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Chemical Composition, Antioxidant Potential and Antibacterial Activity of *Pistacia atlantica* Desf. Essential Oil Leaves, with A Focus on Variations in The Main Trunk Diameter

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Abstract: The aim of the present study is to assess the chemical compounds, antibacterial potential, and antioxidant qualities of essential oils as extracted from dried leaves of five *Pistacia atlantica* of different trunk diameters from Rommani (a rural area in the west of Morocco). The DPPH and FRAP methods are used to measure antioxidant activity. Essential oils have been tested on *Acinetobacter baumannii*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The essential oils were analysed using gas chromatography coupled with mass spectrometry (GC/MS) analysis. The obtained results show that the tree's diameter, which is proportional to its age, was related to the original components. Terpinen-4-ol (19.00%–22.33%) with a diameter greater than or equal to 23.73 cm and α-Pinene (18.49%–37.51%), smaller than or equal to 22.45 cm, are the two primary components. The findings of the DPPH and FRAP tests indicate that the IC_{50} values range from 8.70 ± 0.02 mg/mL to 11.46 ± 0.01 mg/mL and the EC_{50} values range from 8.27 ± 0.04 mg/mL to 12.76 ± 0.16 mg/mL, respectively. The interval of the zone of inhibition [8.77 ± 1.48 mm – 12.07 ± 2.51 mm], according to the results of antibacterial activity testing, shows that MIC and MBC vary between 4 and 10 µL/mL.

Keywords: *P. atlantica*; DPPH; chemical compounds; antibacterial activity; secondary metabolites; biological activity.

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1. Introduction

Herbal remedies are natural medicinal plants used to treat and prevent disease. They belong to the field of traditional medicine, sometimes known as complementary and alternative medicine. Thousands of herbal over-the-counter medications are available in every nation [1]. *P. atlantica*, sometimes called "Elbetoum'' in Arabic and "Iggh" in Amazigh, is a part of the Anacardiaceae family [2,3]. Masego in the Canary Islands, Milengic in Turkish, and *P. atlantica* Baneh in Persian [4]. It is one of several extant species of Pistacia found in Mediterranean countries [5], reaching a height of 18 metres [6]. Unlike other pistachio trees, it has typical evergreen leaves that end in two leaflets, while the leaves of other species end in a single leaf [7].

The fruit colour of such a category of plants varies from green to dark brown. Most people are aware of the medicinal and anti-inflammatory properties of *P. atlantica* trees [4]. Besides its medicinal properties, *P. atlantica* is used to treat eczema, diarrhoea, asthma, and throat infections [2]. It is also used in perfumes, the cosmetics industry, and artistic objects made of metal, wood, and glass [8]. The current study seeks to compare the findings of five samples from the Rabat region in order to evaluate the biological activities and examine the components of the essential oil extracted from the plant's leaves according to the diameter of the main stem of the tree.

GC-MS is used to identify the chemical components of the essential oil. The evaluation of their antibacterial activity against seven bacterial strains and antioxidant activity using FRAP and DPPH is the second goal.

2. Materials and Methods

2.1. Plant Material

In the commune of Rommani (33° 32′ 00″ N and 6° 36′ 0″ W), 81 km from Rabat at an altitude of 306 m, an area of 23 metres wide and 37 metres long (an area of 851 m^2), containing several *P.atlantica* trees, was targeted to select five trees with different main trunk diametres. The different leaves were uprooted at the end of October 2021 and then kept in separate bags. The leaves were then transferred to the scientific institute of Rabat to be identified by Professor OUFAE BENKHNIGUE, taking the voucher code RAB 113647 (Figure 1). Some samples were kept in the herbarium of the institute, whereas others were transported to the laboratory.

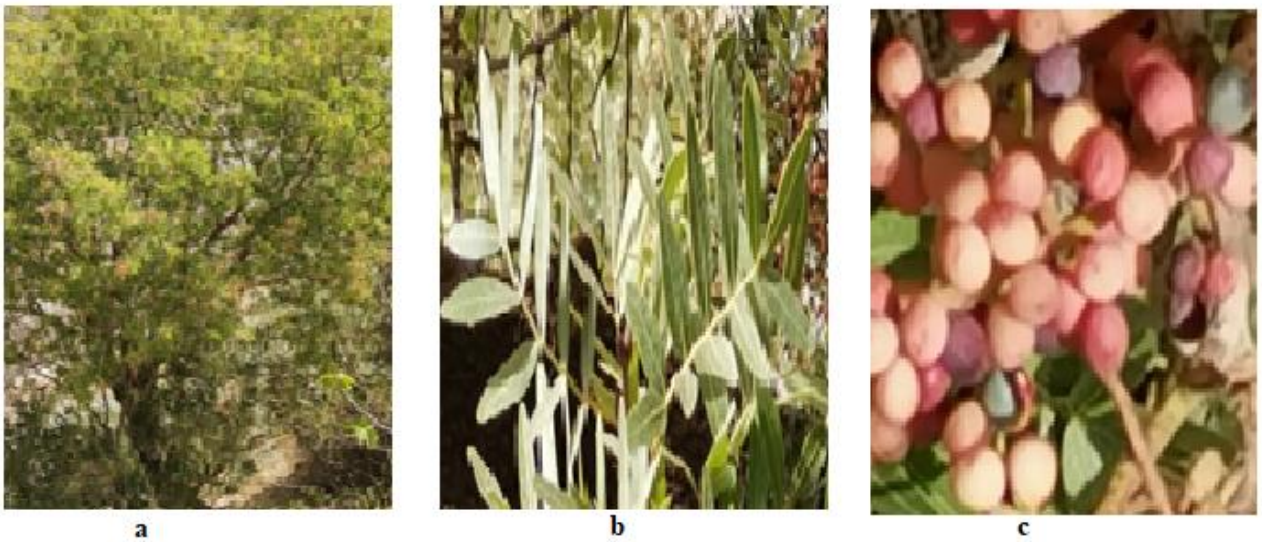

Figure 1. Pictures a, b, and c depict the tree, foliage and fruit of the *Pistacia atlantica*, respectively

2.2. Essential Oil Extraction

For the five samples, we followed the same protocol described by Otaifah et al. [9], with some modifications. 200 g of dried leaves were subjected to hydrodistillation in 375 mL of water for five hours. After the recovery of the organic phase, it was dried by anhydrous sodium sulfate and kept at 5 °C until its use. According to Beniaich et al. [10], the yield "ρ" of the essential oil is calculated by the following expression:

$$\rho = (W_f / W_i)*100$$

W_f is the weight of the extracted oil expressed in gram (g).
W_i is the weight of the initial dried vegetal material in gram (g).

