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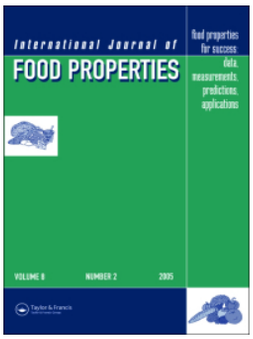

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Phytochemical composition analysis, antioxidant, antimitotic, and anti-inflammatory effects of leaf and stem extracts of *Pistacia lentiscus* L

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ABSTRACT

This study explored the phytochemical composition, antioxidant properties, anti-germinative effects, and anti-inflammatory actions of methanolic leaf extracts (MLE) and methanolic stem extracts (MSE) derived from *Pistacia lentiscus* L. species native to the eastern region of Morocco. Using Ultra-high-performance liquid chromatography (UHPLC),16 and 17 components were discovered inMLE and MSE, respectively, with Epigallocatechin being the most prevalent in both extracts. Polyphenols and flavonoids were found to be more abundant in MLE. Furthermore, DPPH assays revealed that it had a higher free radical scavenging activity (IC50 = 37.5 mg/L) compared to ascorbic acid while ferric-reducing power tests showed that MSE had a greater potency (IC50 = 86.14 mg/L) than MLE. Both MLE and MSE inhibited sorghum seed germination in a dose-dependent manner, with MSE being slightly more effective. Mitotic index determination revealed that both extracts had concentration-dependent antimitotic effects, with MSE showing similarity to colchicine, indicating its potential as an antimitotic agent. *In vitro*, anti-inflammatory assays showed that it had similar properties to indomethacin in protein denaturation and membrane stabilization, while MLE displayed distinct characteristics. Furthermore, in a carrageenan-induced rat model, both extracts demonstrated anti-inflammatory potential, with MSE showing a trend of slightly better efficacy. These results highlight the importance of further investigation into MSE's potential as an anti-inflammatory and antimitotic agent.

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Introduction

For centuries, traditional medicinal plants have played an important role in treating numerous ailments, and even today, an estimated 80% of the global population continues to depend on plant-

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based remedies for their healthcare needs.[1] Morocco boasts an exceptionally diverse vascular flora, standing out as one of the most varied in the Mediterranean region and the most abundant in North Africa. The country is home to an extensive array of plant taxa, totaling around 5211, encompassing both species and subspecies.[2,3] Notably, approximately 22% of this botanical wealth is endemic to Morocco, highlighting the nation's unique and rich plant biodiversity.[4] *Pistacia lentiscus* L., a perennial plant native to the Mediterranean region, is renowned for its nutritional, medicinal, and pharmacological attributes. Numerous studies have explored the chemical composition and antioxidative capacities,[5–13] as well as the anti-inflammatory potential of *Pistacia lentiscus* L.'s various plant parts. This includes investigations into its ability to regulate inflammatory responses.[14–16] Both aqueous and ethanolic extracts of *Pistacia lentiscus* L. have demonstrated potent antioxidant activity, assessed through DPPH and FRAP assays.[17] This antioxidant capacity is largely attributed to gallic acids and their galloylated derivatives.[18,19] Moreover, the high polarity of extraction solvents like ethanol and methanol has been linked to enhanced antioxidant activity, likely due to the presence of polar compounds acting as hydrogen atom donors or singular atom transfer agents.[7] In addition, numerous studies have shown that extracts of *Pistacia lentiscus* L. contain bioactive compounds capable of inhibiting the growth of cancer cells,[14,20,21] notably ethanolic leaf extracts demonstrated promising anticancer potential against several cell lines, including lung cancer A549, breast cancer MCF7, prostate cancer PC3, and liver cancer HepG2 in vitro.[19] Studies have also shown antiproliferative activities against human neuroblastoma cell lines.[14] Additionally, leaf and fruit extracts inhibited the growth of melanoma B16F10 and breast EMT6 cells.[14] Furthermore, the ethanolic extract demonstrated notable selectivity in cytotoxicity evaluations, yielding promising outcomes in animal models by reducing micronucleated cell count while preserving crucial cellular parameters.[22] These findings underscore the potential of *P. lentiscus* extracts as antioxidant, anti-inflammatory, and anticancer agents, warranting further exploration for therapeutic applications, opening new avenues for future studies. Additionally, other biological activities[23–27] have been explored, further supporting its traditional use in folk medicine. However, studies investigating the chemical composition and biological activities of *Pistacia lentiscus* L. from Morocco's eastern region are limited. To address this gap, the current study aims to thoroughly examine the chemical and biological properties of *Pistacia lentiscus* L. from the eastern region of Morocco. The research involves acomprehensive analysis of the chemical composition of methanolic extracts from leaves and stems using Ultra High-Performance Liquid Chromatography (UHPLC), as well as the evaluation of their antioxidant, anti-inflammatory, and antimitotic activities.

