Dioecy impacts the chemical profile and biological activities of essential oils from leaves of *Pistacia lentiscus* L.

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Abstract

A comparative study on the chemical composition and biological properties of essential oils from male and female plants of *Pistacia lentiscus* essential oils is presented. The analysis highlights the predominance of hydrocarbon monoterpenes across all samples, with the essential oil from female plants exhibiting the greatest diversity of molecules and the highest antioxidant activity, as assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays. The antimicrobial tests reveal that essential oils from female plant show the strongest activity against Gram-negative bacteria, particularly *Escherichia coli* and *Klebsiella pneumoniae*, while essential oils from male plant are most effective against *Staphylococcus aureus* and *Aspergillus niger*. Antileishmanial assays demonstrate a moderate activity against axenic amastigotes of *Leishmania major* and *Leishmania donovani*, with essential oils from female plant showing the strongest activity. Furthermore, cytotoxicity tests showed low toxicity for both oils. These results underscore the significant influence of dioecy on the chemical and biological properties of essential oils, emphasizing the need for gender-specific selection of plants for targeted applications.

Key words

Antileishmanial activity; Antimicrobial activity; Chemical composition; Dioecy; Essential oil; *Pistacia lentiscus* L..

1. Introduction

Plant species of the genus *Pistacia*, particularly *P. lentiscus* L. are known for their significant therapeutic potential. This dioecious plant, with distinct male and female individuals, exhibits considerable variation in traits depending on its geographical origin and gender [1–3].

The traditional uses of this plant are highly diversified. In Tunisia, oil from fruits of *P. lentiscus* L. is used to treat various pains of inflammatory origin [4]. Seed oil is also recognized for its hepatoprotective propreties [5–7]. Leaves and seeds of *P. lentiscus* L. are traditionally used to address issues such as burns, asthma, arthritis, hypertension and hyperuricemia [8,9]. In Chios, mastic gum is employed by local populations to treat gastrointestinal disorders [10] while in Palestine, *P. lentiscus* L. mastic serves as an anti-inflammatory, antipyretic and antispasmodic agent. Additionally, leaves, seeds, and young branches of this plant are used to treat throat infections, diarrhea, jaundice, and renal stones[11]. Several studies have investigated the pharmacological activities of the resin, known as mastic, derived from different parts of the *P. lentiscus* L. species. Mastic gum, obtained from male plants, is the most economically significant product in cosmetic and pharmaceutical industries [7]. Its therapeutic potential extends to cancer prevention and treatment [12], digestive disorders [13], neuroprotection [14], antibacterial properties [15], blood sugar regulation [16], and anti-inflammatory effects.

Essential oils obtained from *P. lentiscus* L. are also a rich source of bioactive molecules, with hydrocarbons and oxygenated monoterpenes being the main constituents. Indeed, α-pinene has been reported as the main compound in leaf essential oils, which contain significant amounts of limonene, myrcene, sabinene and terpinen-4-ol, among others [17–19]. These compounds are recognized for their valuable biological properties, such as antioxidant, anti-inflammatory, anticancer, anticholinesterase, antidiabetic, antihyperlipidemic, antiviral, and antimicrobial activities, as well as their use in treating gastrointestinal disorders [9,20] Additionally, *P. lentiscus* L. essential oils exhibit notable antiparasitic activity [20] .

Since antiquity, the therapeutic virtues of plants have been an integral part of traditional pharmacopoeias in many countries. Understanding the biological activities of these plants in relation to their traditional uses remains a central focus of numerous studies [21,22]. While parameters such as phenological stages, growth area, and targeted plant organs have been extensively studied [19,21,23], little is known about the impact of the plant gender on secondary metabolites production and associated biological activities [24,25]. Gender-based qualitative and/or quantitative differences in secondary metabolite levels in the natural environment may ultimately influence the overall efficacy and yield of these active ingredients. Thus, the consideration of plant gender in phytochemistry-related research is of high interest [24].

For example, previous studies have reported variations in the composition and content of phenolic compounds in plant extracts based on gender [7,26,27]. One study showed that phenolic compound levels in the branches of female *Salix myrsinifolia* were significantly higher, while certain molecules were more abundant in the leaves of male plants of the same species [27]. Similarly, Zaouali et al. [7] reported gender-based differences in phenol, flavonoid, and tannin content in *P. lentiscus* L., with lower polyphenol levels in female individuals, potentially linked to physiological and biochemical differences, such as growth regulation mechanisms. Ecological factors, such as chemical defense indices and growth potential, may also serve as estimators, with females generally exhibiting greater reproductive investment [28].

Species of the genus *Pistacia*, which belong to the Anacardiaceae family [29], include eleven dioecious plant species that grow wild in the warm temperate regions [30]. These resinous shrubs, whether evergreen or deciduous, are classified as xerophytic plants [31] typically reaching heights of 8–10 m and displaying a wide distribution in the Mediterranean basin and circum-Mediterranean areas [9].

However, it has been highlighted that the composition and biological properties of *P. lentiscus* L. essential oils may vary depending on several factors, such as the harvesting region, targeted

organ, environmental conditions and developmental stages of the plant [7]. For instance essential oil obtained from *P. lentiscus* L. leaves has demonstrated strong cytotoxic effects compared to oil derived from its fruits, as well as remarkable leishmanicidal activity [32]. Furthermore, another study indicated that essential oils extracted from leaves harvested during the flowering and dormant vegetative stages exhibited greater antioxidant activity and polyphenol content compared to other organs and developmental stages [7].

Bacterial and fungal infections pose a significant public health challenge, exacerbated by increasing antimicrobial resistance and the limited availability of effective therapeutic options. Pathogens such as *Escherichia coli*, responsible for intestinal and extra-intestinal diseases [33], *Klebsiella pneumoniae*, which causes severe infections such as pneumonia and septicemia [34], and *Staphylococcus aureus*, a major agent of foodborne intoxications [35] highlight the urgent need for innovative treatments. Additionally, enteric bacteria from the *Salmonella* genus frequently cause zoonoses, ranging from gastroenteritis to systemic infections [36], while opportunistic fungi such as *Aspergillus* further complicate therapeutic management[37].

In this context, plant secondary metabolites are emerging as promising alternatives, with numerous studies investigating their antimicrobial potential. While *P. lentiscus L.* is widely recognized for its traditional therapeutic properties, little is known about the efficacy of its secondary metabolites against a broader spectrum of pathogenic microorganisms. Furthermore, understanding intrinsic factors, such as plant gender, that influence the production of secondary metabolites and their biological activities remains crucial.

The present study aims to provide a scientific foundation for the traditional uses of this medicinal plant in Tunisia by investigating gender-dependent differences in the phytochemical profile, as well as the antioxidant, antibacterial, antifungal, and antileishmanial potency of essential oils extracted from the leaves of *P. lentiscus* L.

2. Material and methods

2.1. Plant material

Leaves of *P. lentiscus* L. were harvested during their productive stage in early spring (February and March) in a natural ecosystem in Bizerte, northern Tunisia (37° 14' 59" North, 9° 55' 12" East). This region has a Mediterranean climate, characterized by hot, dry summers and mild, wet winters, with an average annual temperature of 18°C during the study year.

Leaves were collected from twelve fully expended mature plants (six males and six females) distributed across a plot no larger than 1000 m^2, located 500 meters from sea at an altitude of 1–2 meters above sea level. Sampling was conducted early in the morning under dry weather conditions, at a temperature of 12°C. The freshly harvested plants were cut and dried in a dry, dark place at room temperature for three weeks.

The botanical identification and distinction between male and female plants were conducted out by Mohamed Dridi, a senior agricultural engineer at Rimmel Forest (Menzel Jemil East, Bizerte region, Tunisia). Voucher specimens of the leaves from each plant (male or female) were deposited at the herbarium of the laboratory (LBE, Life Sciences Department, 7021 Bizerte) under the following numbers: *P.l.L.-F/LBE 20-001* to 20-006 for female plants and *P.l.L.-M/LBE 20-001* to *20-006* for male plants, respectively.

