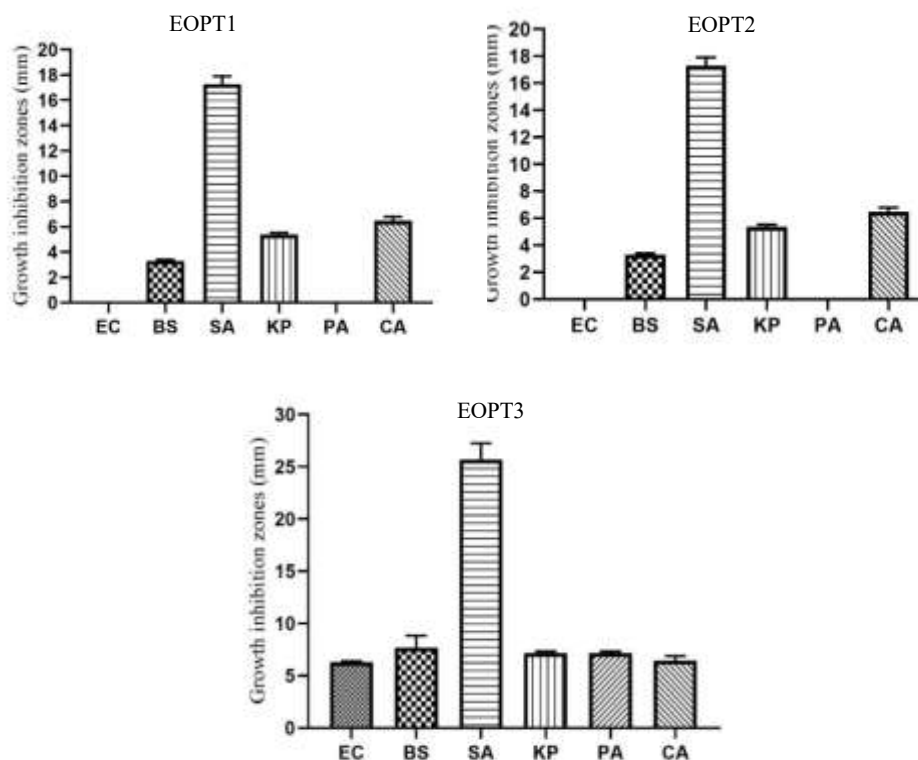
terpinen-4-ol engaged Ala11 and Ala303 via alkyl bonds, Gly281 via a C–H bond, and His10, Ala280, and Asp282 through hydrogen bonding (Figure 4B).  $\tau$ -Murolol generated one hydrogen bond to Asp282, a C–H bond with Gly114, and alkyl contacts at Ala11 and Ala300 (Figure 4C). These recurring interactions particularly at Ala11, Asp282, and Ala300 corroborate the proposed mechanism by which these terpenoid and hydroxylated monoterpene compounds inhibit NADPH oxidase activity, thereby underpinning the observed antioxidant effects.<sup>58</sup>

#### activity of essential oil of *Pistacia terebinthus* L

Figure 5 illustrates the inhibition zones observed for the various strains tested with the different essential oils. The antimicrobial activity showed considerable variation depending on the bacterial and fungal species. Among the oils tested, EOPT3 exhibited particularly strong effects against several microbial strains. The antibacterial potential of the three essential oils (EOPT1, EOPT2, and EOPT3) was evaluated against a panel of bacterial strains. EOPT3 exhibited the strongest activity overall, showing a pronounced inhibition against *Staphylococcus aureus* ( $25.7 \pm 1.572$  mm), moderate activity against *Pseudomonas aeruginosa* ( $7.167 \pm 0.1528$  mm), and a measurable effect on *Escherichia coli* ( $6.3 \pm 0.1$  mm), indicating broad-spectrum efficacy. In contrast, EOPT1 and EOPT2 showed no inhibitory effect on *E. coli* and *P. aeruginosa* (0 mm). However, EOPT2 displayed moderate activity against *Klebsiella pneumoniae* ( $8.167 \pm 0.1528$  mm). Regarding *Bacillus subtilis*, an increasing gradient of inhibition was observed: EOPT3 exerted the strongest effect ( $7.7 \pm 1.127$  mm),