Materials and methods

Chemical compounds and reagents used

The study utilized several reagents, including Folin-Ciocalteu, sodium hydroxide (NaOH), ferrous chloride ($FeCl_2$), Dragendorff, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), aluminumtrichloride ($AlCl_3$), colchicine, potassium hydroxide (KOH), and lead acetate ($(C2H3O_2)_2$) linoleic acid. The solvents used were methanol (CH_3OH) and Chloroform.Additionally, chemicals such as hydrochloric acid (HCl), sulfuric acid (H_2SO_4), gallic acid ($C_7H_6O_5$), quercetin ($C_{15}H_{10}O_7$), Colchicine, acetocarmine, sodium citrate, dextrose, citric acid, isosaline, indomethacin, carrageenan were used. All these chemicals and reagents were obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA) and were of analytical grade.

Collection and processing of plant extracts

The leaves and stems of *Pistacia lentiscus* L. were collected in the "Jerrada" region of Northeastern Morocco in March 2022. After collection, the plant material was kept at room temperature until further processing. The material was then air-dried at room temperature and ground into powder (100 g), which was then macerated in methanol (1000 mL) for 48 hours at ambient temperature with

constant stirring. The extract was filtered after each extraction process and stored at 4°C for future use. To determine the extraction yield, the following formula was employed[28]:

$$Yield\,\% = \frac{M\ Extract}{M\ Sample} * 100\%$$

Where M extract refers to the weight of the extract obtained from the plant sample, expressed in grams. M sample represents the weight of the plant sample used for the extraction process, also expressed in grams.

Phytochemical screening of plant extracts

The phytochemical screening was carried out in accordance with established procedures as outlined by Obouayeba et al., Pandey and Tripathi, Junaid R Shaikh, and MK Patil.[29–31] The screening tests conducted included coumarin (Alkaline Test), alkaloids (Dragendorff Test), tannins (Lead Acetate Test), flavonoids (Alkaline Reactivity Test), anthocyanins (HCl Test), steroids (Sulfuric Acid Test), and phenols (Ferric Chloride Test).

LC-MS/MS profiling of methanolic extract from different parts of P. lentiscus L

The extraction process for sample aliquots (80 mg) involved adding 1 mL of ethanol and then subjecting the mixture to agitation and sonication in a bath at 45°C for 1 hour. The qualitative analysis was performed using a Shimadzu Ultra-High-Performance Liquid Chromatography (Nexera XR LC 40, Kyoto, Japan) equipped with an MS/MS detector (LCMS 8060, Shimadzu Italy, Milan, Italy). The MS/MS analysis was conducted using electrospray ionization, regulated by Lab Solution software (ver. 5.6, Kyoto, Japan), with a low-energy scan of 4 V (full scan MS) and a high-energy scan (10–60 V ramping) within a single LC cycle. The source was set up with a nebulizing gas flow of 2.9 L/min, a heating gas flow of 10 L/min, an interface temperature of 300°C, a DL temperature of 250°C, a heat-block temperature of 400°C, and a drying gas flow of 10 L/min. The analysis employed flow injection without chromatographic separation, using a mobile phase of a mixture of acetonitrile and water with 0.01% formic acid in a ratio of 5:95 (v/v).[32,33] Molecule identification was achieved by comparing the characteristic fragments with those in the in-house-developed library. A molecule was considered positive if its area under the curve exceeded that of the blank. Additional details regarding retention time and typical fragment m/z values can be found in the Supplementary Materials File.