It can be expressed as a percentage (%).

2.3. Analysis of The Chemical Components of Essential Oils Using GC-MS

The chemical compositions of the essential oils were determined by gas chromatography coupled with mass spectrometry at CNRST in Rabat, Morocco. Gas chromatography used a TSQ 8000 EVO coupled to a mass spectrometer and column class TR-35MS (30 m x 0.25 mm x 0.25 µm). Helium was used as a carrier gas at a pressure of 1.5 mL/min. The injection temperature is 200°C, the partial flow is 50 mL/min, and the split ratio is 33-3. Prior to being injected into the column, 20 µL of the essential oil was dissolved in 1 mL of hexane. Sample injection operates in split mode, and the system is linked to a computer system that oversees a NIST mass spectrum library. Components are identified based on their mass spectrum acquired through gas chromatography coupled with mass spectrometry (GC-MS) and their Kovat indices (KI).

2.4. Determination of Antibacterial Activity

2.4.1. Method of Diffusion in Gelose

The disc diffusion method is used to assess the antibacterial activity of *P. atlantica* essential oil because it is simple and effective in determining which strains are susceptible to it [11]. A volume of 20 mL of supercooled Mueller-Hinton agar medium is poured into Petri dishes. A microbial suspension with an optical density of 10^6 CFU/mL is applied to the surface after the culture medium has solidified. Whatman absorbent paper discs, 6 mm in diameter, are sterilised in the autoclave. They are soaked in essential oil and placed on the surface of the agar. Petri dishes are kept at 4 ºC for one hour so that the essential oil can diffuse before the start of the multiplication of germs [12]. The whole is incubated for 24 hours at 37 °C. The essential oil uniformly diffuses as soon as the impregnated discs are used. As individual positive controls, 25 µg of amoxicillin and 5 µg of penicillin were used.

2.4.2. Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC)

By using the agar dilution technique, the minimum inhibitory (MIC) and bactericidal (MBC) values are calculated [13]. The essential oil is emulsified by a 0.2% agar solution. The prepared dilutions are: 100 µL/mL, 40 µL/mL, 20 µL/mL, 10 µL/mL, 5 µL/mL, 3,3 µL/mL, and 2 µL/mL in this agar solution. 1, 5 ml of each dilution is aseptically added to test tubes containing 13.5 ml of the solid medium that have been sterilised in an autoclave at 121°C to produce the final concentrations of 10 µL/mL, 4 µL/mL, 2 µL/mL, 1 µL/mL, 0.5 µL/mL, 0.33 µL/mL, and 0.2 µL/mL. The contents of each tube are immediately poured into a sterile Petri dish. In addition, controls are carried out using the 0.2% agar solution and the culture medium. To start, stries are used with a calibrated anse of palatine to prelever the same amount of inoculum. This last one is presented as 24 hours of cultured broth.

Incubation takes place for 24 hours at 37°C. The CMI was always stated in microliters per millilitre (µL/mL). Each experiment is run three times to reduce experimental error.

2.5. Antioxidant Activity

2.5.1. Diphenyl Picrylhydrazyl (DPPH)

The DPPH test is a frequently used method for evaluating antioxidant activity [14]. One of the main features of DPPH is its ability to produce stable free radicals. This stability is caused by the internal relocation of free electrons. The existence of these DPPH radicals gives the solution a dark purple hue. The reduction of DPPH radicals by an antioxidant causes the solution to discolor. The antioxidant capacity of vegetable essential oils is measured by spectrophotometry at 517 nm. 50 µL of essential oil at different concentrations (100, 50, 25, and 12.5 µg/ml) were mixed with 2 mL of methanol solution containing DPPH (60 µM). After 30 minutes of incubation at 25°C, absorbance was measured against a blank [15]. The percentage inhibition (I%) of free radicals is determined by the following formula:

$$I\ (\%) = 1 - (A_{sample} / A_{balnc})*100$$

A_{sample}: is the value of test solution; A_{blanc} : is the record value of the blank sample.

Ascorbic acid (A.A) is used as a positive control. The curve representing the percent inhibition variation as a function of the essential oil concentration was used to calculate the EO concentration, which provides a 50% inhibition (IC_{50}), knowing that IC_{50} (A.A) = 4.85 ± 0.58 mg/mL. All tests were performed three times.

2.5.2. Ferric Ion (Fe^{3+}) (FRAP)

The reducing power determined by FRAP technique is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) [16]. The presence of reducing agents in plant essential oil enables the Fe^{3+} ferricyanide complex to be reduced to the ferrous form. Consequently, the increase in the density of cyan blue in the reaction medium makes it possible to evaluate the formation of Fe^{2+} ions. The FRAP reagent was made using a modified version of one of the procedures reported in the literature. 1 mL (10 mM) of 2,4,6-tripyridyl-s-triazine (TPTZ) solution (dissolved in 40 mM hydrochloric acid) and 1 mL (20 mM) of hexahydrate ferric chloride (dissolved in distilled water) are combined with 10 mL (300 mM) of acetate buffer solution, pH = 3.6 (3.1 g sodium acetate trihydrate), and 10 µL of essential oil at various concentrations (100, 50, 25, and 12.5 mg/mL) were added to 190 µL of FRAP solution. After 30 minutes of rest at 25°C, the absorbance was measured at 593 nm using a UV-Vis spectrophotometer. Ascorbic acid was used to establish a calibration curve in the concentration range of 0–200 µg/mL, and the results were expressed in mg EAA/g dry weight [17]. Measurements are performed in three steps to minimize errors.