2.2. Isolation of the essential oils

Essential oils were individually extracted from 100g of dried leaves from each sample, which were immersed in 300 mL of distilled water and subjected to hydrodistillation using a Clevenger apparatus for 3 hours [7]. This duration was chosen as no further recovery of the volatile fraction was observed, in accordance with the guidelines of the European Pharmacopoeia [38]. The essential oils were separated by decantation, transferred to small opaque glass flasks, sealed, and stored at 4°C to preserve their integrity.

We determined the average leaf essential oil yield from six different plants of each sex. The yield of essential oil was determined in triplicate based on the dry plant material weight (Yield % V/W) using the following formula:

$$Y_{EO} = \frac{V_{EO}}{W_D} \times 100 \qquad (1)$$

Where V_{EO} = essential oil volume (g), W_D = weight of dried plant material (g), and Y_{EO} = essential oil yield (% V/W).

2.3. Determination of the chemical composition of the essential oils

The chemical characterization of essential oils from leaves of male and female *P. lentiscus* L. was determined by gas chromatography coupled with mass spectrometry. The analyses were performed on a HP 7890 N series gas chromatograph that was controlled by the ChemStation software and paired with an HP 5975 series mass spectrometer (GC-MS). Two µL of each essential oil diluted in hexane at a 1% V/V ratio were injected into the GC-MS system. The molecules were separated using a non-polar HP-5MS capillary column (30 m × 0.25 mm which was coated with 5% phenyl methyl silicone and 95% dimethylpolysiloxane, Hewlett-Packard, CA, USA) with a film thickness of 0.25 µm. The column temperature was set to rise from 50°C to 250°C at a rate of 5°C per minute. Helium was used as the carrier gas, with a flow rate of 1 mL/min and a split ratio of 60:1. The separated compounds were then ionized using electronic impact at 70 eV. Scan time and mass range were set at 1 s and 40–300 m/z, respectively.

The components were identified by analyzing the chromatographic retention indices (RI) and the obtained spectra, which were compared to the computerized data from the library (Pal 600K®), as previously described [21]. Retention indices (RI) were determined experimentally using a series of n-alkane homologues (C7–C30) and compared to values reported in the

literature [39]. Identification was further achieved by comparing with authentic standard compounds (Sigma, Darmstadt, Germany) which were analyzed under the same conditions as the essential oils.

2.4. Fourier-transform infrared spectroscopy (FTIR)

A qualitative analysis using FTIR was performed to characterize the essential oils extracted from the leaves of male and female *P. lentiscus* L. The mid-infrared spectra were measured in the range of 400–4000 cm^{-1} using the VERTEX 70/70v infrared spectrometer. Five to ten microliters (µL) of the essential oils were deposited on the surface of the diamond-ZnSe ATR crystal (0.5 mm^2 in diameter), and the spectrum was recorded using the OPUS software. The infrared spectrum is expressed as absorbance (A) versus wavenumber (cm^{-1}).

2.5. Determination of the biological activities of essential oils

2.5.1. Antioxidant assay

The radical scavenging activity of the essential oils against DPPH (1,1-diphenyl-2-picrylhydrazyl) was assessed as previously described [21], with some minor changes. A 1 mL aliquot of methanolic essential oil solutions at concentrations of 25, 50, 75, and 100 µg/mL was combined with 1 mL of a 0.004% w/V methanolic DPPH solution. After 30 minutes of incubation at room temperature, the absorbance was measured at 517 nm using a dual-beam Ultrospec 7000 UV-Vis spectrophotometer (GE Healthcare, Chicago, IL, USA), with ascorbic acid serving as the control in this analysis. All tests were performed in triplicate. The percent inhibition of DPPH free radicals (% Inhibition) was calculated using the following formula (Hazzit et al., 2009):

$$\% \text{ Inhibition} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) * 100 \qquad (2)$$

Where $\text{Abs}_{\text{control}}$ = absorbance of the control sample, $\text{Abs}_{\text{sample}}$ = absorbance of the test sample.

2.5.2. Test FRAP (Ferric Reducing Antioxidant Power)

The reducing power of *P. lentiscus* L. essential oils was evaluated using Lee's method [41], with minor modifications. In a test tube, 1 mL of sample solution at various concentrations (0.5, 1, 5, 10, 25, 80, and 100 mg/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (10 g/L). The mixture was incubated in a water bath at 50°C for 20 minutes.

After incubation, 2.5 mL of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. Subsequently, 2.5 mL of the supernatant was mixed with distilled water (2.5 mL) and 0.1% ferric chloride ($FeCl_3$) solution (0.5 mL). A blank sample, prepared without essential oil, was used as a control under the same conditions. Absorbance was measured at 700 nm using a UV spectrophotometer (Ultrospec 7000). Ascorbic acid served as the positive control.

2.5.3. Antibacterial activity

Ten bacterial strains were use for the antibacterial activity assays. Seven of these were isolated from sick animals provided by the bacteriology laboratory of the Veterinary Research Institute of Tunis, Tunisia. Three reference strains known to infect and cause diseases in both humans and animals were obtained from the Pasteur Institute of Tunis (Table 1). The Bromocresol Purple agar medium (BCP; Biokar, France) was used to subculture all strains [42].

The antibacterial activity was conducted according to the agar disk-diffusion method [43]. The bacteria involved in the experiment were grown in Muller Hinton Broth until their turbidity aligned to a concentration of 1.5×10^8 CFU/mL equivalent to the 0.5 McFarland standard. Essential oils from the female and male plants derived from *P. lentiscus* L. were tested at a concentration of 5 µL per disc (reference disc: MN 640w Filter Paper, MACHEREY-NAGEL Gmbh & Co. KG, Germany, diameter 6 mm). After sealing the petri dishes, they were allowed to diffuse at ambient temperature for 30 minutes, then incubated at 37°C for 24 hours for all bacteria, positive control Cephalothine (30 µg), and negative control DMSO. The inhibition zone was measured in mm. Each test was performed in triplicate, and the results were interpreted by measuring of the diameter of the inhibition zone in mm, including the disc diameter.

The Minimal Inhibitory Concentration (MIC) of the essential oils was determined using by microdilution assay on 96 wells microplates. A volume of 100 µL of Mueller Hinton Broth (MHB) and 50 µL of essential oil diluted in 25% (v/v) DMSO were added to each well. Then, 50 µL of bacterial suspension were added. Positive controls consisted of wells with bacterial suspensions in MHB-DMSO, while negative controls were represented by wells containing essential oils mixed with MHB-DMSO.

The plates were incubated at 37°C for 24 hours. The change in color from yellow to violet blue is indicative of bacterial growth. As a result, the MIC corresponds to the lowest concentration of essential oil, where there is no visible growth. All determinations were performed in triplicate.

The Minimal Bactericidal Concentration (MBC) was estimated by taking 5 µL from each well that did not contain a bacterial pellet, along with two control wells, and streaking the samples onto MH agar. The inoculated plates were then incubated for 24 hours at 37°C. The MBC of

the essential oil is identified as the lowest concentration at which no bacterial colonies are observed [43].

2.5.4. Antifungal activity

The antifungal proprety of essential oils was evaluated against three fungal strains (Table 1), that were provided by the laboratory of the Veterinary Research Institute of Tunis, Tunisia. The antifungal assays were performed as for the antibacterial assays, with minor modifications. Concerning the disk diffusion method, the paper disks were impregnated with 5 µL of essential oils and incubated 48 hours at 35°C. Amphotericin (5 µL) was used as the positive control. The MIC and the Minimum Fungicidal Concentration (MFC) were assessed using the microdilution method, where wells were seeded with 100 µL of the inoculum. Fungal plates were then incubated for 48 hours at 35°C. All tests were conducted in triplicate for each essential oil and each fungal species.

2.5.5. Antileishmanial activity and cytotoxicity assay

The antileishmanial effect of the essential oils from male and female *P. lentiscus* L. was evaluated against *L. major* and *L. donovani* following a previously described protocol [44] .

The cytotoxic potential was assessed based on their effects on cell viability. The CC_{50} value, related to the proliferation of RAW 264.7 mouse monocyte/macrophage cells, is calculated following the methods outlined by Maaroufi et al., 2021. We calculated the CC_{50} using nonlinear regression with the ICEstimator website 1.2 version, and miltefosine was used as positive control [44,45].