Quantifying the total content of polyphenols

The quantification of total polyphenols was performed following the spectroscopic method adapted from Singleton et al. 1999.[34] with some modifications in the volume measurements. The reaction mixture consisted of 0.2 mL of plant extracts (1000 mg/L), 1 mL of 10% Folin-reagent Ciocalteu's, and 0.8 mL of 7% Na_2CO_3 solution. After thorough mixing, the blend was stored in the dark room temperature for 1 hour. The absorbance was then measured at 760 nm using a spectrophotometer. This process was repeated three times to obtain the average absorbance value. The total polyphenol content was determined using a gallic acid solution, with the results expressed as gallic acid equivalent (mg GAE).

Quantifying the total content of flavonoids

The total flavonoid content of *P. lentiscus* L. Was determined using the aluminum chloride colorimetric method, as described by N. V. Matyushchenko1 and T. A. Stepanova (2003)[35] with minor adjustments in the volume measurements. Specifically, 0.5 mL of the methanolic extract (1000 mg/L) combined with 0.5 mL of sodium nitrate (5%) and 0.12 mL of 10% aluminum chloride in 1.5 mLof

distilled water. A calibration curve was established using quercetin as the standard. The absorbance of the blends was recorded at 415 nm using a UV-spectrophotometer. The total flavonoid concentration was then quantified in milligrams of quercetin equivalent (mg QE/g of sample). All experiments were performed in triplicate, and the average absorbance value was calculated.

Antioxidant potential of methanolic extracts derived from different parts of P. lentiscus L

The antioxidant activity of *P. lentiscus* L. leaves and fruits were tested using two in-vitro methods: DPPH assay and Ferric Reducing Antioxidant Power (FRAP) assay.

Assay for scavenging DPPH radicals

To assess the capacity of our plant extracts against the DPPH radical, we adopted the methodology described by Djidel et al.[36] with minor modifications in the volume measurements. Dissolve 4 mg of DPPH in 100 mL of methanol to prepare the DPPH solution. Subsequently, we prepared various concentrations of methanolic extracts (25, 50, 100, 200, 400, 800, 1000 mg/L/). For each concentration, 0.8 mL of the DPPH solution was incorporated, resulting in the ultimate volume of 1 mL for each mixture. The blend was thoroughly vortexed and then left to incubate in darkness for 30 minutes. After the incubation period, the absorbance at 517 nm was promptly determined by employing a spectrophotometer. As a reference, the standard antioxidant ascorbic acid was utilized. Each analysis was performed three times. The percentage of DPPH scavenging ability was computed using the formula[37]:

$$DPPHscavengingability\,(\%) = \left(\frac{AB_{DPPH} - AB_{sample}}{AB_{DPPH}}\right) \times 100$$

The absorbance of the DPPH solution in the absence of is denoted as AB_{DPPH}, otherwise, the absorbance of the test sample combined with the DPPH solution is denoted as $AB_{sample.}$

FRAP assay of methanolic extracts

The extracts' ability to reduce iron was evaluated using the procedure outlined by Hemma et al.[38] with slight modifications. Multiple dilutions of the extracts were prepared. To each extract, 1.250 mL of phosphate buffer (0.2 M, pH 6.6) and 1.250 mL of potassium ferricyanide (K_3Fe (CN) 6) (1% w/v) were added. Incubate the blend at 50°C lasting 20 minutes. Once cooled to room temperature, the process was stopped by including 1.250 mL of trichloroacetic acid (10% w/v). Subsequently, centrifugation was carried out at 3000 rpm for exactly 10 minutes. After ward, the supernatant solution (1.250 mL) was combined with an equal volume of distilled water (1.250 mL), and 0.25 mL of a 0.1% w/v ferric chloride solution was added. The spectrophotometer was utilized to measure the absorbance at 700 nm. Ascorbic acid was employed as a control substance. All experiments were conducted in duplicate. The IC50 value, representing the sample concentration required to attain 0.5 absorbance at 700 nm, was determined based on the sample concentration.