2.6. Data Processing and Statistical Analysis

The Tidyverse package was used for data processing and analysis in RStudio. The "diameter" variable was changed to a factor and the dataset was imported from a CSV file. To see the data distribution of various variables over various diameters, we used boxplots and jitter plots for descriptive statistics. Yield, DPPH, FRAP, and numerous bacterial species (*S. aureus*, *S. epidermidis*, *S. aureus* methicillin-resistant, *K. pneumoniae*, *A. baumannii*, and *E. cloacae*) were among the variables that were taken into consideration. The Shapiro-Wilk test was used to determine whether the data for each variable was normal. Non-parametric tests were utilized if the assumption of normality was broken. For each variable, Kruskal-Wallis tests were run to evaluate the variance in distributions over various diameters. The Kruskal-Wallis tests were followed by post-hoc Dunn's tests to identify particular groups that differed, with Benjamini-Hochberg multiple comparisons correction to reduce the false discovery rate. Using Pearson's correlation approach, correlation analysis was also carried out among all variables

(diameter was excluded) and was shown as a correlation plot. To minimize the dimensionality and pinpoint the crucial variables that contribute the greatest variance to the dataset, principal component analysis (PCA) was applied to the data. To show the observations and variables in the PC space and to examine how diameter is related to other variables, biplots were created using the ggbiplot and factoextra programmes. For more comprehensive PCA results, the FactoMineR package was used. This involved calculating the eigen values, cos^2 (a measure of the factor map's quality), and the contributions of each variable to each principal component. The variables were grouped using the k-means method, and correlations between them were also shown. To show the quality of the variables and individuals on the factor map, the individual cos^2 values were shown. All statistical tests were two-sided, and statistical significance was defined as a p-value of less than 0.05.

3. Results and Discussion

3.1. Yield of Essential Oils Extraction

Essential oil yield is measured in millilitres per 100 grammes of dry weight. *P. atlantica* has a modestly low production of volatile chemicals, according to the yields found in the leaves of the five trees. The production of essential oils ranges from 0.06 to 0.11 percent. Its colour ranges from light yellow to light brown, and it smells strongly. Below are the EO yields for each sample (diameter), which are reported as average values with standard deviations (Table 1). The results demonstrate that samples 1, 4, and 5 all produced the same amount of essential oil. Sample 2 (20.86 cm), on the other hand, shows a yield of 0.11 ± 0.03%. According to studies [18–20], the yield of essential oils from the leaves of *P. atlantica* is 0.13%, 0.24%, and 0.2%, respectively. Yet, our results don't go over 0.11%. Studies have revealed that between 0.02% and 0.12% of essential oils were produced at An-Oussera, Algeria [21]. This variation is likely brought on by habitat, age, harvest season, and extraction technique.

Table 1. Yields of essential oils

Mean Diameter (Ø 10^{-2} .m)	**Mean ± SD (%)**
Ø1: 11.46	0.06 ± 0.01
Ø2: 20.86	0.11 ± 0.03
Ø3:22.45	0.09 ± 0.02
Ø4: 23.73	0.06 ± 0.02
Ø5: 25.12	0.06 ± 0.02

3.2. Antioxidant Activity

Five essential oils were the subject of antioxidant investigations using two techniques: DPPH and FRAP. We utilized ascorbic acid and trolox as standards. Our results showed that the initial oil concentration and the rate of free radical inhibition changed in phase. The IC_{50} values of the antioxidant activity of the essential oils are collated in Table 2 for the five samples, with an IC_{50} ranging from 8.70 ± 0.02 to 11.46 ± 0.01 mg/mL. Several articles have studied the antioxidant properties of essential oils of *P. atlantica,* such as leaves, oleoresins, and fruits. Our results are different for some but equivalent for others. The five samples showed significant effectiveness against the oxidants used in tables 2 and 3. This difference is due to several factors, including the harvest period, the typical climate, the nature of the soil, and retention conditions [19–22].

Table 2. Antioxidant activity of essential oil extracted from *P. atlantica* leaves of different diameters of the main trunk using the DPPH protocol

Mean Diameter (Ø 10^{-2} .m)	**IC_{50} (mg/mL)**		
	Max	**Min**	**Mean ± SD**
Ø1: 11.46	08.73	08.68	08.70 ± 0.02
Ø2: 20.86	09.24	09.20	09.22 ± 0.02
Ø3:22.45	11.16	11.14	11.15 ± 0.01
Ø4: 23.73	11.20	11.24	11.22 ± 0.03
Ø5: 25.12	11.47	11.45	11.46 ± 0.01

The 50% inhibitory concentrations for diameters 3, 4, and 5 are almost identical (IC_{50} = 11.15, 11.23, and 11.46 mg/mL, respectively). The activity of essential oils for diameters 1 and 2 is greater than that of the first three diameters, with IC_{50} values of 08.70 ± 0.02 and 9.22 ± 0.02 mg/mL, respectively. In general, the IC_{50} values for the five samples were relatively close. It was also found that the antioxidant activity of various essential oils was significantly lower than the standard antioxidant compounds tested in various experiments. Oil may also be considered a good antioxidant, according to the studies of [19] and [22], which found 06.02 ± 0.10 and 1.71 ± 0.05 mg/mL for FRAP and 25.20 ± 0.05 and 82.85 ± 1.02 mg/mL for DPPH, respectively. The antioxidant effectiveness of *P. atlantica* essential oils was evaluated using the ferric reducing capacity test (FRAP), using ascorbic acid as a reference antioxidant. The range of EC_{50} inhibition concentrations varies from 8.27 ± 0.04 to 12.76 ± 0.16 mg/mL (Table 3).

Table 3. Antioxidant activity of essential oil extracted from *P. atlantica* leaves of different diameters of the main trunk using the FRAP protocol

Diameter	**EC_{50} (mg/mL)**	**EC_{50} (mg/mL)**	**EC_{50} (mg/mL)**	**Mean ± SD (mg/mL)**
Ø 1:(P4)	8.27	8.24	8.32	8.27 ± 0.04
Ø2:(P1)	8.65	8.35	8.29	8.43 ± 0.19
Ø3 :(P5)	12.37	12.27	12.13	12.26 ± 0.12
Ø4 :(P3)	12.51	12.39	12.18	12.36 ± 0.16
Ø5 :(P2)	12.76	12.91	12.6	12.76 ± 0.16

For DPPH IC_{50} values, the lower the value, the more potent the antioxidant. In this case, Morocco (Rommani) - Leaves exhibit the lowest DPPH IC_{50} (8.70 ± 0.02 mg/mL), suggesting that the extract from these leaves is the most effective at inhibiting DPPH free radicals compared to other samples. For FRAP values, a higher value indicates stronger antioxidant potential. In this context, the extract from Morocco (Rommani) leaves shows the highest FRAP value (12.76 ± 0.16 mg/mL), indicating a robust ability to reduce ferric ions and thereby possessing the strongest antioxidant power among the samples. Therefore, if we consider both DPPH IC_{50} and FRAP values together, the plant extract from Morocco (Rommani) leaves stands out as particularly potent in terms of antioxidant activity. It demonstrates both the most efficient radical scavenging with the lowest DPPH IC_{50} and the highest FRAP value, suggesting good reducing power. It is essential to note that the choice of the "most potent" extract may depend on the specific antioxidant properties one prioritizes, such as radical scavenging or reducing power. The obtained results show that the two most active diameters are 1 and 2. These results confirm the assertions of the DPPH method. We will examine the chemical composition of each sample individually to determine the cause of this difference. Based on the chemical compound composition, α-Pinene could be considered the main compound responsible for the antioxidant action; these results are consistent with those obtained by [23–24]. In general, the essential oils of *P. atlantica*

leaves show significant and higher antioxidant activity than those found in the literature for various test organs (Table 4).