2.6. Data analysis

Quantitative data were expressed as mean ± SD or ± SEM as appropriate, and comparisons of means between males and females were made using the Student's *t*-test for unpaired series. When the number of groups was greater than two, a one-way ANOVA was used, followed by Dunnett's post hoc test to assess the differences between the pairs of means of the groups to be compared. IC_{50} values were obtained by fitting experimental data according to the sigmoidal dose-response model. Statistical significance was set at a P-value ≤ 0.05 (two-tailed).

3. Results and Discussion

3.1. Extraction and chemical characterization of *P. lentiscus* L. essential oils

Essential oils were hydrodistillated from female and male plant leaves of *P. lentiscus* L. with yields of respectively 0.71 ± 0.10% (V/W)and 0.49 ± 0.02% (V/W). As the literature does not extensively report yields for *P. lentiscus* L. essential oils from distinct male and female plants, the results were compared to these reported for *P. lentiscus* L., where the plant's gender was not specified. The results showed a higher extraction yield for female plant of *P. lentiscus* L. in comparison to male plants, that is similar to the yield described by [44], Moreover, all these results remain overall higher than those reported compared to these found by [7] which had a yield around 0.15%. However, as the essential oil yield is highly depending to environmental conditions[46], which may explain the observed variations, particularly concerning harvest periods. Seasonal fluctuations are often correlated with changes in oil yield, resulting from various bioclimatic and environmental factors including ambient temperature, wind exposure, relative humidity, precipitation, sunlight, and UV radiation levels [47]. Furthermore, annual environmental changes influence the morphology, physiology, and productivity of plants, thereby enabling their adaptation and resilience to stressful conditions. [44].

Both essential oils were firstly characterized by FTIR (Fig.1) to highlight potential variations in the infrared spectra of essential oils extracted from male and female *P. lentiscus* L. leaves. Results highlighted two similar spectra, with the presence of aromatic products, as indicated by the elongation vibration band of the C-H aromatic bond at 3039.73 cm^{-1}. This was also confirmed by the appearance of two σ deformation bands at 750-990 cm^{-1}, which is distinctive of a di- or tri-substituted benzene rings. The latter also contained unsaturated compounds (C=C terpene compounds) since the characteristic σ C=C vibration band was highlighted at 1652 cm^{-1} in the two spectra.

To gain more insight on the individual components and differences between the two essential oils, they were subsequently analyzed using GC-MS. Respectively 34 and 26 differents compounds (Table 2, Fig.2 and Fig. S4) were detected in the essential oils of female and male *P. lentiscus* L. plants, with α-pinene being the main coumpound (31.11 and 38.80 %), followed by β-phellandrene (13.36 and 10.77 %), β-pinene (8.11 and 6.49 %), α-phellandrene (7.31 and 6.28 %), and terpinen-4-ol (6.66 and 6.66 %). While the main components of both essential oils were the same, the mean percentages of several major and minor compounds varied significantly, indicating differences between essential oils extracted from male and female plants. In addition, several compounds found in lower proportions were not common in both oils. It is notably the case of alloaromadendrene, β-elemene and α-cadinol, which were present in oil from female but not from male plants. It is widely recognized that environmental biotic and abiotic factors strongly influence the expression of secondary metabolism[48]. The highlighted differences could be explained by varying levels of sex-related enzymes, and notably the polyphenol oxidase and guaiacol peroxidase, which exhibit crucial roles in various metabolic processes in response to abiotic and biotic factors [49–52] resulting in the synthesis of secondary metabolites [7]. Additionally, it can also be due to distinctions in the energy requirement related to reproduction, as female plants have a generally higher

reproductive effort [28]. Furthermore, given that the plants grew under the same ecological conditions and were of the same age. These differences can be interpreted as attributable to their genetic variation associated with sexual differentiation, rather than the result of environmental factors [7]. These results are supported by the fact that some dioecious plants have already been reported to have intraspecific variations in their primary and secondary metabolites depending on their gender, as an example, previous studies have highlighted differences in the content and composition of essential oils from male and female *Juniperus scopulorum* Sarg [53]. Nagy et al [54] also noted different distributions in the compositions of essential oils from the leaves of females and males *Cannabis sativa* L. The former was indeed found to be richer in cannabinoids and poorer in monoterpene hydrocarbons and in oxygenated sesquiterpenes. Similarly, [55] highlighted a significant difference in the chemical composition of essential oils between male and female specimens of *Baccharis punctulate*, similar to the observations of Samaneh et al [56], distinctions were noted between hermaphrodite and female specimens of two endemic thymes species from Iran.

Due to limited previous investigations studying the impact of dioecy on the phytochemical profile of *P. lentiscus* L. leaves, results were compared to data obtained with the same plant species, but that did not take gender into consideration as blends of both male and female plants were studied. In these previous studies, 3-carene [57], α-pinene, terpinene-4-ol, myrcene [58], α-pinene [59] and β-myrcene [60–62] were identified as the main molecules. Surprisingly, leaves from the present study produced the highest amount of monoterpene hydrocarbons, with values of 91.81% and 92.54%, respectively, surpassing the levels reported by [7]. These differences could be explained by potential changes in the chemical composition from one year to the next, as well as variations related to the geographical region of harvest.

3.2. Evaluation of the impact of gender on the biological activities of *P. lentiscus* L. essential oils

The significant differences observed in the composition of essential oils extracted from the leaves of *P. lentiscus* L., particularly the gender-dependent variability and the notable presence of monoterpene hydrocarbons known for their antibacterial properties [63], prompted a deeper investigation into their biological potentials. This study seeks to better understand these properties in the context of traditional medicine and to explore the possible impact of dioecy on these biological activities.

3.2.1. Antioxidant activity

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay

The antioxidant potential of essential oils extracted from male and female plants was assessed using the DPPH radical assay [64]. This evaluation was chosen due to the extensive traditional uses of *P. lentiscus* L. in medicine and food preservation. Historically, extracts from this plant have been used for their medicinal properties, particularly in treating various gastric, renal, and hepatic disorders [65], as well as a natural preservative in traditional Cypriot sausages [66]. Testing the antioxidant activity of the essential oils allows for a better understanding of the scientific mechanisms behind these traditional uses and validates their efficacy.

Results (Table 3) first highlighted the good radical-scavenging activity of all samples, with EC_{50} values of respectively 125.93 ± 0.50 $\mu g.mL^{-1}$ and 129.81 ± 0.89 $\mu g.mL^{-1}$ for essential oils extracted from female and male plants. These values are comparable to those previously reported by Zaouali et al. [7] for blends of male and female plants and correlate with the high levels of oxygenated monoterpenes and sesquiterpenes found in the current study's samples. These compounds contribute redox potential by neutralizing radicals and chelating metal ions, which enhances the antioxidant ability of *P. lentiscus* L. essential oil [7,67].

Interestingly, the differences in the radical-scavenging activity between the essential oils extracted from female and male plants were shown to be statistically significant ($P<0.05$); the lowest EC_{50} value being obtained with female plants. This can be explained by the impact of dioecy on the composition of the essential oils and may be related to the highest quantities of β-phellandrene in the essential oil from female plant, as this compound is known for its strong antioxidant activity [68], or it could be due to the presence of proton-donating molecules, such as monoterpene alcohols like terpinene-4-ol and α-terpineol, which have been found to be much more concentrated in the essential oils of the female plant than in the male [69]. However, several other factors may also explain the higher antioxidant activity of female leaf essential, that has a more complex composition in comparison to the essential oil from male plant as respectively 34 and 26 differents molecules were identified. It is indeed possible that not only the main compounds contribute to the antioxidant potential of the essential oils. Other less common compounds, such as camphene, β-myrcene, p-cymene, etc. could also play a role [32,70] and even act synergistically [71].

FRAP (Ferric reducing antioxidant power) assay

The FRAP assay represents a key mechanism of action for phenolic antioxidants [72]. It relies on the ability of reducing agents present in oils to reduce Fe^{3+} to Fe^{2+}, leading to the formation of a ferrous complex characterized by an increase in the intensity of the blue color in the reaction medium. This reaction was assessed for the essential oils extracted from the leaves of male and female *P. lentiscus* L., and the results are presented in Fig. 3.

For each essential oil, a concentration-dependent effect was observed, with a significant increase in antioxidant activity proportional to concentration ($p < 0.001$).