Antimitotic activity of methanolic extracts from P. lentiscus L

Inhibition of germination in sorghum seeds by methanolic extracts of P. lentiscus L

In this study, various concentrations of methanolic extracts from the leaves and stems of *Pistacia lentiscus* L. were employed to assess their anti-germinative effects on sorghum seeds (2 mg/mL, 3 mg/mL, and 6 mg/mL). The evaluation process involved preparing Petri boxes that were previously cleaned and sterilized. The bottom of each box was lined with filter paper soaked in water for the Control, Colchicine at a concentration of 2 mg/mL for the Positive control, and different concentrations of methanolic extracts from the stems and leaves of *P. lentiscus* L. Subsequently, 30 sorghum seeds, carefully selected based on shape and physiological condition, were placed in each petri box.

Germination was observed, and the number of germinated seeds was counted after 96, 120, and 144 hours, using sterile forceps for accuracy. The determination of the inhibition rate is calculated as the difference between 100 and the germination rate. Alternatively, it represents the percentage of ungerminated seeds.[39]

Antimitotic activity

To evaluate the antimitotic potential of methanolic extracts from the leaves and stems of *P. lentiscus* L, we employed a modified assay described by Saboo et al.[40,41] using onion bulbs. Radical germination was induced through a 24-hour immersion in water, leading to the selection of onion bulbs with radicals measuring 1 to 2 cm for the study, then the chosen onion bulbs were subjected to different concentrations of methanolic extracts derived from both leaves and stems specifically at 2 mg/mL, 6 mg/mL, and 10 mg/mL. This experimental setup also included positive controls using colchicine at concentrations of 2 mg/mL and 6 mg/mL, and a negative control involving water.

After exposure periods spanning 12, 24, 48, and 72 hours, a meticulous excision of the apical regions of onion bulb roots (5 apices for each extract) was conducted. The excised tissue underwent procedures involving immersion in 1 M HCl for 5 minutes, a gentle rinse in distilled water, and subsequent fixation with acetocarmine for chromosome staining. Following this, the samples underwent delicate squashing, with the quantification of mitotic and total cells occurring in 5 to 8 fields (400 to 500 cells) within each radical tip. This holistic microscopic examination allowed for a nuanced calculation of the mitotic index (Optical microscopy was performed using an Optika microscope (Italy), and images were captured with an Infinity 1 camera microscope.), providing insightful perspectives into the percentage of meristematic cells actively undergoing mitosis. The mitotic index was calculated by the following formula[39,40]:

$$Mitotic\ index = Number\ of\ dividing\ cells Total\ Number\ of\ cells * 100$$

In vitro and in vivo anti-inflammatory activity

In vitro anti-inflammatory activity

The reaction solution (0.5 mL; pH 6.3) included 0.45 mLof bovine serum albumin (5% aqueous solution) and 0.05 mL of distilled water. A modest amount of 1 N HCl was added to obtain a pH of 6.3. Following that, 0.5 mL of each MSE and MLE extract was introduced into the reaction blend, which was then allowed to rest for 30 minutes at 37°C. The samples were then heated for 5 minutes at 57 degrees Celsius. After cooling, 2.5 mLof phosphate buffer solution was added and turbidity was determined spectrophotometrically at 660 nm. For the negative control, 0.45 mL of bovine serum albumin and 0.05 mLof distilled water were used.[17] The method for calculating the protein denaturation inhibition percentage is as outlined below[17]:

$$\text{Percentage inhibition}\% = \frac{\text{Control} - \text{Treated Sample}}{\text{Control}} * 100$$