Table 4. Compares the IC_{50} and FRAP assay values found in various literature searches and in our research.

Origin	Organ	Harvest period	DPPH IC_{50} (mg/mL)	FRAP (mg/mL)	References
Morocco (Khenifra)	Leaves	May	82.85 ±1.02	1.71 ± 0.05	[22]
Algeria	Leaves	October	13.91 ± 1.79 18.95 ± 6.67	05.70 ± 2.78 09.02 ± 0.92	[21]
Iran	Fruit	October	25.20 ± 0.03	06.02 ± 0.10	[19]
Morocco (Rommani)	Leaves	October	08.70 ± 0.02 11.46 ± 0.01	08.27 ± 0.04 12.76 ± 0.16	Our study

On the other hand, EOs from *P. atlantica* leaves showed low antioxidant activity compared to standard antioxidant (Ascorbic acid and Trolox) (Table 5).

Table 5. The antioxidant activity of a few synthetic antioxidants as measured by the DPPH test and FRAP assay.

Artificial antioxidant	IC_{50} (mg/mL)	EC_{50} (mg/mL)
Ascorbic Acid	4.85 ± 0.58	0.093 ± 0.01
Trolox	4.47 ± 0.54	-

3.3. Chemical Constitution of Essential Oils

Table 6 summarizes the results of the GC-MS analysis of essential oil from *P. atlantica* leaves. The composition data reveals the presence of 35 compounds in the essential oil. Using gas chromatography coupled with mass spectrometry makes it possible to identify the latter qualitatively and quantitatively. It is noted that the five oils are all rich in hydrocarbon monoterpenes first, followed by oxygenated monoterpenes at 70.28% and 26.62%, respectively. Where α-Pinene (18.49%, 24.67%, and 37.51%) is the majority compound for Ø3, Ø2, and Ø1, respectively, and Terpene-4-ol for the other two Ø4 (19.00%) and Ø5 (22.33%) (Table 6).

This rise in hydrocarbon monoterpenes can be thought of as a protective strategy for photosynthesis against heat oxidation [25]. By comparison with other studies, we find that our results are in agreement with those indicated by [19] (10.9%), (32.6–54.7%), and (15.33–40.47%) for α-Pinene [21], and (26.20%) [23] and (15.3%) for Terpene-4-ol [26], but with differences in the proportions of compounds. This is explained by several parameters, notably those that we cite: the time of harvest, the age of the tree, the nature of the soil, the climate, and fresh or dried leaves.

Where each oil is characterized by three primary compounds which have a significant predominance, which are present as follows: α-Pinene (37.51%), α-Sabinene (12.03%) and Camphene (11.58%) for leaves harvested from the tree with whose diameter Ø1=11.46 cm, as we find α-Pinene (24.67%), Spatulenol (14.23%) and Terpinen-4-ol (9.17%) for Ø2 and we also find α-Pinene (18.48%), Terpinen-4-ol (16.67%) and γ-Terpinene (10.33%) for diameter Ø3 and Terpinen-4-ol (19.00%), α-Pinene (15.71%) and p-Cymene (13.74%) for Ø4, as for the last oil diameter 25.12cm we have Terpinene-4-ol (22.33%), p-Cymene (13.97%) and α-Pinene (13.38%).

Table 6. The chemical composition of the essential oil of *P. atlantic*a leaves of different diameters for the main trunk

N	Compounds	RT (min)	KI	Area (%) Ø1=11.46 cm	Area (%) Ø2=20.86 cm	Area (%) Ø3=22.45 cm	Area (%) Ø4=23.73 cm	Area (%) Ø5=25.12 cm
1	α-Pinene (M)	6.72	936	37.51	24.67	18.49	15.71	13.38
2	Camphene (M)	7.57	955	11.58	6.33	2.20	-	1.08
3	α-Sabinene (M)	8.69	977	12.03	5.26	7.06	9.84	4.10
4	β-Pinene (M)	9.12	980	4.26	0.43	-	1.34	0.94
5	α –Phellandrene (M)	9.85	1006	-	0.11	1.30	0.42	0.85
6	α-Terpinene (M)	10.19	1020	0.23	0.42	7.15	2.59	4.56
7	Limonene (M)	10.65	1022	1.84	0.59	-	-	-
8	p-Cymene (M)	11.21	1025	1.87	6.71	6.65	13.74	13.97
9	γ-terpinene (M)	11.99	1059	0.45	1.28	10.33	5.94	7.59
10	Isoterpinolene (M)	12.99	1064	0.51	0.48	2.68	1.71	2.30
11	Nonanal(C9H18O) (A)	14.82	1072	-	0.07	0.07	-	0.10
12	3-Terpinen-1-ol (OM)	15.86	1088	-	-	-	-	0.12
13	Camphenol (OM)	16.13	1109	0.22	-	-	-	-
14	trans-Pinocarveole (OM)	16.35	1136	0.47	1.11	0.17	0.28	0.37
15	α-Phellandrene-8-ol (OM)	17.03	1151	0.12	0.11	-	-	-
16	Terpinen-4-ol (OM)	17.60	1179	1.68	9.17	16.27	19.00	22.33
17	3-Cyclohexene-1-methanol, α,α,4-trimethyl-(R) (OM)	18.40	1183	2.75	2.06	1.41	3.21	3.47
18	p-Cymen-8-ol (OM)	18.99	1197	-	-	-	0.37	-
19	Myrtenal (OM)	19.42	1220	0.46	0.58	-	-	0.28
20	Verbenone (OM)	20.24	1229	0.50	-	-	-	-
21	Bornylacetate ($C_{12}H_{20}O_2$) (A)	20.96	1250	0.09	4.04	1.03	0.72	0.61
22	α-Cubebene (S)	21.23	1257	0.16	-	-	-	-
23	Cumal (OM)	21.30	1276	-	0.18	-	0.10	0.33
24	Copaene (S)	22.32	1289	0.69	-	-	0.48	0.89
25	β-bourbenene (S)	22.77	1321	0.82	-	-	-	0.70
26	α-Terpinenylacetate ($C_{12}H_{20}O_2$) (A)	23.07	1347	-	0.76	0.64	0.98	0.47
27	1, 1, 5-Trimethyl-1,2-dihydronaphthalene ($C_{13}H_{16}$) (A)	23.75	1382	0.17	0.70	-	0.27	0.43
28	Caryophyllene (E) (S)	24.24	1431	-	2.42	4.15	-	0.37
29	cis-β-Copaene (S)	24.51	1449	0.72	-	-	0.17	0.26
30	Alloaromadendrene (S)	24.76	1467	-	1.37	1.53	-	-
31	α-Gurgujene (S)	25.37	1469	-	0.63	0.75	-	-
32	α-guaiene (S)	25.66	1473	-	1.58	1.22	-	0.48
33	cis-Muurola-4(15),5-diene (S)	26.52	1494	7.75	-	-	4.53	-
34	Leden (S)	26.64	1510	-	5.32	3.53	-	-
35	α-Muurolene (S)	27.01	1524	-	-	-	1.76	6.33
36	Bicylogermacrene (S)	27.13	1529	-	-	3.87	-	-
37	δ-Cadinene (S)	27.70	1532	4.84	-	-	3.58	2.84
38	Epizonarene (S)	27.92	1537	-	-	-	0.12	0.12
39	(Z)-Calamenene (S)	28.41	1540	0.87	-	-	0.54	0.75
40	Germacrene B (S)	29.25	1546	0.43	-	0.64	-	1.34
41	Palustrol (SO)	29.69	1554	-	1.68	-	-	-
42	Spatulenol (SO)	30.60	1563	0.89	14.23	3.83	1.02	1.32
43	Junenol (SO)	31.57	1570	0.48	-	-	0.55	0.49
44	β-Eudesmol (SO)	31.72	1576	-	0.69	0.30	-	-
45	β-guainene (S)	32.04	1580	-	-	-	-	0.36

