Scientific paper

RSM-optimized extraction of essential oils from *Pistacia lentiscus* sap, leaves, and berries: Comparative study of composition and activity against gastric bacteria

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Abstract

Pistacia lentiscus essential oil is widely recognized for its gastroprotective and antimicrobial properties; however, there is no clear consensus on which plant part exhibits the strongest activity against *Helicobacter pylori*. The objective of this study was to compare essential oils extracted from leaves, berries, and sap in terms of extraction yield, chemical composition, and antibacterial activity. Essential oils were extracted, characterized by GC-MS, and evaluated for antibacterial activity against *H. pylori* strains J99 and F29, as well as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The sap showed the highest yield (9.218%). GC-MS analysis revealed monoterpenes and sesquiterpenes as the main constituents, with α-pinene present in all samples. The leaf essential oil exhibited the strongest inhibition against *H. pylori*, exceeding the tested antibiotics against the F29 strain. These findings indicate that *P. lentiscus* leaves represent the most promising natural source for anti-*H. pylori* activity and suggest that the effect is due to synergistic interactions rather than α-pinene alone.

Keywords: Central composite design; Gas chromatography; *Helicobacter pylori*; *Pistacia lentiscus*; terpenoids

1. Introduction

Pistacia lentiscus, an evergreen shrub of the Anacardiaceae family native to the Mediterranean region, has been widely used in traditional medicine.[1] In Italy and North Africa, it has been traditionally employed to treat inflammatory conditions and gastric disorders, including dyspepsia and peptic ulcers.[2,3] Recent studies on the genus *Pistacia* have highlighted the strong antibacterial potential of terpene-rich essential oils, supporting the pharmacological relevance of these species as natural antimicrobial agents.[4,5] However, despite its extensive use, no consensus exists regarding which part of *P. lentiscus* leaves, berries, or sap exhibits the highest biological activity.

Helicobacter pylori (H. pylori) is a Gram-negative bacterium belonging to the *Helicobacteraceae* family and represents a major contributor to gastric disorders worldwide. It is notably responsible for approximately 90% of gastric cancer cases and 70–90% of gastric ulcers.[6]

The persistence of *H. pylori* in the highly acidic gastric environment, combined with its increasing ability to develop resistance to conventional antibiotics, makes eradication particularly challenging.[7] Consequently, the rising prevalence of antibiotic-resistant strains has intensified the need to identify and develop alternative therapeutic strategies based on natural antibacterial agents.

Hydrodistillation, specifically of the Clevenger type, is one of the simplest yet most effective techniques for the extraction of essential oils.[8] It is customary to use response surface methodology (RSM) to identify the optimum extraction conditions.[9,10] Previous studies on essential oil extraction from *Pistacia lentiscus* have focused on both

extraction methods and compositional analysis. For instance, Belhachat et al.[11] investigated ultrasonic pre-treatment for essential oil extraction from the berries, while Ayub et al. optimized essential oil yield from *Pistacia lentiscus* oleo-gum resin using superheated steam extraction and response surface methodology.[12]

Gas chromatography–mass spectrometry (GC-MS) is the most commonly used technique to analyze the composition of essential oils.[13] Since essential oils are primarily composed of terpenes,[14] and other volatile compounds, GC-MS is particularly suited for their identification and quantification. Identifying the components of essential oils is important, as it can help to determine those responsible for specific biological activities. Previous studies on *Pistacia atlantica* essential oil, such as those by Memariani et al.[15] and Khoramjouy et al.[16] have suggested that α-pinene, a major component, plays a key role in antibacterial and anti-ulcer activity. Similarly, Ramezani et al.[17] identified α-pinene as the primary antibacterial compound in *Pistacia vera*.

Antibacterial activity can be evaluated using the disk-diffusion method, where bacteria are exposed to a substance that inhibits or stops their growth. The results are expressed as the diameter of the inhibition zone.[18] A complementary method, known as the minimum inhibitory concentration (MIC), provides insight into the lowest concentration of a substance required to exhibit antibacterial activity.[19] Previous studies have reported the antibacterial properties of *Pistacia lentiscus*. Marone et al.[20] conducted both disk-diffusion and MIC assays on *Helicobacter pylori* using gum essential oils from *Pistacia lentiscus*. Additionally, Meriem et al.[21] investigated the antibacterial activity of *Pistacia lentiscus* leaves essential oil. *Pistacia lentiscus* essential oil has been previously studied. For example, Beraich et al.[22] examined both traditional and modern extraction methods. Aouinti et al.[23] studied the effect of the harvesting season on extraction yield, and Ayoub et al.[12] optimized essential oil yield from *Pistacia lentiscus* oleo-gum resin using superheated steam extraction and response surface methodology.

However, these studies focused on only one part of the plant. Some works investigated several parts of *Pistacia lentiscus*, such as Boelens et al.[24] who studied the composition of different plant parts including leaves and berries, and Dob et al.[25] who analyzed the chemical composition of essential oils from the aerial parts of the plant. Nevertheless, even these studies did not provide a comparative analysis of more than two plant parts. When more parts were considered, the extractions were not optimized and were conducted under a single set of extraction conditions. Consequently, the compositions of essential oils from different parts of the plant were studied separately and cannot truly be compared.

Among the previously cited articles, some also investigated the antibacterial activity of *Pistacia lentiscus* essential oils against different bacteria. Notably, studies by Marone et al.[20] and Meriem et al.[21] tested the effects of *Pistacia lentiscus* gum and leaf essential oils, respectively, on *Helicobacter pylori*. Yet, once again, these studies only explored

Figure 1. Geographical location of the *Pistacia lentiscus* collection site in the Goraya Mountains, northern Algeria.

the essential oil from a single part of the plant in isolation.