Alsever's solution was formulated by dissolving the following components in distilled water: 20 mg/mL dextrose, 8 mg/mL sodium citrate, 0.5 mg/mL citric acid, and 4.2 mg/mL sodium chloride. The solution was then sterilized for use. Healthy rat blood[42] was collected via retro-orbital puncture into heparinized centrifuge tubes and blended with an equivalent volume of sterilized Alsever's solution. After centrifugation at 3000 rpm, the concentrated cells were washed with isosaline, resulting in the preparation of a 10% (V/V) isosaline suspension. The experimental blend (4.5 mL) comprised 1 mL of phosphate buffer, 2 mL of hyposaline, 0.5 mL of both MLE and MSE extracts, and 0.5 mL of red blood cell (RBC) suspension. Indomethacin was employed as the benchmark pharmaceutical, and a control reaction blend was established. Following a 37°C incubation for 30 minutes and subsequent centrifugation, the analysis of the liquid above was performed Utilizing UV absorbance measurement at 560

nm.[42] The calculation of membrane stabilization activity percentage was determined utilizing the following formula[42]:

$$\text{Percentage inhibition } \% = \frac{\text{Control} - \text{Treated Sample}}{\text{Control}} * 100$$

In vivo,inflammation in rat paws induced by Carrageenan

To assess the inflammation-inhibiting effect of MSE and MLE, we adhered to the procedure outlined in the investigation carried out by Rajasree et al.[43] with some modifications. The rats were divided into 4 groups, each comprising 5 rats. All rats were male.

Group 1 (negative control): received an oral gavage of physiological saline (0.9% NaCl) using a gavage tube.

Groups 2 and 3: were treated with MLE and MSE extracts via oral gavage at a dose of 500 mg/kg.

Group 4: (The standard group) was administered indomethacin orally at a dose of 10 mg/kg.

Before injection of carrageenan, the circumference of the right paw of all rats was measured. One hour after the administration of the extracts or indomethacin, inflammation was induced by intraplantar injection of 1% carrageenan into the right paw of each rat. The paw circumference was then measured at 3, 4, 5, and 6 hours after the carrageenan injection to evaluate the anti-inflammatory effect. The calculation of inflammation inhibition percentage was determined by the following formula[43]:

$$PI\,\% = \frac{(\text{Ct} - \text{C0})\text{Control} - (\text{Ct} - \text{C0})\text{treated}}{(\text{Ct} - \text{C0})\text{Control}} * 100$$

C0: The average paw diameter before injection.

Ct: The average paw diameter following carrageenan injection at a specified time

Statistical analysis

Statistical representations, with the mean and standard error of the mean (SEM), were utilized for data representation. Group differences in the antigermination and antimitotic test were assessed through analysis of variance (one-way ANOVA) and Dunnett's test. A significance value of 0.05 was used. The statistical interpretation was conducted utilizing SPSS software, and GraphPad Prism was used for generating graphs.

Results and discussion

Yields of extraction

The extraction yields of Pistacia lentiscus L. leaves and stems were expressed in % and are presented in Figure 1. Results showed that the highest extraction yield was presented by the methanolic extract of leaves 33% followed by the methanolic extract of stems 22%. A study conducted by Maha et al.[44] comparing the methanolic and aqueous extracts of various parts of Pistacia lentiscus, including leaves, stems, and roots revealed that the methanolic extract exhibited higher yields compared to the aqueous extract. Specifically, the methanolic extract demonstrated a yield improvement ranging from 13% to 33.3%, where as the aqueous extract showed an increase ranging from 10% to 18%. Furthermore, the comparison between different plant parts indicated that the extract yield from leaves was the highest, followed by that from stems, which is consistent with our findings (Figure 1).

Phytochemical screening of P. lentiscus L. methanolic extracts

Table 1. shows the phytochemical screening of P. lentiscus L. methanolic extracts. As revealed by the results, all the phytoconstituents are presented in the methanolic extracts for both stems and leaves.

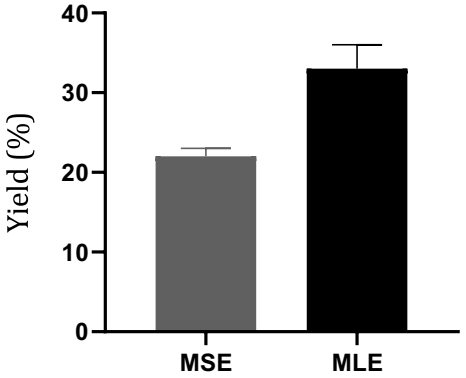

Figure 1. The yield extract of methanolic extract of *Pistacia lentiscus* L. leaves and stems. Data were presented as Mean ± SEM, and experiments were done in triplicates. **MES**: Methanolic stems extract; MLE: Methanolic leaves extract

Table 1. Phytochemical screening of the methanolic extract of *P. lentiscus* L. leaves and stems.