Whatever the concentration, the essential oil extracted from the female plant leaves of *Pistacia lentiscus* L. showed remarkable antioxidant activity, superior to that of the essential oil from the male plant leaves and to that of ascorbic acid, used as a reference antioxidant. However, the antioxidant activity of the essential oil from male plant remains very close to that of ascorbic acid. This exceptional activity could be explained by the richness of both oils in phenolic compounds, molecules known for their strong correlation with biological activities [73,74]

The variation in antioxidant activity between the essential oils of female and male plants can be attributed to differences in their chemical composition. The essential oil from female plants, in particular, appears to be richer in monoterpenes—compounds well-known for their potent antioxidant potential [21]. This abundance of monoterpenes in female plants may be linked to their distinct physiological and biochemical activities, such as higher photosynthetic rates and greater reproductive investment compared to male plants [28].

However, it is challenging to attribute these differences to a single compound. It is likely that the observed antioxidant activity results from the synergistic interactions between multiple constituents rather than the effect of individual components [32]

These findings, compared with those reported for other dioecious species, underscore the influence of gender on antioxidant activity. For example, FRAP values indicate that the essential oil from female *Pistacia atlantica* leaves harvested in Algeria exhibits significantly higher antioxidant activity than that of male plants, likely due to the accumulation of monoterpenes during their seasonal peak in female plants [75]. Conversely, no sex-related differences were observed in the antioxidant properties of *Juniperus rigida* essential oils, suggesting that separating oils by gender is unnecessary for this species [76].

Additionally, Zaouali et al [7] reported that male extracts of *P. lentiscus* L., particularly those collected during the vegetative and flowering stages, displayed the highest antioxidant values. Such differences could be attributed to environmental factors or the development stage, significantly influence the chemical composition of essential oils from both male and female plants [7,76].

3.2.2. Antibacterial activity

As previous work highlighted the antibacterial potential of *P. lentiscus* L. leaf essential oils, notably against *S. aureus*, *K. pneumoniae*, *E. coli* and *P. aeruginosa* [77,78], this study aimed to better understanding the impact of dioecy on these antibacterial properties. The *in vitro* antibacterial potentials of essential oils extracted from male and female *P. lentiscus* L. were therefore compared against both ATCC reference strains and clinical strains. The targeted bacterial strains were isolated from sick livestock of various species and are likely responsible for foodborne infections in humans, thus posing a major public health concern. These strains are known to cause severe gastrointestinal disorders, such as vomiting, diarrhea, and abdominal pain [35,79,80]. Given that *P. lentiscus* L. leaves are traditionally utilized in medicine as a remedy for gastrointestinal ailments, particularly diarrhea [11,81,82], a scientific assessment was undertaken to evaluate the antibacterial efficacy of essential oils extracted from both male and female *P. lentiscus* L. against these pathogens.

Results (Table 4) of the agar-well diffusion assays firstly highlighted the growth inhibition of all tested bacteria by both essential oils, at different levels depending on the strain, targetted microorganism, and the type of plant (male or female) from which the essential oil was derived. The antibacterial activity of an essential oil may be due to the ability of the lipophilic molecules to penetrate and destabilize the bacterial membrane [83]. The differences obtained with the strains and essential oils may therefore be due to the fact that each essential oil has its

own unique composition, and each bacteria has its own specific metabolism and membrane structures [84].

Both oils therefore exhibited interesting antibacterial activities (Tables 4 and 5), with inhibition zone diameters (IZ) varying from 8.00 ± 1.73 mm to 13.33 ± 1.52 mm, as well as minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) ranging from 3.5 to 224.5 mg/mL, respectively. The MBC/MIC ratio, which reveals whether the antibacterial action is bacteriostatic or bactericidal, was lower than 4 against almost all strains, showing the bactericidal potential of both *P. lentiscus* L. essential oils.

Comparison with cephalothin (IZ 14 to 36 mm with MIC and MBC respectively in the range 2 - 86 mg/mL), a standard antibiotic, confirmed the promising antibacterial properties of these essential oils. For both oils, *S. aureus* ATCC 29213 was a highly sensitive strain, displaying IZ of 13.33 ± 1.52 mm (male) and 11.33 ± 1.15 mm (female), which were not significatively different to that of the reference drug (14 ± 0.30 mm). In addition, essential oil from female *P. lentiscus* L. was found to have interesting bactericidal activity, and especially against gram-negative bacteria like *E. coli* 131 (IZ 12 mm; MIC = MBC = 28.06 mg/mL) and *K. pneumonia* 3172 (IZ 12 mm; MIC = MBC 3.5 mg/mL). The highest antibacterial activity of male *P. lentiscus* L. essential oil was reported against *S. pneumonia* 6305 with an MIC of 6.82, while the lowest activity was reported against *S. enteritidis* 2559 and *S. aureus* 935 with MIC values of 54.62 mg/mL.

The results of the present study therefore highlighted important differences in the antibacterial properties of essential oils from male and female *P. lentiscus* L., which probably originate from differences in their composition as the essential oil from female plant had a more complex chemical composition than the male one. Indeed, some compounds, such as α-cadinol, β-elemene, and allo-aromadendrene, were exclusively present in the essential oil from female plants, may have a strong impact on its bactericidal properties, which were in

most of the case stronger than for the essential oil from male plant. This heightened activity could also be ascribed to the potential synergy among the essential oil constituents [85].

Furthermore, the higher antibacterial activity observed in female trees can be linked to their greater investment in defense mechanisms. Female trees generally grow more slowly than males, allocating more resources to reproduction and chemical defenses. This heightened investment in defense appears to promote the production of essential oils with higher concentrations of bioactive compounds, leading to stronger antibacterial activity [86].

3.2.3. Antifungal activity

In the next part of the current study, the antifungal effect of essential oils extracted from male and female *P. lentiscus* L. was evaluated against three different strains of *A. niger* which are common fungi responsible for food spoilage [87] and human gastrointestinal problems [88]. The results (Table 6) highlighted the strong antifungal properties of both essential oil from female and male plants, with IZ ranging from 13.20 to 15.66 mm depending on the tested oil and strain. Compared with amphotericin B, a standard antifungal agent offering a ZI of 14 to 18 mm, both essential oils showed comparable antifungal activities against one of the tested strains (2999/13) while the activity was lower, but still high against the two other strains.

Although low differences were highlighted regarding the IZ measurements for essential oils from female and male plants, high differences were observed in their MIC, which ranged from 6.93 mg/mL to 109.25 mg/mL. These differences are likely due to disparities in their chemical compositions as previously highlighted. The fungicidal effect of both essential oils from female and male plants against the three *Aspergillus* species may be caused by the presence of terpinene-4-ol and α-terpineol, two terpene compounds with already reported antifungal properties, notably against *Candida albicans* [89,90].

When compared to previous studies, this is the the inaugural report of the antifungal propreties of essential oil from *P. lentiscus* L. as Barra et al. [91] showed the absence of antifungal activity against *Rhizoctonia solani*, *Penicillium commune, Aspergillus flavus* and *Fusarium oxysporum.* These differences may be attributed to the distinct chemical composition of the essential oils from male and female plants, or to the variation in bioactive compounds present in each sex, such as α-terpinene[92]. They may also be related to the variation in fungal strains, which likely exhibit different sensitivities to the components of the essential oils, or to the composition of the essential oils themselves. Indeed, is is known that the essential oil chemotype is highly dependent on various factors such as the plant origin [44,93]. The method used to evaluate antifungal activity may have also influenced the results, as suggested by Boyanova et al. [94].

3.2.4. Cytotoxicity and antileishmanial activity

The final objective of the present study was to assess how dioecy influences the antileishmanial potential of *P. lentiscus* L. essential oils as previous studies already highlighted the antileishmanial properties of *P. lentiscus* L. extracts [23]. Notably, Bouyahya et al. [32] revealed the presence of promising antiparasitic effects of essential oils extracted from the leaves of *P. lentiscus* L. from the mountains of Morocco towards three Leishmania species, including L. major. In contrast, no interesting antileishmanial effects were detected with essential oils of *P. lentiscus* L. from Tunisia against either *L. major* or *L. donovani* LV9 WT [44]. However, there is no report of the impact of plant sex on this biological activity. As the present study, as well as previous reports, highlight the differencial composition of essential oil dirived from male and female plant of the same species, and considering that the composition of essential oil is fundamental to its biological properties, this study is of high

importance when selecting the proper plants that may be used for targeted therapeutic activities and, in this case, to cure leishmaniasis.