46	τ-Muurolol (SO)	32.47	1589	0.78	-		2.44	-
47	α-Cadinol (SO)	32.90	1625	1.49	0.75	-	5.01	1.91
48	Phytone ($C_{18}H_{36}O$) (A)	36.23	1667	-	0.31	-	-	0.09
49	Benzoicacid($C_{14}H_{12}O_2$) (A)	38.02	1680	-	-	-	-	0.29
50	Phthalicacid($C_{16}H_{22}O_4$) (A)	39.75	1684	-	-	-	0.40	-
Total identifier (%)				**96.66**	**94.72**	**95.20**	**96.32**	**95.51**

RT: Retention time (min).
KI: Kovats index.

Moreover, the constituents have been identified from the essential oil of *P. atlantica* leaves, which accounts for 94.72% to 96.66% of the overall content of this oil. Monoterpene hydrocarbons (46.28 to 70.28%) and oxygenated monoterpenes (6.2 to 26.62%) predominate, followed by sesquiterpene hydrocarbons (11.18 to 16.28%) and oxygenated sesquiterpenes (3.64 to 17.35%). The other compounds are represented in small quantities (Table 7). These results correspond to those obtained by [27]. The yield, quantity and quality of essential oils vary according to geographical origin, climatic circumstances, and maturity phases [28]. Our oils often differ in the concentration of compounds rather than the content. Because the only variable that varies is the age (diameter) of the tree.

Table 7. *Pistacia atlantica* essential oil terpene subclasses

Class	**Area%** (Ø1=11.46cm)	**Area%** (Ø2=20.86cm)	**Area%** (Ø3=22.45cm)	**Area%** (Ø4=23.73cm)	**Area%** (Ø5=25.12cm)
Hydrocarbon monoterpenes (%)	70.28	46.28	55.86	51.29	48.77
Oxygenated monoterpenes (%):	6.20	13.21	17.85	22.86	26.62
Hydrocarbon sesquiterpenes (%):	16.28	12.07	15.69	11.18	14.44
Oxygenated sesquiterpenes (%)	3.64	17.35	4.13	9.02	4.08
Other (%):	0.26	5.81	1.67	1.97	1.60

3.4. Antibacterial Activity

Using the disc diffusion technique on a solid agar medium, we examined the laboratory's in vitro antibacterial properties of five essential oils of *P. atlantica* leaves versus the following against seven bacterial strains: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus aureus* methicillin-resistant, *Escherichia coli*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, and *Enterobacter cloacae.*

Table 8. Comparison of diameter of the zone of inhibition.

Microorganisms	**Diameter of the zone of inhibition (mm)**				
	Ø1	**Ø2**	**Ø3**	**Ø4**	**Ø5**
S. aureus	15.25 ± 0.35	12.02 ± 0.22	10.12 ± 0.26	09.33 ± 0.29	08.00 ± 0.20
S. epidermidis	14.66 ± 0.58	12.67 ± 0.58	12.67 ± 0.58	11.02 ± 0.14	09.33 ± 0.29
S. aureus Methicillin resistant	14.67 ± 0.58	14.83 ± 0.29	09.33 ± 0.29	10.00 ± 0.81	10.00 ± 0.24
K. pneumonia	10.00 ± 0.5	09.52 ± 0.26	09.83 ± 0.29	08.33 ± 0.29	06.50 ± 0.41
A. baumannii	11.02 ± 0.14	09.00 ± 0.16	07.33 ± 0,29	08.00 ± 0.16	07.12 ± 0.16
E. coli	10.67 ± 0.58	10.83 ± 0.29	10.00 ± 0.16	08.50 ± 0.12	06.50 ± 0.24
E. cloacae	10.50 ± 0.71	10.00 ± 0.24	08.00 ± 0.41	08.33 ± 0.29	07.00 ± 0.16

The method of disc diffusion was used to assess antibacterial activity. The findings of the antibacterial activity of *P. atlantica* leaves' essential oils are listed in Tables 8 and 9. The antimicrobial effectiveness of *P. atlantica* leaf essential oils was tested in vitro on six bacterial sores from five different-sized trees in northwest Morocco (*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia, Enterobacter cloacae, and Acinetobacter baumannii*).