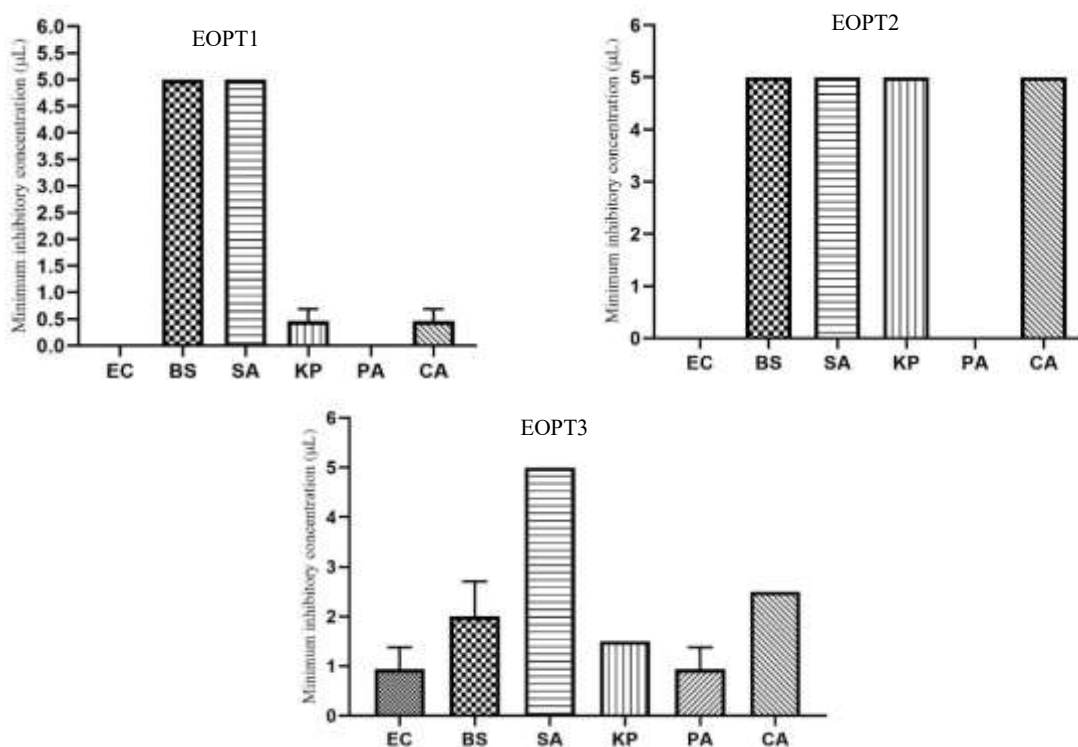
followed by EOPT2 ( $5.2 \pm 0.2646$  mm) and EOPT1 ( $3.3 \pm 0.1$  mm). These results highlight both the differential antibacterial spectra among the oils and the particularly potent activity of EOPT3 (Figure 6). All three essential oils exhibited similar antifungal activity against *Candida albicans*, with inhibition zones around 6.467 mm, indicating a modest effect. Overall, EOPT3 emerged as the most effective essential oil in terms of antimicrobial properties, while EOPT1 and EOPT2 displayed more selective and strain-dependent activities.

However, *P. terebinthus* oil demonstrated a broader antimicrobial spectrum, showing activity not only against *S. aureus* and *E. coli*, but also against *P. aeruginosa*, *K. pneumoniae*, *B. subtilis*, and *Candida albicans*, with notable MIC values below 1 mg/mL for several strains. Unlike other *P. lentiscus* study, which focused mainly on antibacterial effects, the present findings also confirmed significant antifungal potential, supported by prior studies against phytopathogens and *C. albicans*<sup>61</sup>. In a previous study, the antimicrobial activity of *Pistacia atlantica* subsp. *atlantica* was evaluated against the bacterial strains *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and the fungus *Candida albicans*. Significant differences were observed between the essential oils of *P. atlantica* and *P. terebinthus*. Among the tested oils, the Afourgagh leaf oil (EOAA2) showed the strongest activity, particularly against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans*, highlighting its remarkable antimicrobial potential



**Figure 6:** Antimicrobial activity (Growth inhibition) of essential oils extracted from *Pistacia terebinthus* leaves collected from three distinct locations in the Middle Atlas region of Morocco

EC: *Escherichia coli*, BS: *Bacillus subtilis*, SA: *Staphylococcus aureus*, KP: *Klebsiella pneumoniae*, PA: *Pseudomonas aeruginosa*, CA: *Candida albicans*, EOPT1: Essential oil of *Pistacia terebinthus* collected from site 1 (Amghas), EOPT2: Essential oil of *Pistacia terebinthus* collected from site 2 (Lac Afourgagh), EOPT3: Essential oil of *Pistacia terebinthus* collected from site 3 (Aït Naamane).



**Figure 7:** Antimicrobial activity (Minimum inhibitory concentration) of essential oils extracted from *Pistacia terebinthus* leaves collected from three distinct locations in the Middle Atlas region of Morocco

EC: *Escherichia coli*, BS: *Bacillus subtilis*, SA: *Staphylococcus aureus*, KP: *Klebsiella pneumoniae*, PA: *Pseudomonas aeruginosa*, CA: *Candida albicans*, EOPT1: Essential oil of *Pistacia terebinthus* collected from site 1 (Amghas), EOPT2: Essential oil of *Pistacia terebinthus* collected from site 2 (Lac Afourgagh), EOPT3: Essential oil of *Pistacia terebinthus* collected from site 3 (Aït Naamane).

## Conclusion

This study explored the chemical diversity of *Pistacia terebinthus* L. leaf essential oils collected from three distinct bioclimatic zones in the Moroccan Middle Atlas, spanning sub-humid to semi-arid regions. Gas chromatographic analysis identified 98 compounds, revealing notable regional variations in the essential oil profiles. These differences were reflected in the oils' antioxidant and antimicrobial activities, which varied according to the bioclimatic origin. The findings suggest that environmental and climatic conditions significantly influence the chemical composition and bioactive properties of the oils. Notably, samples from semi-arid zones appeared to produce more distinct chemotypes with enhanced therapeutic potential. Overall, the observed variability in chemical composition, mineral content, and biological activity underscores the pharmaceutical relevance of *P. terebinthus* L. and highlights the importance of its sustainable cultivation and valorization, particularly in Morocco's ecologically diverse landscapes. To further exploit this potential, future research should focus on isolating key active molecules, evaluating their efficacy and safety through *in vivo* studies, and developing cultivation strategies adapted to specific bioclimatic conditions. Additionally, sustainable harvesting and conservation efforts are essential to preserve the unique genetic and chemical diversity of wild populations, while promoting the integration of this species into local medicinal and agro-industrial value chains.

## Conflict of interest

The author's declare no conflicts of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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