The choice to test this biological activity was also motivated by the traditional uses of *Pistacia lentiscus* L. The leaves of this plant are indeed used to treat various infections, including bacterial and fungal infections [95]. This antimicrobial property suggests a potential activity against leishmaniasis. By selecting *L. major*, which causes cutaneous leishmaniasis, and *L. donovani*, which causes visceral leishmaniasis, the efficacy of treatments can be evaluated against two clinically significant and biologically distinct forms of leishmaniasis prevalent in North Africa [96]. Axenic amastigotes enable rapid large-scale assessments to evaluate the viability of parasites in response to essential oils. This approach allows for a quick and dependable initial identification of new active compounds against Leishmania. While intramacrophage amastigotes reflect the state of the parasite within the host organism, inside they are essential for evaluating the capacity of essential oils to effectively penetrate host cells and act in an intracellular environment, which is crucial for developing effective treatments. Utilizing both axenic and intramacrophage amastigotes allows for the analysis of the direct antileishmanial activity of essential oils, as well as the validation of their efficacy in the actual physiological context of the infection [97].

The antileishmanial activity results reported in Table 7, firstly highlighted the moderate sensitivity of axenic amastigotes of *L. major* and *L. donovani* LV9 to the two tested essential oils, with IC_{50} between 24 to 40 µg/mL, whereas the reference drug miltefosine had lower IC_{50} values (1.21 ± 0.11 µg/mL and 2.53 ± 0.15 µg/mL). It should be however noted that none of the tested essential oils demonstrated activity against intracellular amastigote forms of both parasites.

IC_{50} values for the essential oils of female and male *P. lentiscus* L. were of 24.31 ± 4.94 µg/mL and 31.03 ± 8.77 µg/mL, respectively, against *L. major* axenic amastigotes. Interestingly, the latter showed a slight higher sensitivity to both essential oils than *L. donovani* LV9 (IC_{50} = 32.03 ± 3.30 µg/mL and 40.10 ± 2.05 µg/mL), although this difference was only significant for the essential oil extracted from male *P. lentiscus* L. In addition, it was highlighted that essential oil extracted from female plants was slightly effective than that from male *P. lentiscus* L., which further emphasizes the influence of dioecy on the biological activities of these essential oils. This slight difference between their activity profiles could be explained by variations in the proportions of major and minor compounds between the two oils. Indeed, the essential oil from female plant had notably higher proportions of β-pinene and β-phelandrene, which may correlate with the variability observed in antileishmanial activity.

Aditionnally, as promising extract with antilesmanial properties should also exhibit no cytotoxicity, the absence of toxicity of both essential oils towards macrophages was confirmed[98], indeed, the absence of cytotoxicity is crucial not only for optimizing treatment efficacy by specifically targeting the parasite without damaging host cells, but also for providing a safer alternative to existing treatments. This, in turn, offers promising therapeutic prospects.

The results obtained therefore indicate the absence of cytotoxicity as well as the moderate antiparasitic potential of both essential oils against axenic amastigotes *L. major* and *LV9 WT* and the absent antileishmanial power against intracellular amastigotes, that could result from the low quantity of sesquiterpene hydrocarbons (caryophyllene, germacrene D) in both oils, although the quantity in females oils was practically twice that in males. Indeed, several researchers have already demonstrated the effectiveness of sesquiterpenes against leishmaniasis. Notably, Do Carmo et al. [99] reported that the inhibition of amastigote form of

L. amazonensis by the essential oil of *Piper duckei* was due to the presence of sesquiterpene hydrocarbons. Similarly, Santos et al. [100] showed that essential oils rich in sesquiterpenes, mainly germacrene D, had strong activity against promastigote forms of *Leishmania*. In addition, Bouyahya et al. [32]demonstrated the importance of synergy between the different bioactive compounds containing essential oils.

4. Conclusion

The present study emphasized the unique characteristics of dioecious plants and notably the important impact of gender on their chemical composition and biological activities. With regard to essential oils, it was revealed that male and female *P. lentiscus* L. extracts display significant differences in the nature of their individual components as well as in their proportions, indicating a consequential difference in their biological effects. More specifically, this study highlighted the differential antibacterial, antioxidant and antileishmanial activities of essential oils from female and male plants of *P. lentiscus* L. Overall, the findings obtained demonstrate that the essential oil from female exhibits greater compound diversity, but also a higher scavenging activity against free radicals and reducing power antioxidant potential than the essential oil from male plant, as well as substantial antibacterial potential, and slight stronger antileishmanial activity.

The distinctions observed between the genders, in the chemical composition and biological activities should help optimizing the use of *P. lentiscus* L. in the food and pharmaceutical industries, depending on the variation in its chemical composition. In addition, it is also of high importance when it comes to the use of this plant, and to a greater extend, to the use of dioecious plants in traditional medecine.

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Conflicts of Interest: The authors declare no conflicts of interest.

Supplementary data:

Figure S4. **(**GC-MS spectrum of essential oils from male and female plant leaves of *P. lentiscus* L.**)** is given as supplementary data

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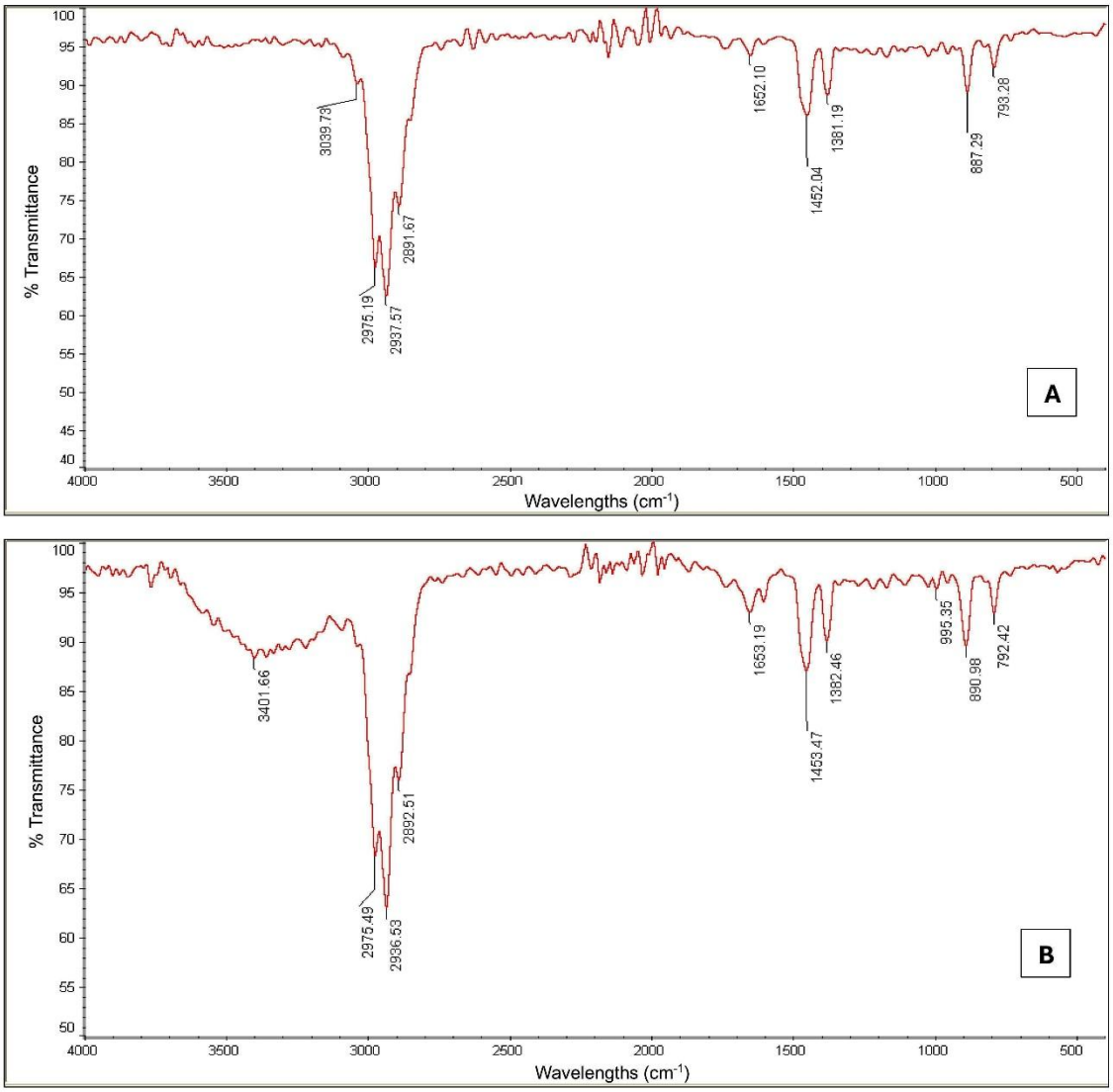


Figure 1. FTIR spectrum of essential oils from the male (A) and female (B) plant leaves of *P. lentiscus* L.