Table 9. Mean diameter of the zone of inhibition

Microorganisms	**Mean diameter of the zone of inhibition (mm)**					
	Ø1	**Ø2**	**Ø3**	**Ø4**	**Ø5**	**Mean**
Staphylococcus aureus	15.25	12.02	10.12	9.33	8.00	10.67 ± 3.17
Staphylococcus epidermidis	14.66	12.67	12.67	11.02	9.33	12.07 ± 2.51
Staphylococcus aureus Methicillin resistant	14.67	14.83	9.33	10.00	10.00	11.77 ± 2.49
Klebsiella pneumonia	10.00	9.52	9.83	8.33	6.50	8.84 ± 2.54
Acinetobacter baumannii	11.02	9.00	7.33	8.00	7.12	8.49 ± 2.49
Escherichia coli	10.67	10.83	10	8.50	6.50	9.3 ± 2.23
Enterobacter cloacae	10.50	10.00	8.00	8.33	7.00	8.77 ± 1.48

The results obtained showed that all the products tested were effective, with inhibition zones ranging between 6.50 ± 0.24 and 15.25 ± 0.35 mm. The largest zone of inhibition was seen in *Staphylococcus aureus* (15.25 mm) (Table 8). Compared to positive controls, the essential oil of tree number 4 had a moderate ability to stop the growth of Gram-positive sputum. The measured inhibition zones for *Staphylococcus epidermidis* and *Staphylococcus aureus* were 14.66 mm and 15.25 mm, respectively. Penicillin is ineffective against both of these bacteria because the essential oil of tree number 4 makes these areas larger than amoxicillin can detect. The essential oil of tree number 2, however, has no effect on Gram-negative bacteria; it has a zone of inhibition ranging from 06.50 mm against *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter cloacae*, and *Acinetobacter baumannii*, but classifies less than 8 mm as non-sensible [29].

These findings indicate that the growth inhibition varies depending on the bacterial species, the concentration, and the nature of the extracted natural product. The experiments revealed that all strains are hypersensitive to the various plant extract concentrations obtained. The strongest antibacterial action was demonstrated by monoterpenes and sesquiterpenes, which are oxygenated hydrocarbons. In order to increase food safety and lower the risk of foodborne diseases, *P. atlantica* essential oil can be used as a preservative in the food business.

The α-pinene content of trees 1 and 4 is 24.67% and 37.51% respectively. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of essential oil are equal to a value of 4 µL/mL for gram-positive strains and 10 µL/mL for gram-negative strains respectively. On the other hand, trees 2, 3 and 5 including (13.38%), (15.71%) and (18.49%), of α-Pinene respectively, MIC and MBC have the same 10 µL/mL value for all strains (Table 10).

Table 10. Comparison of CMI and CMB of studied bacterial strains using *P. atlantica* EOs

Microorganisms		**Tree 1**	**Tree 2**	**Tree 3**	**Tree 4**	**Tree 5**
	MIC (µL/mL)	4	10	10	4	10
Staphylococcus	MBC (µL/mL)	4	10	10	4	10
Aureus	MIC/MBC	1	1	1	1	1
	MIC (µL/mL)	4	10	10	4	10
Staphylococcus	MBC (µL/mL)	4	10	10	4	10
Epermidis	MIC/MBC	1	1	1	1	1
	MIC (µL/mL)	10	10	10	10	10
Klebsiella	MBC (µL/mL)	10	10	10	10	10
Pneumoniae	MIC/MBC	1	1	1	1	1
	MIC (µL/mL)	10	10	10	10	10
Acinetobacter	MBC (µL/mL)	10	10	10	10	10
Baumannii	MIC/MBC	1	1	1	1	1
	MIC (µL/mL)	10	10	10	10	10
Escherichia Coli	MBC (µL/mL)	10	10	10	10	10
	MIC/MBC	1	1	1	1	1
	MIC (µL/mL)	10	10	10	10	10
Enterobacter	MBC (µL/mL)	10	10	10	10	10
Cloacae	MIC/MBC	1	1	1	1	1

The differences observed for MIC values can be explained not only by the presence of antibacterial compounds in *P. atlantica* essential oil at different concentrations but also by the choice of techniques used. The sensitivity of microorganisms to various chemical compounds that depend on the characteristics of the plant species, chemiotypes, and climatic circumstances is probably what causes the variability in the antibacterial power of essential oils. On the other hand, the fact that some essential oils from aromatic plants can stop Gram-positive bacteria from growing is not because of their main ingredients [30–33], but because of small compounds [34]. Also, the antibacterial potency of essential

oils has a broad range of action because of the variety of chemical components they contain [35–37]. This activity is also variable from one essential oil to another and from one bacterial strain to another. Their antibacterial activity is mainly dependent on their chemical composition and, in particular, on the nature of their major volatile compounds [38–41].

3.5. Correlations Matrix

Table 11 and Figure 2 exhibit a strong correlation among the various examined factors. The correlation matrix and p-values of coefficients illustrate an inverse relationship between antioxidant and antibacterial activity. Both Table 11 and Figure 2 show the Pearson correlation for the five oil samples. This shows that there is a strong relationship between the many study variables and the p-values for the coefficient of the correlation matrix across all parameters. A significant positive correlation ($p \leq 0.01$) was noticed between the two methodologies employed for antioxidant activity assessment (DPPH and FRAP), with an R^2 value of 0.99. Moreover, a considerable correlation ratio ($p \leq 0.05$) was found between DPPH and other factors, such as *S. aureus* methicillin-resistant ($R^2 = -0.97$) and *E. cloacae* ($R^2 = 0.95$). Furthermore, there was a substantial positive correlation ($p < 0.05$) between the FRAP index and several bacteria, including *S. aureus* methicillin-resistant ($R^2 = - 0.98$), *K. pneumoniae*, *E. coli* ($R^2 = 0.98$), and between *A. baumanii* and *S. aureus* ($R^2 = 0.97$). The observed strong correlation between the different variables for the five essential oils indicates mutual confirmation among the variables.