To our knowledge, no previous work has simultaneously extracted essential oils from three parts of *Pistacia lentiscus* leaves, berries, and sap using a hydrodistillation process optimized by Response Surface Methodology (RSM). Furthermore, no study has tested all three oils against *H. pylori* to determine which part yields the most effective antibacterial activity against this bacterium, known to be responsible for gastric and duodenal ulcers. Therefore, the aim of this work is to extract essential oils from three different parts of the *Pistacia lentiscus* plant leaves, berries, and sap using hydrodistillation, with extraction yield optimized through RSM to maximize efficiency. The extracted oils are then analyzed and compared using GC-MS, and their antibacterial activity is evaluated against gastric bacteria, with a focus on *Helicobacter pylori*.

2. Materials and methods

2. 1. Plant material

The plant was collected from the Goraya Mountains in northern Algeria (latitude 36.7694° N, longitude 5.0892° E). In December 2023, leaves, berries, and sap were harvested from a healthy, undamaged specimen. A representative plant sample containing all major parts was preserved and submitted to the Botanical Department of the Algerian School of Agronomy (ENSA) for proper taxonomic identification. The plant material was shade-dried for several days until constant weight was achieved. The dried material was then ground into powder and sieved through a 250 µm mesh. The plant material was stored in sealed containers and kept at 4 °C in the dark until further use.

2. 2. Essential oil extraction

Hydrodistillation using a Clevenger-type apparatus was employed for the extraction of essential oils from the sap (SEO), leaves (LEO), and berries (BEO) of *Pistacia lentiscus*. For all extractions, the volume of water was kept constant and adjusted according to the plant material: 600 mL for leaves and berries and 60 mL for sap. The distillate mass was measured, and the extraction yield was calculated using the following equation:

$$\text{Extraction Yield (\%)} = \frac{\text{Mass of distillate (g)}}{\text{Mass of plant material (g)}} \times 100$$

To determine the optimal extraction conditions, a two-factor Central Composite Design (CCD) was performed using JMP Pro software (version 14.3). Two factors were studied: the extraction time (X_1), varying from 3 to 5 hours, and the plant-to-water ratio (X_2), varying from 01:06 to 02:06 (g/mL).

The conditions of the experimental design, including both coded and real values for each run, are presented in Table 1.

Table 1: Essential oil extraction conditions based on central composite design

Run	X_1 (coded)	X_2 (coded)	Extraction time (h)	P/W (mL/g)
1	−1	−1	3	01:06
2	−1	1	3	02:06
3	1	−1	5	01:06
4	1	1	5	02:06
5	0	−1	4	01:06
6	0	1	4	02:06
7	−1	0	3	1.5:06
8	1	0	5	1.5:06
9	0	0	4	1.5:06
10	0	0	4	1.5:06

2. 3. Gas chromatography-mass spectrometry (GC-MS) analysis

The protocol used is the one described by Netopilova et al.[27] with slight modifications. The analysis was performed on a SHIMADZU GCMS-QP2020 using an Rx-i®-5ms capillary column (30 m × 0.25 mm, 0.25 µm film thickness, 5% diphenyl/95% dimethyl polysiloxane). A 0.5 µL sample diluted in *n*-hexane was injected in split mode (1:50). The injector and detector temperatures were set at 250 °C and 310 °C, respectively. The column temperature was programmed from 50 °C (2 min), ramping to 310 °C at 3 °C/min, and held for 2 min. Helium (99.995%) at 1 mL/min was used as the carrier gas. Mass spectrometry employed 70 eV ionization, a 200 °C ion source, and scanned 45–600 *m*/*z*.

2. 4. Antibacterial activity

The antimicrobial activity of the three essential oils has been evaluated using the disk-diffusion method as prescribed in M45 of CLSI,[28] with slight modifications. The primary target bacteria was *Helicobacter pylori (H. pylori)*, with two strains tested: J99, an ATCC 700824 reference strain, and F29, an internal control strain. Suspensions of *H. pylori* at 3 McFarland (approximately 9×10^8 CFU/mL) were prepared and spread onto Mueller-Hinton agar supplemented with 5% horse blood using a cotton swab. Filter paper disks (Whatman No. 3, 6 mm in diameter) are soaked with 3 µL of each essential oil and placed on the inoculated plates. DMSO serves as the negative control. In parallel, three antibiotics (ampicillin, metronidazole, and clarithromycin) were evaluated using standardized commercial disks (10 µg per disk). The plates are incubated at 37°C for 48 hours under microaerophilic conditions. Zones of inhibition are measured in millimetres,

The essential oils were also tested against *Staphylococcus aureus (S. aureus)* ATCC 25923, *Escherichia coli (E. coli)* ATCC 25922, and *Pseudomonas aeruginosa (P. aeruginosa)* ATCC 27853, the tests were performed on simple

Mueller-Hinton agar using a 0.5 McFarland suspension. The plates were incubated at 37 °C for 24 hours and zones of inhibition were measured in millimeters. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (n = 3).

To determine the Minimum Inhibitory Concentration (MIC) against *Helicobacter pylori*, we followed the protocol described in M45 of CLSI,[28] with slight modifications. Stock solutions of the tested substances (LEO, BEO, SEO, ampicillin, metronidazole, and clarithromycin) were initially prepared at a maximum concentration of 1024 µg/mL. Serial dilutions were then performed to obtain a range of concentrations, with the lowest final concentration reaching 0.0156 µg/mL.

Each well received 70 µL of Mueller-Hinton broth supplemented with 5% blood, 5 µL of the previous *H. pylori* suspension, and 25 µL of the antibiotic or essential oil solutions. The plates were incubated at 37 °C for 48 hours under microaerophilic conditions. Control wells contained no test substance. The MIC was defined as the lowest concentration at which no visible growth was observed. All experiments were conducted in triplicate.