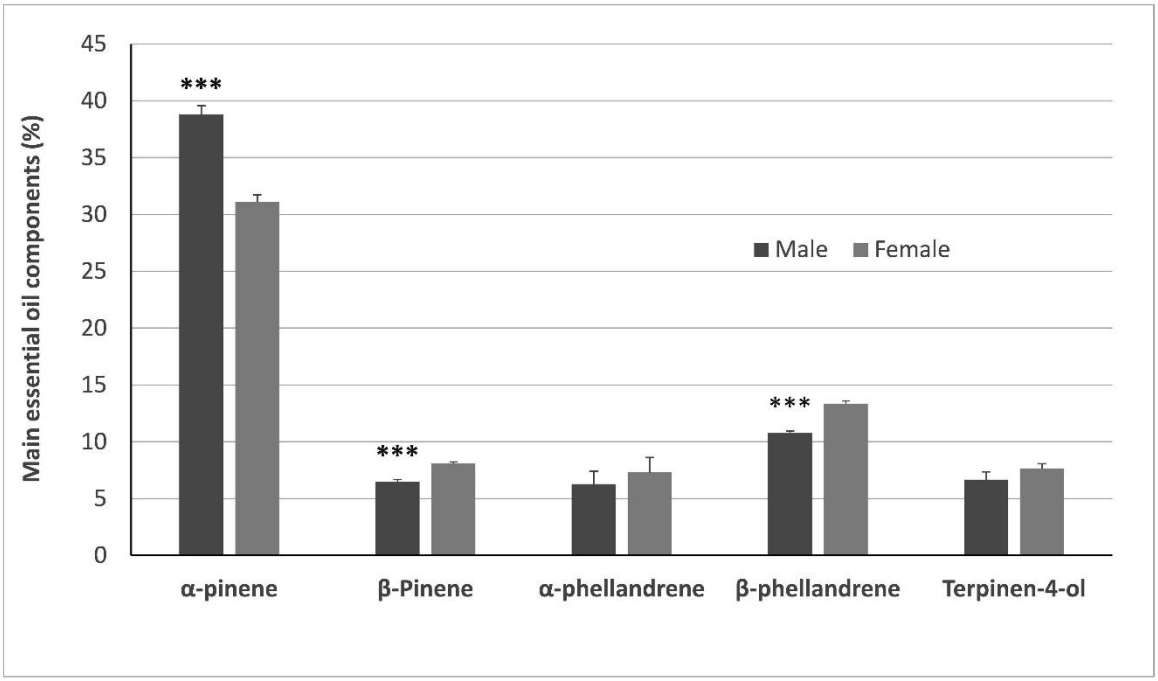


Figure 2. Main constituents of essential oils from the male and female plant leaves of *P. lentiscus* L.

Values are expressed as mean ± standard deviation for 3 replicates and are statistically compared by an unpaired, two-tailed Student's t-test.

***$p < 0.001$ vs. essential oil of female plant leaves of *P. lentiscus* L.

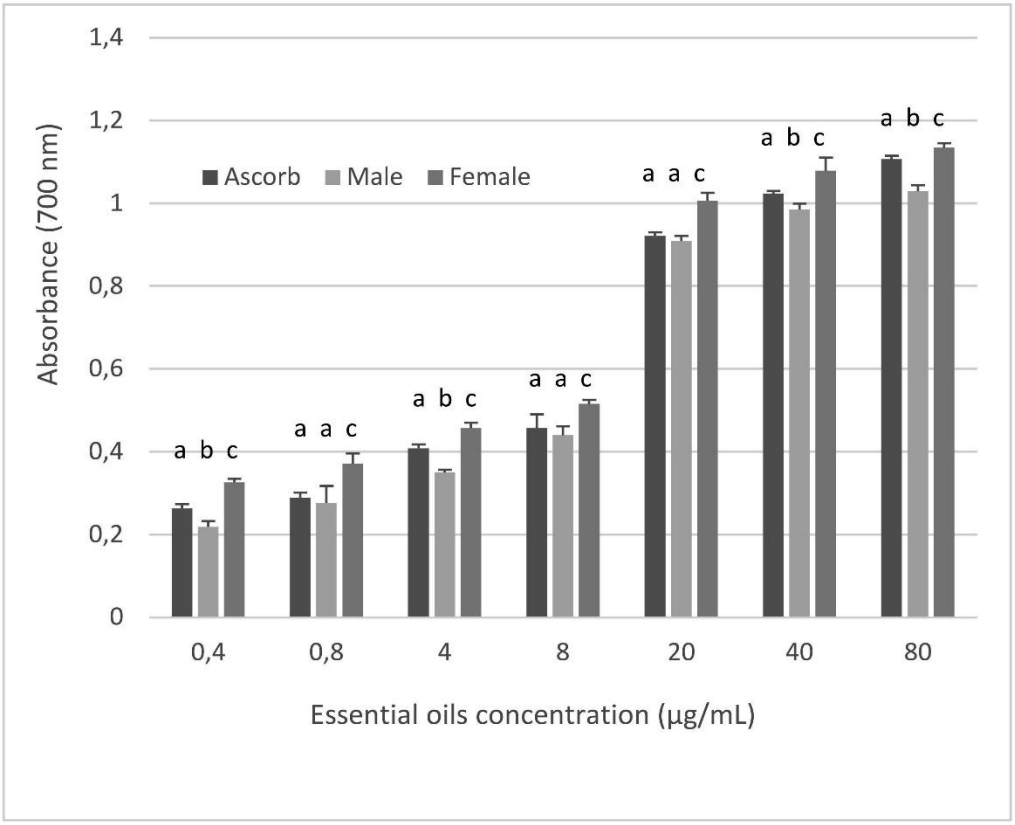
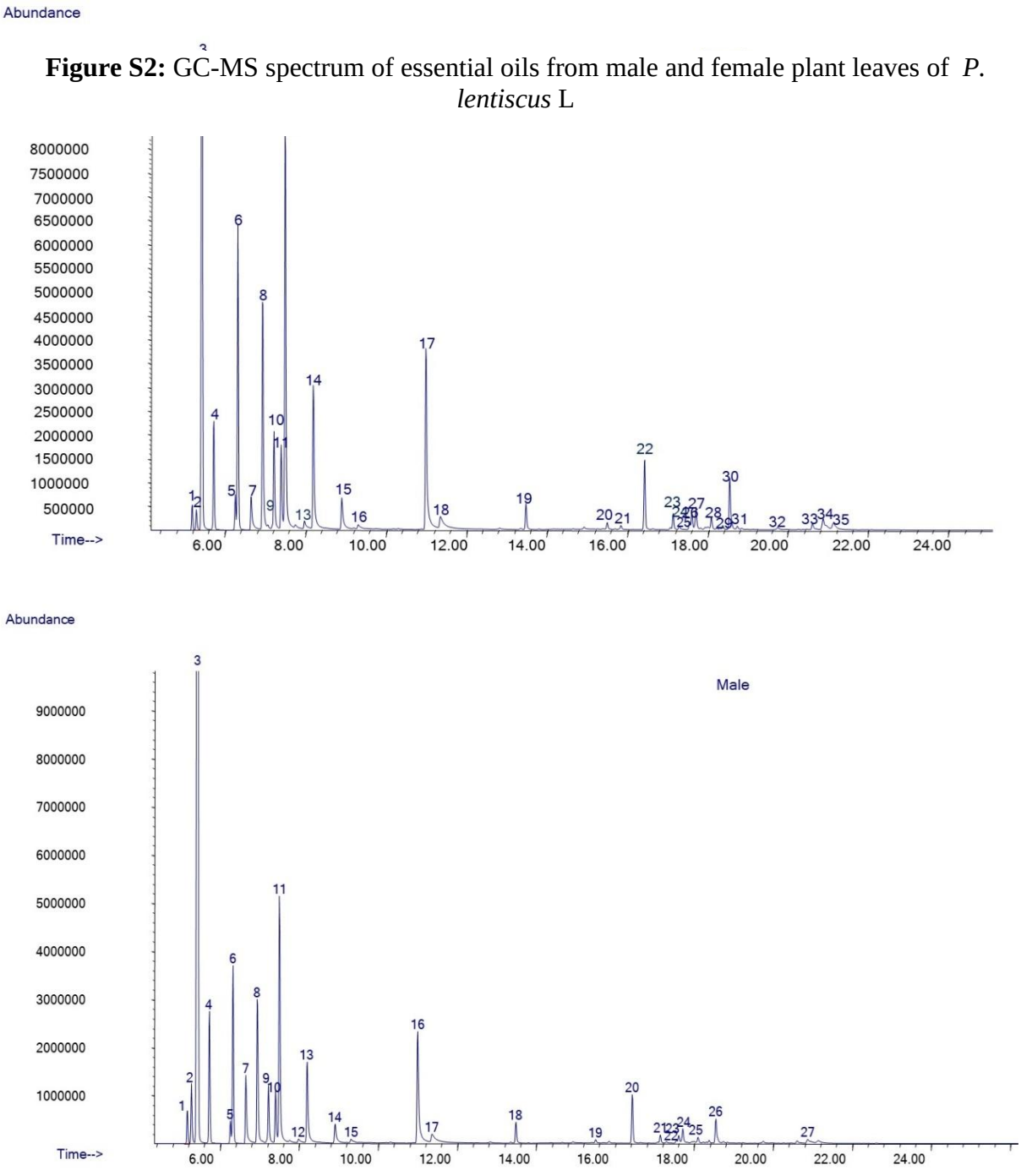