Table 11. Coefficient of Pearson's correlation matrix and p-values between quality indices (*S. aureus, S. epidermidis, S. aureus. methicillin resistant, K. pneumonia, A. baumannii*), DPPH and FRAP of the five oils studied

	Yield	**DPPH**	**FRAP**	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Staphylococcus aureus Methicillin resistant***	***Klebsiella pneumoniae***	***Acinetobacter baumannii***	***Escherichia coli***	***Enterobacter cloacae***
Yield	**1.00**	-0.26	-0.35	0.09	0.25	0.30	0.46	-0.06	0.47	0.27
DPPH	-0.26	**1.00**	0.99	-0.94	-0.80	-0.97	-0.64	-0.93	-0.73	-0.95
FRAP	-0,35	0,99	**1.00**	-0.90	-0.76	-0.98	-0.63	-0.89	-0.72	-0.94
Staphylococcus aureus	0.09	-0.94	-0.90	**1.00**	0.91	0.84	0.75	0.97	0.80	0.93
Staphylococcus epidermidis	0.25	-0.80	-0.76	0.91	**1.00**	0.65	0.91	0.81	0.91	0.85
Staphylococcus aureus Methicillin resistant	0.30	-0.97	-0.98	0.84	0.65	**1.00**	0.50	0.86	0.61	0.89
Klebsiella pneumoniae	0.46	-0.64	-0.63	0.75	0.91	0.50	**1.00**	0.61	0.98	0.72
Acinetobacter baumannii	-0.06	-0.93	-0.89	0.97	0.81	0.86	0.61	**1.00**	0.69	0.92
Escherichia coli	0.47	-0.73	-0.72	0.80	0.91	0.61	0.98	0.69	**1.00**	0.80
Enterobacter cloacae	0.27	-0.95	-0.94	0.93	0.85	0.89	0.72	0.92	0.80	**1.00**

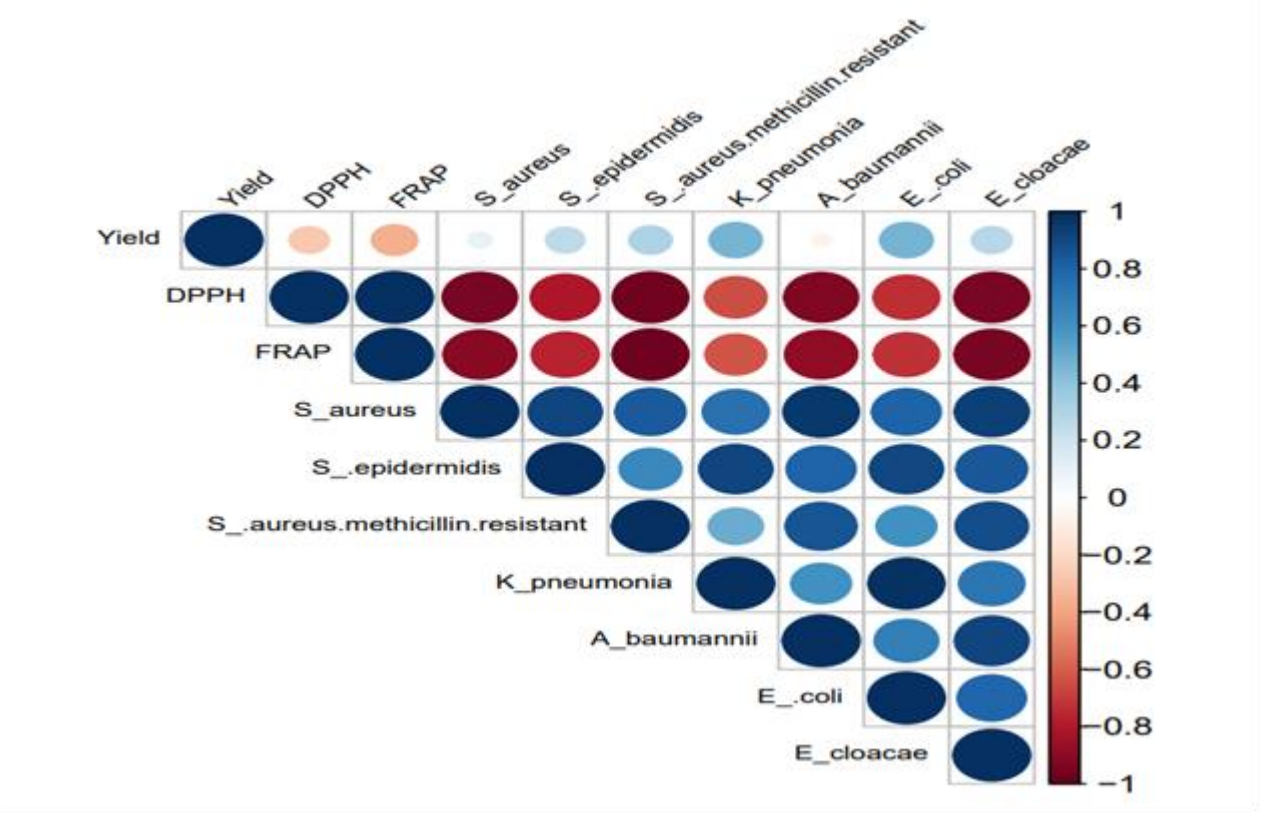

Figure 2. Correlation between essential oil experimental parameters

3.5.1. Principal Component Analysis

The results derived from relevant biological and phytochemical studies, which include *S. aureus*, *S. epidermidis*, *S. aureus* methicillin-resistant, *K. pneumonia*, *A. baumannii*, *E. coli*, *E. cloacae*, DPPH, and FRAP, serve as active variables. These variables are portrayed on the factorial plane (PC1-PC2) using principal component analysis (PCA), as displayed in Figure 3. PC1 represents 77.10% of the total variance, whereas PC2 accounts for 13.1%, cumulatively making up 90.20% of the total inertia, signifying a notable linear combination since it exceeds 50%. The PC1 axis is primarily established by the inverse positive correlation between the antioxidant tests (DPPH, FRAP) and three antibacterial tests (*K. pneumonia, E. coli, and S. epidermidis)* and a correlation among another set of four tests (*E. cloacae*, *S. aureus*, *S. aureus* methicillin-resistant, and *A. baumannii*). Two distinct groups emerge from this analysis. The first group contains products obtained through hydrodistillation of leaves from the Atlantic pistachio tree with main trunk diameters of 3, 4, and 5. Conversely, the second group involves diameters 1 and 2, extracted through the same method as demonstrated in Figure 4. In line with the theoretical results shown in Figure 3, the first group (1 and 2) has lower levels of oxygenated monoterpenes (6.2% and 13.21%, respectively), but the DPPH and FRAP tests show that they are powerful antioxidants. On the other hand, the oils taken from trunks with diameters of 3, 4, and 5 have a higher concentration of oxygenated monoterpenes (17.85%, 22.86%, and 26.62%, respectively), which means they are less effective at fighting free radicals. The squared cosine analysis highlights the importance of a component for a specific observation by indicating its contribution to the square distance of that observation from the origin. Specifically, it points out that most variables, with the sole exception of yield, hold considerable significance for all samples, as visualized in Figures 3 and 4.