3. Results and discussion

3. 1. Essential oils extraction

Essential oils were successfully extracted from the sap, leaves, and berries of *Pistacia lentiscus*. The extraction yields are summarized in Table 2. The extraction yields obtained from the leaves and berries are comparable to those reported in the literature (Table 3). The extraction yields from the sap in the present study are higher than those reported in the literature ranging from 4.451 to 9.218%. These results can be explained by the fact that, in the present study, viscous sap was used, which contains approximately 13% essential oil.[29] In contrast, studies reported in the literature typically used dried sap or mastic containing about 3% essential oil and reported extraction yields of around 2%.[30]

Table 4 shows the derived equations for the three extractions and also highlights the high R^2 and R^2 adjusted values, indicating that the equations successfully represent the effects of the different parameters and their interactions on the extraction process.

The ANOVA results for the three extraction models are presented in Table 5. All three models showed a non-significant lack-of-fit ($p > 0.05$) and were therefore considered statistically adequate. The analysis also indicates that experimental errors were minimal for the leaf and berry extractions, whereas larger errors were observed for the sap. However, these did not compromise the overall significance of the model. The higher variability in the sap extraction can be attributed to differences in the volumes of water used and the inherent difficulties associated with handling viscous sap, which is extremely sticky and can adhere to the surfaces of the flasks, potentially increasing experimental error.

The study of the effects of extraction time and plant-to-water ratio on the essential oil yield from *Pistacia lentiscus* leaves, summarized in Table 6, showed a distinct behavior. Both quadratic effects were statistically significant, with *F*-values of 74.01 for extraction time and 20.00 for the plant-to-water ratio, indicating a strong non-linear dependence of the extraction yield on these parameters. Regarding the linear terms, only extraction time was found to be significant ($F = 22.98$), whereas the linear effect of the plant-to-water ratio was not statistically significant.

Table 3: Comparison of essential oil yields (%) from leaves, berries, and sap of *Pistacia lentiscus* reported in the present study and in the literature, including plant part, physical state, extraction method, and optimization approach.

Plant part	Study	Physical state & extraction method	Optimization approach	EO Yield (%)
Leaves	Present study	Hydrodistillation on fresh leaves	RSM (CCD)	0.205–0.273
	Jaradat et al.[31]	Hydrodistillation on dried leaves	Conventional	1.81
	Belkessam et al.[32]	Hydrodistillation on dried leaves	Conventional	0.49–0.71
	Haloui et al.[33]	Hydrodistillation on dried leaves	RSM (CCD)	0.40–0.58
	Bampouli et al.[32]	Hydrodistillation on fresh leaves	Conventional	0.06
	Labhar et al.[34]	Hydrodistillation on dried leaves	Seasonal monitoring	0.15–0.30
	Nouar et al.[35]	Hydrodistillation on dried leaves	Conventional	0.1
Berries	Present study	Hydrodistillation on fresh berries	RSM (CCD)	0.136–0.353
	Belhachat et al.[11]	Ultrasound-assisted hydrodistillation on ripe berries	RSM (BBD)	0.66
	Boelens et al.[24]	Hydrodistillation on ripe berries	Conventional	0.5
	Boelens et al.[24]	Hydrodistillation on unripe berries	Conventional	0.4
	Congiu et al.[36]	Supercritical CO_2 extraction on unripe berries	Conventional	0.2
Sap	Present study	Hydrodistillation on fresh liquid sap	RSM (CCD)	4.451–9.218
	Pachi et al.[37]	Hydrodistillation on fresh liquid sap	Fluid collection	13
	Ayub et al.[12]	Superheated steam extraction on fresh resin	RSM (CCD)	5.7
	Ayub et al.[12]	Hydrodistillation on fresh resin	Conventional	2
	Pachi et al.[37]	Hydrodistillation on solid resin	Conventional	0.35–2.48
	Fahmy et al.[38]	Hydrodistillation on solid resin (Ph. Eur.)	Conventional	1

Table 4: Response surface models, fit statistics (R^2 and adjusted R^2), and optimal extraction conditions for essential oil yield from leaves, berries, and sap of *Pistacia lentiscus*.

	Derived Equation	R^2	R^2 adj	Optimal X_1	Optimal X_2
Leaves	$0.2669785714 + 0.010566\ X_1 + 0.0125\ X_1X_2 - 0.030407143\ X_1^2 - 0.015807143\ X_2^2$	0.976	0.945	4.23 h	0.2726
Berries	$0.2420214286 - 0.023633\ X_1 - 0.056\ X_2 - 0.009875\ X_1X_2 - 0.078092857\ X_1^2 + 0.0648071429\ X_2^2$	0.988	0.972	3.82 h	0.285
Sap	$9.1383571429 - 0.511383\ X_1 + 0.4615\ X_2 - 0.9485\ X_1X_2 - 2.429714286\ X_1^2 - 1.171714286\ X_2^2$	0.973	0.940	3.84 h	0.257

Table 5: ANOVA results for the central composite design models of essential oil extraction from *Pistacia lentiscus* leaves, berries, and sap.

Sample	Degrees of freedom (DF)	Sum of squares (SS)	Mean square (MS)	*F*-value	*p*-value	R^2 max
Leaves	3	0.00010255	0.000034	2.4338	0.4329	0.9971
Berries	3	0.00029959	0.0001	0.4203	0.7794	0.9946
Sap	3	0.57165633	0.190552	59.5475	0.0949	0.9999

Table 6: Study of the effects of extraction time, plant-to-water ratio, and their interactions on essential oil yield from leaves, berries, and sap of *Pistacia lentiscus*.