Figure 3. The ferric-reducing power of essential oils extracted from male and female plant leaves of *P. lentiscus* L.

Data are expressed as the mean ± standard deviation (SD) in triplicate from three independent samples (n =3). Within the same concentration, Dunnett's test indicates that mean values that are followed by the different letters are significantly different ($p < 0.001$).

Figure S2: GC-MS spectrum of essential oils from male and female plant leaves of *P. lentiscus* L



Experimental bacteria strains		
1	*Escherichia coli*	131-17 AV
2	*Escherichia coli*	122-15 AV
3	*Escherichia coli*	3588-17
4	*Salmonella enteritidis*	2559-15
5	*Staphylococcus aureus*	935-14
6	*Klebsiella pneumoniae*	3172-17
7	*Klebsiella pneumoniae*	3295-13
Reference bacteria strains		
1	*Staphylococcus aureus*	ATCC 29213
2	*Streptococcus pneumoniae*	ATCC 6305
3	*Escherichia coli*	ATCC 25922
Fungal strains		
1	*Aspergillus niger*	2999/13
2	*Aspergillus niger*	2514/14
3	*Aspergillus niger*	654/18

Table 1. List of bacterial and fungal strains used in this study

Table 2. Chemical composition of essential oils from male and female plant of *P. lentiscus*

Peak No.	Volatile compounds	RI[a]	RI[b]	Mode of identification	Percentage (%)	
					male *P. lentiscus* L.	female *P. lentiscus* L.
1	α-thujenee	924	924	MS, RI, STD	2.22 ± 0.02***	0.60 ± 0.01
2	Tricyclene	927	920	MS, RI, STD	1.22 ± 0.04***	0.70 ± 0.03
3	**α-pinene**	931	931	MS, RI, STD	38.80 ± 0.78***	31.11 ± 0.62
4	Camphene	949	944	MS, RI, STD	4.81 ± 0.13***	3.01 ± 0.14
5	Sabinene	969	968	MS, RI, STD	0.77 ± 0.03**	0.91 ± 0.02
6	**β-pinene**	970	971	MS, RI, STD	6.49 ± 0.19***	8.11± 0.13
7	β-myrcene	981	986	MS, RI, STD	3.18 ± 0.04***	1.49 ± 0.03
8	**α-phellandrene**	996	999	MS, RI, STD	6.28 ± 1.13	7.31 ± 1.32
9	s-(-)-*α*-pinene	1008	1005	MS, RI, STD	-	0.22 ± 0.01
10	α-terpinene	1017	1012	MS, RI, STD	2.53 ± 0.11*	3.20 ± 0.32
11	P-cymene	1026	1020	MS, RI, STD	2.18 ± 0.04**	2.54 ± 0.13
12	**β-phellandrene**	1027	1025	MS, RI, STD	10.77 ± 0.19**	13.36 ± 0.24
13	β-ocimene	1041	1046	MS, RI, STD	0.26 ± 0.07	0.41 ± 0.07
14	γ-terpinene	1054	1056	MS, RI, STD	4.10 ± 0.40	5.16 ± 0.90
15	Terpinolene	1088	1088	MS, RI, STD	1.18 ± 0.04**	1.47 ± 0.07
16	Isoamyl isovalerate	1105	1107	MS, RI, STD	0.23 ± 0.01	0.27 ± 0.01
17	**Terpinen-4-ol**	1206	1183	MS, RI, STD	6.66 ± 0.70	7.66 ± 0.42
18	α-terpineol	1200	1199	MS, RI, STD	1.43 ± 0.07	1.60 ± 0.09
19	Bornyl acetate	1287	1295	MS, RI, STD	0.97 ± 0.01***	0.89 ± 0.01
20	α-copaene	1382	1386	MS, RI, STD	0.16 ± 0.01***	0.25 ± 0.01
21	β-elemene	1407	1402	MS, RI, STD	-	0.18 ± 0.04

Table 1 continued

22	Caryophyllene	1423	1428	MS, RI, STD	2.11 ± 0.02***	2.31 ± 0.03
23	α-humulene	1460	1461	MS, RI, STD	0.37 ± 0.02***	0.56 ± 0.03
24	Alloromadendrene	1457	1467	MS, RI, STD	-	0.16 ± 0.01
25	2-isopropyl-5-methyl-9- methylene-bicyclo[4.4.0] dec-1-ene	1503	1478	MS, RI, STD	0.14 ± 0.00	-
26	γ-amorphene	-	1479	MS	-	0.25 ± 0.00
27	γ-muurolene	1481	1482	MS, RI, STD	0.39 ± 0.00***	0.58 ± 0.00
28	Germacrene-d	1485	1486	MS, RI, STD	0.79 ± 0.00***	0.88 ± 0.01
29	α-muurolene	1504	1504	MS, RI, STD	0.32 ± 0.00***	0.48 ± 0.00
30	δ-cadinene	1528	1524	MS, RI, STD	1.27 ± 0.01***	2.04 ± 0.03
31	Cadina-1(2).4-diene. cis	1537	1533	MS, RI, STD	-	0.11 ± 0.08
32	Caryophyllene oxide	1578	1578	MS, RI, STD	-	0.15 ± 0.02
33	Naphthalene. 1.2.3.4.4a.7-hexahydro-1.6-dimethyl-4-(1-methylethyl)-	-	1617	MS	-	0.34 ± 0.03
34	Epi-α-cadinol	1634	1629	MS, RI, STD	0.35 ± 0.03***	0.87 ± 0.05
35	α-cadinol	1632	1641	MS, RI, STD	-	0.67 ± 0.05
Non-oxygenated monoterpenes (%)					84.79	78.78
Oxygenated monoterpenes (%)					9.06	10.12
Non-oxygenated sesquiterpenes (%)					5.55	7.8
Oxygenated sesquiterpenes (%)					0.35	1.69
Others (%)					0.23	0.61
Total identified compounds (%)					99.98	99

Identification methods: MS: comparison of mass spectra to those of PAL 600®libraries; RI: comparison of retention indices to those reported in the literature; STD: comparison of retention times and mass spectra for commercially available standards; RI[a]: theoretical kovats indices (Pubchem and Nist); RI[b]: calculated kovats indices.