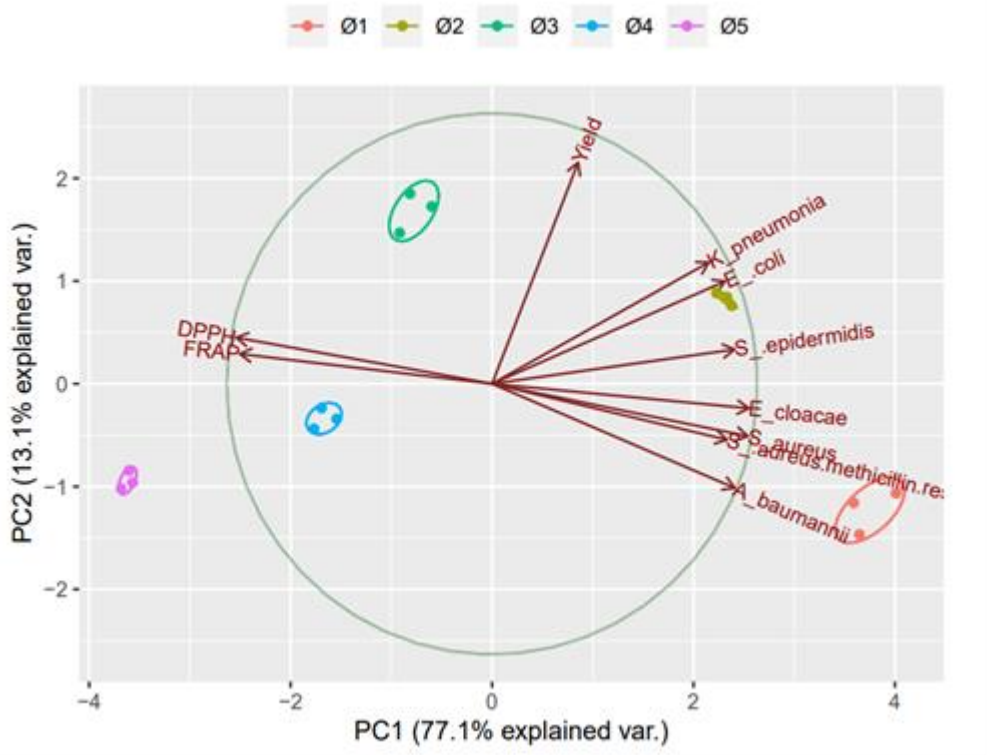

Figure 3. Correlation between variables

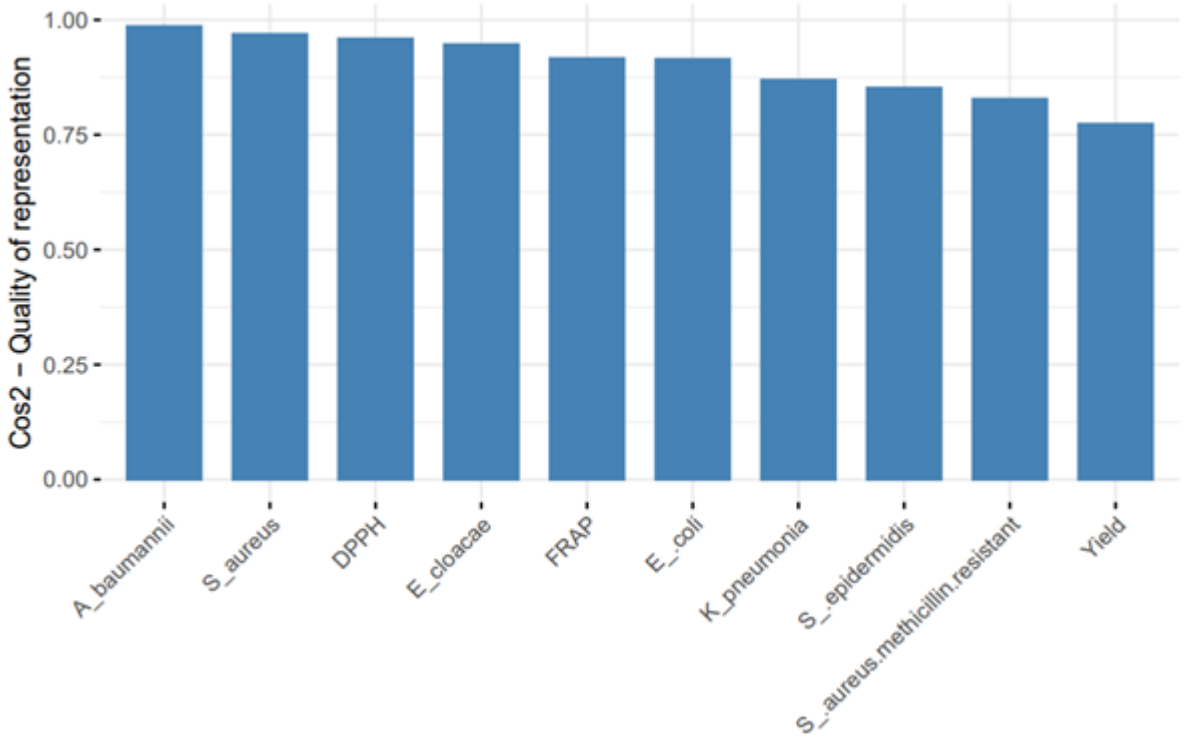

Figure 4. Cos^2 of variables to dim-1-2

4. Conclusion

The primary focus of this study was to assess the yield, chemical composition, antioxidant potential, and antimicrobial characteristics of the essential oil extracted from *Pistacia atlantica* leaves, a wild plant indigenous to the Rommani region of Morocco. Gas Chromatography-Mass spectrometry (GC-MS) analysis revealed that the predominant compounds in the organic phase of the essential oil were α-pinene and terpinen-4-ol. The investigation into the antimicrobial effects of the essential oil against Gram-positive and Gram-negative bacteria demonstrated robust antibacterial activity. The antioxidant test allowed researchers to draw the conclusion that the presence of polyphenols in essential oils is what gives them their potent antiradical action. There was an increase in antioxidant activity. These findings support the idea that *Pistacia atlantica* essential oil has potential applications in the pharmaceutical industry for the prevention or treatment of pathogenesis brought on by microorganisms and free radicals, as well as for use in the food industry as a natural antioxidant and antibacterial substitute for synthetic bactericides. There will need to be more tests.

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Supporting Information

Supporting Information accompanies this paper on a http://www.acgpubs.org/journal/records-of-natural-products

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