	Leaves		Berries		Sap	
Source	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
extraction time	22.9832	0.0087	24.9526	0.0075	11.0287	0.0294
plant-to-water ratio	6.6878	0.0609	141.1037	0.0003	8.8966	0.0406
extraction time * plant-to-water ratio	21.4419	0.0098	2.9043	0.1635	25.0533	0.0075
(extraction time)^2	74.0136	0.001	105.9532	0.0005	95.8997	0.0006
(plant-to-water ratio)^2	20.0017	0.0111	72.9688	0.001	22.3023	0.0092

Consistent with these results, the surface plot presented in Figure 2 exhibits a clear unimodal trend, confirming the predominance of quadratic effects over linear contributions. The corresponding contour plot reveals that the optimal extraction conditions are achieved at an extraction time of 4.23 h and a plant-to-water ratio of 0.273.

Regarding sap, both factors exhibited significant linear and quadratic effects, although the linear effect of extraction time showed a relatively lower *F*-value. The quadratic effect of extraction time (F = 95.90) clearly predominated over its linear effect (F = 11.03), in agreement with findings reported by Ayoub et al.[39] who report-

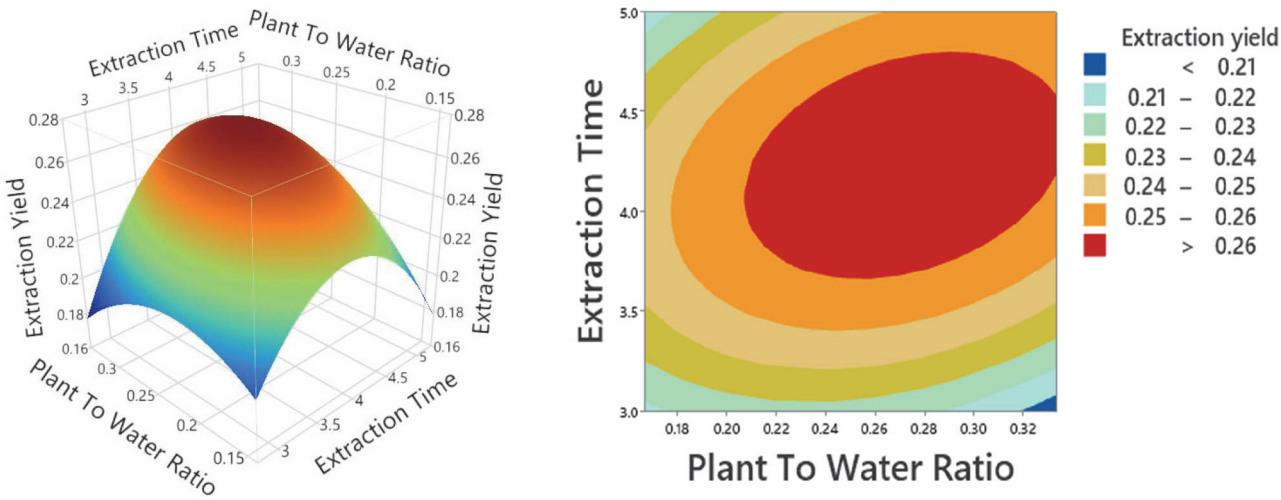

Figure 2: Surface and contour plot of extraction yield of essential oil from *P. lentiscus* leaves.

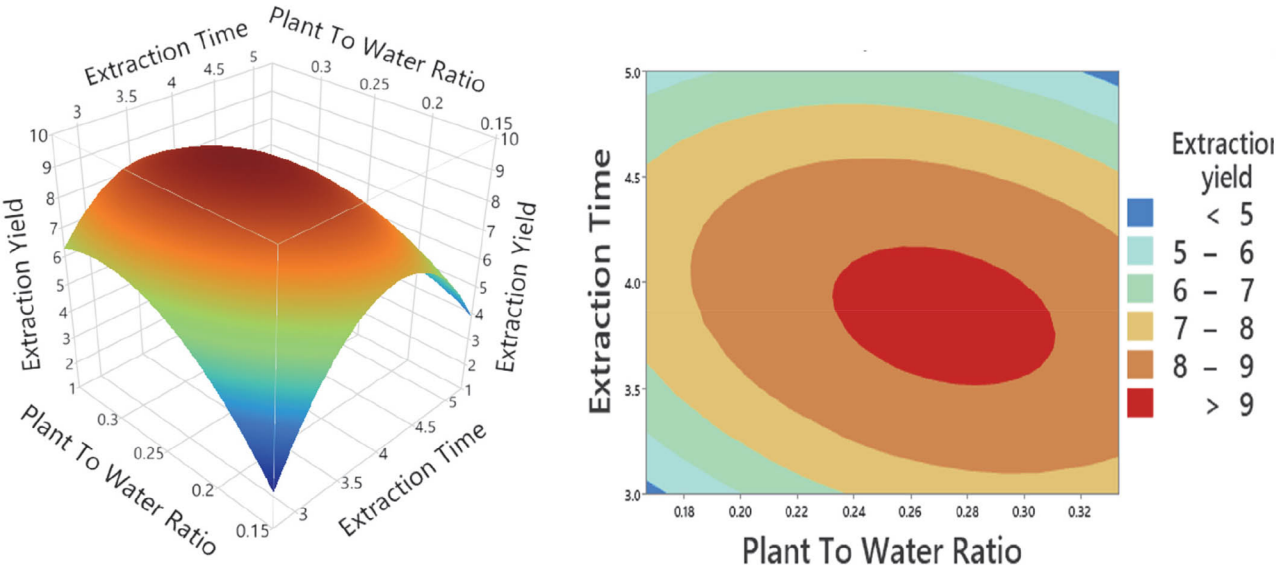

Figure 3: Surface and contour plot of extraction yield of essential oil from *P. lentiscus* sap.

ed a similar trend in the extraction of essential oil from *Pistacia lentiscus* oleo-gum resin using superheated steam.