Values are expressed as mean ± standard deviation for 3 replicates and are statistically compared by an unpaired, two-tailed Student's t-test.

[*]$p < 0.05$ vs. essential oil from female plant leaves of *P. lentiscus*.

[***]$p < 0.01$ vs. essential oil from female plant of leaves *P. lentiscus*.

[***]$p < 0.001$ vs. essential oil from female plant of leaves *P. lentiscus*

Table 3. DPPH radical-scavenging of essential oils from male and female plant leaves of *P. lentiscus* L.

Essential oil	Male *P. lentiscus* L.	Female *P. lentiscus* L.	Ascorbic Acid
EC_{50} [$\mu g.mL^{-1}$]	$129.81 \pm 0.89^{*}$	125.93 ± 0.50	$30 \pm 1.89^{\alpha,\beta}$

Values are expressed as mean ± standard deviation for at least 3 replicates and are statistically compared by an unpaired, two-tailed Student's t-test.

$^{*}p < 0.05$ vs. essential oil from female plant leaves of *P. lentiscus* L.

$^{\alpha}$ $p < 0.001$ vs. essential oil from male plant of leaves *P. lentiscus* L.

$^{\beta}$ $p < 0.001$ vs. essential oil from female plant of leaves *P. lentiscus* L.

Table 4. Antibacterial activity of essential oils from male and female plant leaves of *P. lentiscus* L.

	Inhibition zone diameter (mm ± standard deviation)[b]		
Strains	Male *P. lentiscus* L.	Female *P. lentiscus* L.	Cephalothine
E. coli ATCC 25922	9.66 ± 2.88	11 ± 1.01	26 ± 0.60 $^{\alpha,\beta}$
E. coli 131-17 AV	8 ± 1.73*	12 ± 1.02	24 ± 0.50 $^{\alpha,\beta}$
E. coli 122-15 AV	9.33 ± 2.51	9.33 ± 2.08	22 ± 1.02 $^{\alpha,\beta}$
E. coli 3588	9 ± 1.52	10.66 ± 0.57	38 ± 0.40 $^{\alpha,\beta}$
K. pneumonia 3295-13 CP	9.66 ± 0.57*	11.33 ± 0.57	15 ± 0.81 $^{\alpha,\beta}$
K. pneumoniae 3172	10.33 ± 2.08	12 ± 1.02	18 ± 0.72 $^{\alpha,\beta}$
S. enteritidis 2559-15 OV	6.33 ± 1.02**	9.66 ± 0.57	20 ± 0.67 $^{\alpha,\beta}$
S. aureus 935	8.33 ± 1.52*	11.33 ± 0.57	22 ± 1.10 $^{\alpha,\beta}$
S. aureus ATCC 29213	13.33 ± 1.52	11.33 ± 1.15	14 ± 0.30 ^{NS}
S. pneumonia ATCC 6305	11 ± 1.01**	9.66 ± 3.05	0 ND

Values represent means ± standard deviation for triplicate experiments.
The diameter of the inhibition zone, including the well diameter of 9 mm, was determined by the agar-well diffusion method at a concentration of 50 µL of oil per well.
$^{*}p < 0.05$ vs. essential oil from female plant leaves of *P. lentiscus* L.
$^{**}p < 0.01$ vs. essential oil from female plant leaves of *P. lentiscus* L.
$^{\alpha}$ $p < 0.001$ vs. essential oil from male plant of leaves *P. lentiscus* L.
$^{\beta}$ $p < 0.001$ vs. essential oil from female plant of leaves *P. lentiscus* L.
ND: not determined
NS: not significant

Table 5. Antibacterial power of essential oils from male and female plants leaves *P. lentiscus* L.

Strains	Female *P. lentiscus* L.			Male *P. lentiscus* L.			Cephalothine	
	MIC	MBC	Effect	MIC	MBC	Effect	MIC	MBC
E. coli ATCC 25922	14.03	14.03	Bactericidal	27.32	27.32	Bactericidal	5.20	5.90
E. coli 131-17 AV	28.06	28.06	Bactericidal	27.32	27.32	Bactericidal	9	14
E. coli 122-15 AV	7.01	7.01	Bactericidal	27.32	54.62	Bactericidal	2	15
E. coli 3588	7.01	7.01	Bactericidal	27.32	27.32	Bactericidal	2	8
K. pneumonia 3295-13 CP	7.01	14.03	Bactericidal	27.32	27.32	Bactericidal	10	20
K. pneumoniae 3172	3.55	3.55	Bactericidal	27.32	27.32	Bactericidal	8	18
S. enteritidis 2559	28.06	56.12	Bactericidal	54.62	54.62	Bactericidal	1.20	13.20
S. aureus ATCC 29213	28.06	224.5	Bacteriostatic	27.31	218.50	Bacteriostatic	14.50	34
S. aureus ATCC 935	14.03	112.25	Bacteriostatic	54.62	218.50	Bactericidal	18	86
St. pneumonia ATCC 6305	28.06	112.25	Bactericidal	6.82	27.31	Bactericidal	-	-

MIC: Minimum inhibitory concentration (as % (v/v)) (mg/mL).

MBC: Minimum bactericidal concentration (as % (v/v)) (mg/mL).

Table 6. Antifungal activity of essential oils from male and female plant leaves of *P. lentiscus* L.

Fungal strains	Antifungal activity – Diameter Inhibition zone (IZ ; mm ± SD)			Male *P. lentiscus* L.			Female *P. lentiscus* L.		
	Male *P. lentiscus* L.	Female *P. lentiscus* L.	Amphotericin B	MIC[1] (mg/mL)	MFC[2] (mg/mL)	Effect	MIC (mg/mL)	MFC (mg/mL)	Effect
A. Niger 2999/13	14 ± 0.02	14 ± 1.02	14 ± 0.01	6.93	ND	ND	54.62	ND	ND
A. Niger 2514/14	13.20 ± 0.20	13.33 ± 1.20	15 ± 0.02 $^{\alpha,\beta}$	111	ND	ND	109.25	ND	ND
A. Niger 654/18	15.33 ± 1.01	15.66 ± 0.60	18 ± 0.02 $^{\alpha,\beta}$	28.06	28.06	Fungicide	109.25	218.50	Fungicide

ND: not determinate

$^{\alpha}$ $p < 0.05$ vs. essential oil from male plant leaves of *P. lentiscus* L.

$^{\beta}$ $p < 0.05$ vs. essential oil from female plant leaves of *P. lentiscus* L.

[1] MIC : Minimal Inhibitory Concentration

[2] MFC : Minimum Fungicidal Concentration

Table 7. Antileishmanial and cytotoxic activities of essential oils from male and female plant leaves *P. lentiscus* L.

	IC_{50} axenic amastigotes ($\mu g.mL^{-1}$ ± SD)		IC_{50} intramacrophage amastigotes ($\mu g.mL^{-1}$ ± SD)		CC_{50} ($\mu g.mL^{-1}$ ± SD)	Selectivity index SI= CC_{50}/IC_{50} intramacrophage	
	L. major	*L. donovani LV9*	*L. major*	*L. donovani LV9*		*L. major*	*L. donovani* LV9
Female *P. lentiscus* L.	24.31 ± 4.94	32.03 ± 3.30	> 100	> 100	>100	/	/
Male *P. lentiscus* L.	31.03 ± 8.77	40.10 ± 2.05*	> 100	> 100	>100	/	/
Miltefosine	1.21 ± 0.11 $^{\alpha,\beta}$	2.53 ± 0.15$^{\alpha,\beta}$	4.23 ± 0.38 $^{\alpha,\beta}$	6.75 ± 0.71 $^{\alpha,\beta}$	21.95 ± 1.92 $^{\alpha,\beta}$	5.18	3.25

Values are expressed as mean ± standard deviation for at least 3 replicates and are statistically compared by an unpaired, two-tailed Student's t-test.

$^{*}p < 0.05$ vs. essential oil from female plant leaves of *P. lentiscus* L.

$^{\alpha}$ $p < 0.001$ vs. essential oil from male plant leaves of *P. lentiscus* L.

$^{\beta}$ $p < 0.001$ vs. essential oil from female plant leaves of *P. lentiscus* L.