The plant-to-water ratio also showed significant linear and quadratic effects (F = 8.90 and 25.05, respectively). The surface plot displays a clear unimodal trend, indicating that increasing both parameters enhance the extraction yield up to an optimal point, beyond which a decline occurs. The corresponding contour plot indicates that the optimal conditions are achieved at an extraction time of 3.84 h and a plant-to-water ratio of 0.257.

The berries showed significant effects of extraction time and plant-to-water ratio in both linear and quadratic models. The dominant contributions were the linear effect of the plant-to-water ratio (F = 141.10, p = 0.0003) and its quadratic effect, indicating a strong non-linear dependence of the extraction yield on this parameter. Similar results were reported by Belhachat et al.[11] who observed significant linear and quadratic effects of extraction time and material-to-water ratio during the extraction of essential oil from ripe *Pistacia lentiscus* berries using ultrasonic pretreatment.

In contrast to the surface plots obtained for leaves and sap, the response surface of berry extraction yield presented in Figure 4 exhibits a different behavior. The effect of the plant-to-water ratio appears nearly linear within the studied range, whereas extraction time displays a pronounced arc-shaped trend, reflecting the strong quadratic influence of this parameter. The corresponding contour plot indicates that the optimal extraction conditions are achieved at an extraction time of 3.82 h and a plant-to-water ratio of 0.285.

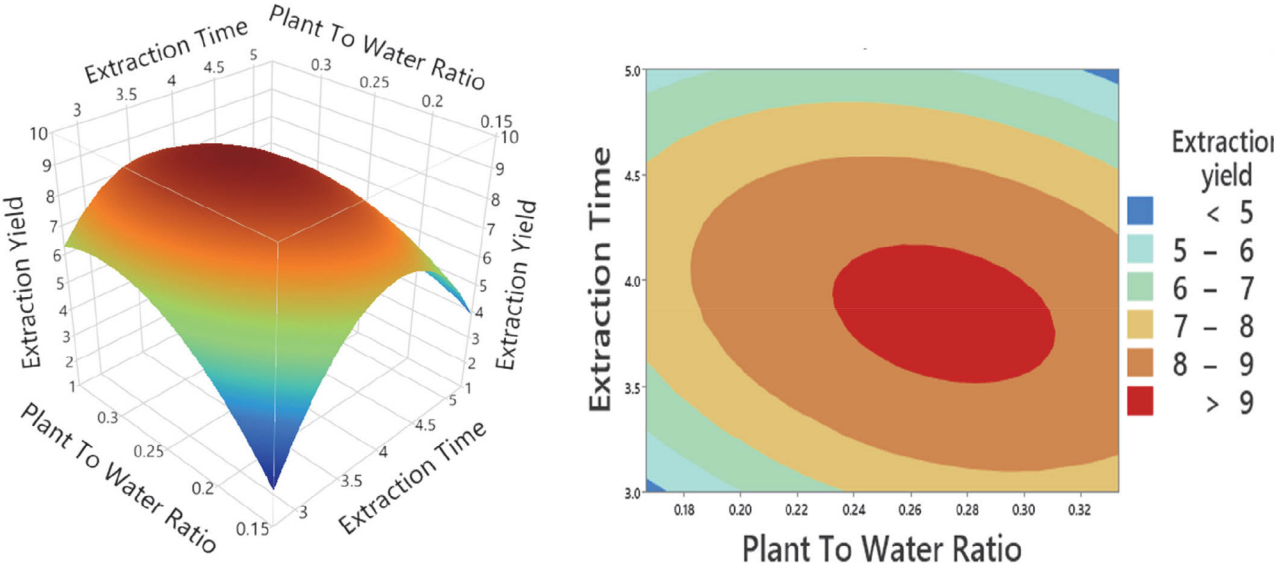

Figure 4: Surface and contour plot of extraction yield of essential oil from P. lentiscus berries.

Extraction time (x_1) and plant-to-water ratio (x_2) were found to have a significant influence on the essential oil extraction yield across all studied matrices. These parameters play a critical role in the extraction process. Water acts both as a vector medium and as an extraction agent, facilitating the disruption of plant fibers. At the same time, water prevents the essential oil from reaching excessively high temperatures, thereby acting as a protective medium.[40] However, when excessive amounts of water are used, certain essential oil components may undergo hydrolysis, leading to their degradation. This explains why the plant-to-water ratio during extraction is a critical parameter: both insufficient and excessive water can negatively affect the extraction yield,[41] accounting for the strong quadratic effects observed across all matrices in the ANOVA analysis.

Increasing the extraction time generally leads to higher yields, as the rise in temperature promotes the breaking of lignin bonds and the release of compounds trapped within the plant matrix.[42] Nevertheless, excessive exposure to elevated temperatures may cause degradation of essential oil components. Indeed, Mokrani et al.[43] reported that α-pinene and β-pinene begin to degrade at relatively low temperatures, which explains the strong quadratic effect of extraction time on the yield.

3. 2. Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis revealed notable differences in the chemical diversity of essential oils extracted from the three parts of *Pistacia lentiscus*. Indeed, a total of 71 compounds were identified in the leaf oil, compared to 49 compounds in the berry oil and only 12 compounds in the sap oil. Despite these variations in chemical diversity, the overall compositions of the three essential oils remain comparable. In all cases, the profiles were dominated by monoterpenes and, to a lesser extent, sesquiterpenes. Moreover, several major compounds were shared among the three oils, including α-pinene, β-pinene, D-limonene, and terpinen-4-ol.

The GC-MS chromatogram of the sap essential oil (Figure 5) revealed a monoterpene dominated profile (86.35% of the total composition). The major component was α-pinene(1) (77.09%), followed by D-limonene(5) (3.08%), camphene(2) (0.25%), β-pinene(3) (1.79%), and *o*-cymene(4) (4.14%). Oxygenated derivatives, such as *trans*-pinocarveol(7) (1.35%) and 2-pinen-4-ol(8) (1%), likely originate from β-pinene oxidation.[44]

Additional components include terpinen-4-ol(10) (1.29%), (–)-myrtenol(11) (0.81%), and (1*R*)-*cis*-verbenol(9) (8.01%), which has demonstrated antibacterial and antifungal effects.[45–47]

These findings are consistent with the composition of *Pistacia lentiscus* gum essential oil reported by Tabanca et al.[30] who found α-pinene levels ranging from 52 to 71%, β-pinene between 2.7 and 6%, myrcene between 2.5 and 20%, and limonene detected as a minor constituent (≈3–4%).

Monoterpenes also dominate the chromatographic profile of the berry essential oil, as shown in Figure 6, making up approximately 85.13% of the total composition. However, in this oil, α-pinene(5) represents only 23.47%, accompanied by myrcene(9) (24.72%) and β-pinene(8) (7.95%). These compounds, particularly myrcene, have been reported to exhibit gastroprotective and anti-inflammatory properties, specifically against *Helicobacter pylori*.[48]

The sesquiterpene content is smaller, constituting 2.25%, and includes caryophyllene(32) and its oxides, as well as γ-muurolene(35), which may contribute additional therapeutic potential.[49]

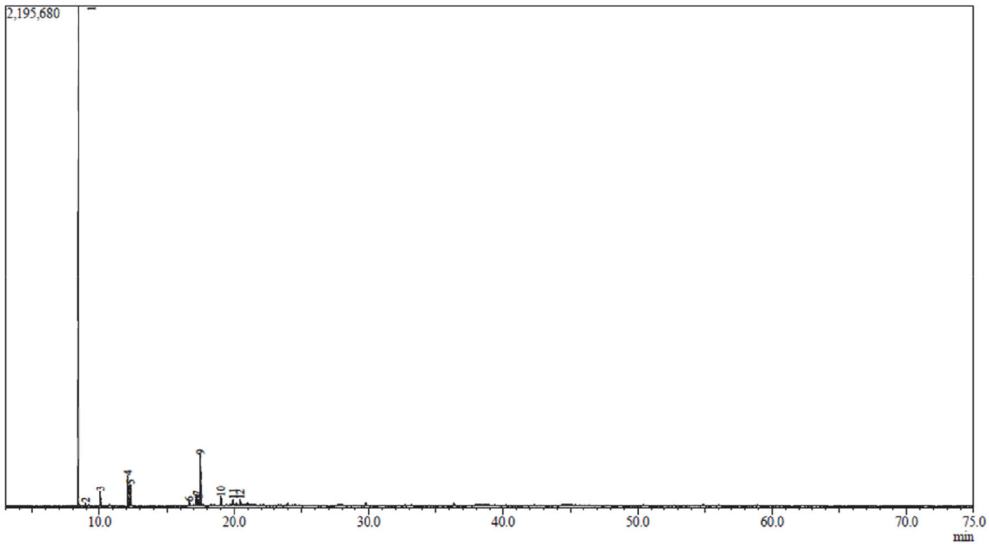

Figure 5: GC-MS chromatogram of *Pistacia lentiscus sap* essential oil

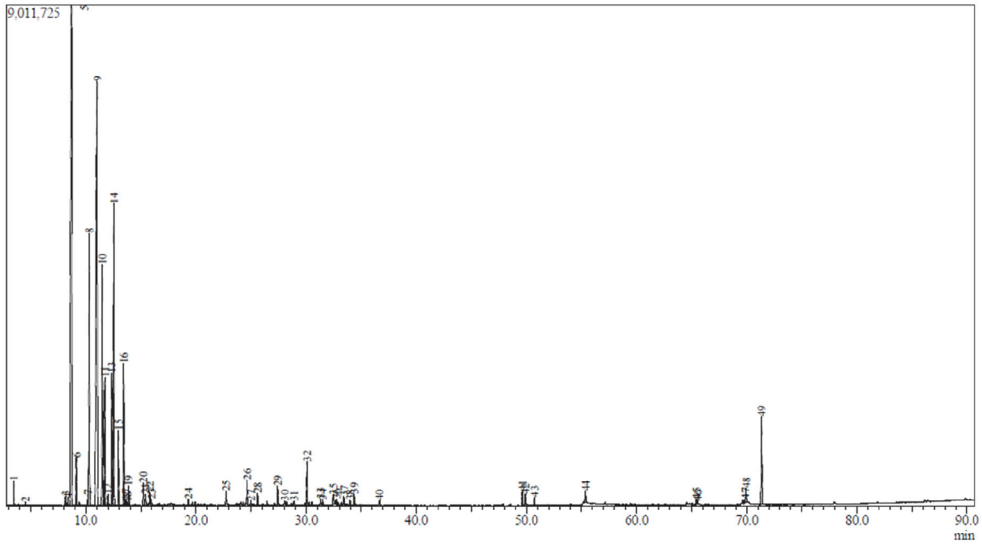

Figure 6: GC-MS chromatogram of *Pistacia lentiscus berries* essential oil

Fatty acids are also present, including oleic acid(44) and *n*-hexadecanoic acid(42) likely reflecting the natural vegetable oils contained in *Pistacia lentiscus* berries, which naturally act as lipid reservoirs within the plant matrix. These results are consistent with previous reports in the literature, which show α-pinene levels around 21.7% in berry essential oil and confirm that the myrcene content is consistently higher in berry oil compared to leaf or gum oils.[24,50]

The leaves essential oils GC-MS profile, as illustrated in Figure 7, highlights its diverse composition. Monoterpenes are present but in lower concentrations compared to the berries oil, accounting for 29.52%. D-limonene(10) (10.69%), cymene(9) (5.06%), and α-pinene(2) (4.35%) are the most prominent. In contrast, the leaf oil is particularly rich in sesquiterpenes (40.39%), including caryophyllene(33) (7.25%) and α-bisabolol(69) (7.65%), both known for their anti-inflammatory and gastroprotective properties.[51] Other sesquiterpenes, such as γ-muurolene(38) (2.5%), α-muurolene(43) (2.65%), and τ-muurolol(66) (3.26%), further contribute to the therapeutic potential, particularly in antimicrobial and anti-inflammatory applications.[52]

Aromatic compounds and diterpenes were also detected. Notably, τ-cadinol(63) (2.76%), α-humulene(35) (1.84%), and germacrene-D(39) (1.65%) enhance its anti-inflammatory and antimicrobial effects. The presence of δ-cadinene(46) (7.79%) adds to the oil's uniqueness.[39]

These results are consistent with those reported in the literature, showing a high presence of α-pinene, D-li-

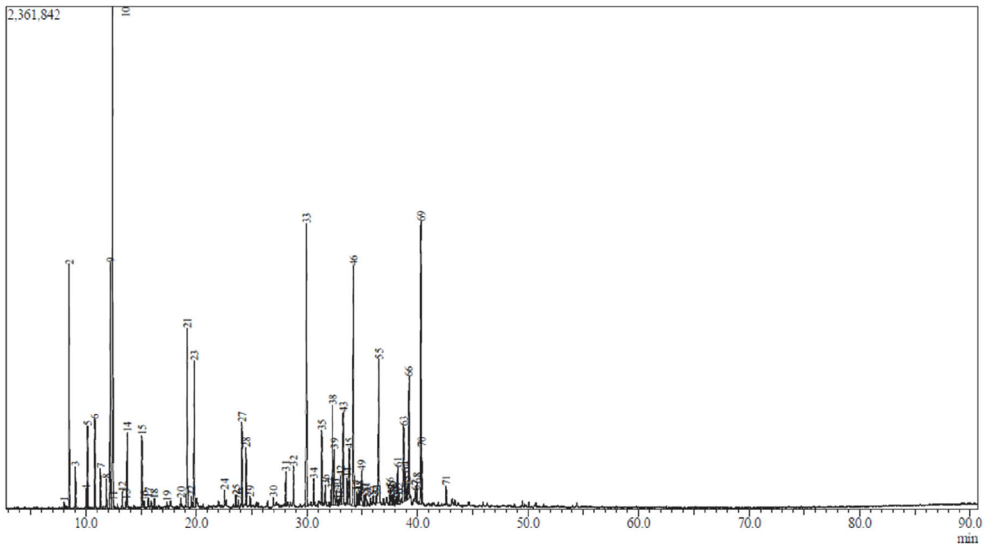

Figure 7: GC-MS chromatogram of *Pistacia lentiscus leaves* essential oil

monene, cadinene, and caryophyllene in *Pistacia lentiscus* essential oils.[23,53] The full qualitative and quantitative GC-MS composition, including minor constituents, is reported in the Supplementary Material (Tables S1–S3).

3. 3. Antibacterial activity

The antibacterial efficacy was evaluated using disk-diffusion and minimum inhibitory concentration (MIC) assays against *Helicobacter pylori* (F29 and J99), *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

3. 4. Disk-diffusion assay

All three *Pistacia lentiscus* essential oils (EOs) exhibited significant inhibitory zones against *H. pylori* (Table 7). The leaf essential oil LEO demonstrated the most potent activity, with zones of 49.76 mm and 33.86 mm against the F29 and J99 strains, respectively surpassing the inhibition diameters observed for the antibiotic controls (ampicillin, metronidazole, and clarithromycin) against the F29 strain. The sap SEO and berry BEO essential oils showed moderate and comparable activity. Notably, all EOs were more effective against the clinical F29 strain than against the reference J99 strain. Conversely, the standard antibiotics were significantly less active against F29, suggesting reduced susceptibility or resistance in this clinical isolate.

Against other pathogens, SEO was most active, particularly against *S. aureus* (28.8 mm) and *E. coli* (23.12 mm). Ampicillin was inactive against *P. aeruginosa*, consistent with its known intrinsic resistance.

3. 5. Minimum inhibitory concentration (MIC)

MIC results against *H. pylori* confirmed the disk-diffusion trends (Table 8). Antibiotics showed lower MICs against J99 (0.0625–0.5 μg/mL), aligning with published ranges for reference strains,[54] but markedly higher MICs against F29, confirming its resistant phenotype. Among the EOs, LEO again showed the lowest MIC values (64 μg/mL for F29; 128 μg/mL for J99), followed by SEO and BEO. Consistent with diffusion data, all EOs displayed greater potency (lower MIC) against the resistant F29 strain than against J99, though their absolute MIC values remained higher than those of antibiotics tested against the J99 reference strain.

Table 8: MIC values (μg/mL) against *Helicobacter pylori* strains

Tested substance	F29 strain (μg/mL)	J99 strain (μg/mL)
DMSO (negative control)	0	0
Ampicillin	0.125	0.0625
Metronidazole	512	0.125
Clarithromycin	512	0.0625
Sap essential oil (SEO)	128	256
Leaves essential oil (LEO)	64	128
Berries essential oil (BEO)	256	512

3. 6. Discussion and structure-activity relationship

The superior activity of LEO can be attributed to a synergistic blend of specialized antimicrobial compounds. Its composition includes α-bisabolol (7.65%), which possesses potent bactericidal activity and enhances membrane permeability,[55,56] and β-caryophyllene (7.25%), which disrupts membrane integrity and downregulates virulence genes (cagA, vacA) and replication machinery (gyrA, dnaE).[57,58] This is complemented by cadinene derivatives (e.g., δ-cadinene, 7.79%) that contribute to a complex synergistic effect.[59]

In contrast, SEO's activity, particularly against *S. aureus* and *E. coli*, is driven by its high α-pinene content (77%), a known antibacterial agent with a reported MIC of 100 μg/mL.[60] This aligns with studies on similar oils, such as *Pistacia atlantica* (>90% pinene), showing MICs of 275–1100 μg/mL against clinical strains.[15] BEO exhibited the weakest activity, consistent with its lower α-pinene concentration (23.7%) and dominance of myrcene (24.72%), which has a higher MIC (~500 μg/mL) and primarily offers gastroprotective rather than strong direct antibacterial effects.[57,60,61]

The differential activity against the two *H. pylori* strains highlights a critical finding. The clinical F29 isolate

Table 7: Inhibition zone diameters (mm) of tested substances.

	Helicobacter pylori F29 (mm)	*Helicobacter pylori J99 (mm)*	*Staphylococcus aureus (mm)*	*Escherichia coli (mm)*	*Pseudomonas aeruginosa (mm)*
DMSO	0	0	0	0	0
Ampicillin	21.54 ± 0.39	61.87 ± 0.74	36.76 ± 0.7	24.81 ± 0.73	0
Metronidazole	0	14.48 ± 0.41	0	0	0
Clarithromycin	0	51.88 ± 0.19	29.38 ± 0.51	16.75 ± 0.48	0
SEO	38.98 ± 0.45	25.63 ± 0.16	28.8 ± 0.54	23.12 ± 0.3	0
LEO	49.76 ± 0.7	33.86 ± 0.79	6.8 ± 0.48	9.77 ± 0.97	0
BEO	25.40 ± 0.15	20.49 ± 0.55	7.14 ± 0.1	8.34 ± 0.02	0

exhibits a multi-drug resistant (MDR) phenotype, a growing issue where 1.36% to >15% of clinical strains show triple resistance,[62,63] often mediated by efflux pumps or target mutations.[63] However, such strains frequently retain sensitivity to EOs.[21,59,61] This is because EOs act via non-specific physical mechanisms, primarily disrupting the lipid bilayer of the bacterial membrane,[59,64] Consequently, genetic resistance to antibiotics (e.g., β-lactams) does not confer cross-resistance to this membrane-lytic action. Paradoxically, membrane alterations in resistant strains can sometimes increase susceptibility to lipophilic terpenes,[65,66] explaining the enhanced efficacy of EOs against the resistant F29 strain.

In conclusion, the EOs, particularly LEO, show promising activity against *H. pylori*, including a clinical MDR strain. Their broad, membrane-targeting mechanism offers a potential advantage over conventional antibiotics in mitigating resistance, warranting further investigation for therapeutic applications.

4. Conclusion

This study highlights the potential of *Pistacia lentiscus* essential oils by evaluating their yields, composition, and antibacterial activity. The sap provided the highest yield, while the leaves and berries yielded significantly less.

GC-MS analysis showed that all three essential oils were mainly composed of monoterpenes and sesquiterpenes, with the leaf oil being the most chemically diverse. Despite differences in composition, common compounds such as α-pinene, D-limonene, and terpinen-4-ol were present in all oils.

The essential oils exhibited strong antibacterial activity, with the leaf oil showing the highest inhibition against *H. pylori*, achieving a larger inhibition zone than ampicillin against the F29 strain in the disk-diffusion test. Importantly, the oils maintained their activity against strains resistant to metronidazole and clarithromycin, highlighting their potential as effective alternatives for individuals allergic to beta-lactam antibiotics. Against other bacteria, the sap oil demonstrated the strongest activity.

Although ampicillin displayed higher potency at lower concentrations, as reflected by its lower MIC values, the leaf essential oil remains a promising antibacterial agent.

While α-pinene contributes to antibacterial effects, it is not solely responsible for the activity against *H. pylori*; rather, the combined action of multiple components plays a key role. These findings highlight the potential of *Pistacia lentiscus* essential oils as natural alternatives for combating *H. pylori*-related infections.

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Povzetek

Eterično olje vrste *Pistacia lentiscus* je splošno prepoznano po svojih gastroprotektivnih in protimikrobnih lastnostih; vendar ni jasnega soglasja o tem, kateri del rastline izkazuje najmočnejšo aktivnost proti bakteriji *Helicobacter pylori*. Namen te študije je bil primerjati eterična olja, pridobljena iz listov, jagod in smole, glede na izkoristek ekstrakcije, kemijsko sestavo in protibakterijsko aktivnost. Eterična olja so bila ekstrahirana, kemijsko okarakterizirana z GC-MS ter ovrednotena glede protibakterijske aktivnosti proti sevoma *H. pylori* J99 in F29 ter proti bakterijam *Staphylococcus aureus*, *Escherichia coli* in *Pseudomonas aeruginosa*. Smola je pokazala najvišji izkoristek (9,218 %). Analiza GC-MS je razkrila, da so glavni sestavni deli monoterpeni in seskviterpeni, pri čemer je bil α-pinen prisoten v vseh vzorcih. Eterično olje iz listov je izkazalo najmočnejšo inhibicijo rasti *H. pylori*, pri čemer je pri sevu F29 preseglo učinek testiranih antibiotikov. Ti rezultati kažejo, da so listi *P. lentiscus* najbolj obetaven naravni vir protibakterijske aktivnosti proti *H. pylori* ter nakazujejo, da je opažen učinek posledica sinergističnih interakcij in ne zgolj delovanja α-pinena.

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