	Samples (methanolic extracts)	
Assay	Leaves	Stems
Alkaloids	_	+
Flavonoids	++	++
Tannins	+++	+++
Anthocyanins	+	+
Steroids	+	+
Coumarin	+	+
Phenol	+++	+++

However, Alkaloids were present only in the methanolic extract of stems. Tannins and Phenols were found to be abundant in leaves and stems. Unlike other plant extracts, such as those from the Caryophyllaceae family, which often contain saponins like gypsogenin or quillaic acid, our analysis did not reveal the presence of these compounds. Gypsogenin saponins, in particular, are triterpene glycosides found in various Gypsophila species.[45] Therefore, although the absence of gypsogenin saponins was noted in our extract, phenolic compounds provide a strong alternative for protection against oxidative stress. These results are similar to other studies that have shown the presence of flavonoids, phenolic acids, and tannins in the leaves and stems of P. lentiscus L.[10,14]

+: present; ++: abundant moderately; +++: present abundantly; -: absent

Phytochemical profiling analysis using LC-MS/MS

It important to mention that our study marks the inaugural utilization of the Shimadzu Ultra-High-Performance Liquid Chromatography (UHPLC) with an MS/MS detector for the qualitative analysis of methanolic extracts of *Pistacia lentiscus* L. from the eastern region of Morocco. This advanced analytical technique was employed to identify and quantify the chemical constituents present in the extracts, which could contribute to their biological activities. The results of the analysis are presented in Table 2, which provides a comprehensive overview of the identified compounds and their respective concentrations.

Table 2 displays the UHPLC chromatography results of the methanolic extracts, revealing that the methanolic extract of *P. lentiscus* L. leaves contains 16 components including Epigallocatechin which is the most abundant component in both methanolic extracts of leaves and stems, Kaempferol-3-O-glucoside, luteolin-7-O-glucoside, epigallocatechin gallate, quercetin, and quercetin-3-O-glucoside are more present in methanolic extracts of leaves than methanolic extract of

Table 2. Principal phenolic compounds revealed in leaf and stem methanolic extract of *Pistacia lentiscus* L. revealed by LC-MS/MS.

				Abundance	
Molecule	Molecular formula	[M-H]+	RT (min)	MEL	MES
Catechin	$C_{15}H_{14}O_6$	289.00	0.331	+	-
Catechin gallate	$C_{22}H_{18}O_{10}$	441.00	0.349	+	+
Epigallocatechin	$C_{15}H_{14}O_7$	305.00	0.332	+++	+++
Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	457.00	0.329	++	+
Gallic acid	$C_7H_6O_5$	168.90	0.330	++	+++
Gentisic acid	$C_7H_6O_4$	153.00	0.334	-	+
Hesperetin	$C_{16}H_{14}O_6$	301.30	0.332	+	+
Hydroxytyrosol	$C_8H_{10}O_3$	153.05	0.335	–	++
Kaempferol	$C_{15}H_{10}O_6$	285.00	0.329	+	++
Kaempferol-3-*O*-glucoside	$C_{21}H_{20}O_{11}$	609.10	0.328	+++	+
Kaempferol-3-*O*-glucuronoside	$C_{21}H_{18}O_{12}$	461.10	0.327	+	+
Luteolin	$C_{15}H_{10}O_6$	284.90	0.330	+	+++
Luteolin 7-*O*-glucoside	$C_{21}H_{20}O_{11}$	447.10	0.328	+++	+
Myricetin	$C_{15}H_{10}O_8$	317.00	0.330	+	-
Oleocanthal	$C_{17}H_{20}O_5$	303.20	0.334	-	+
Procyanidin	$C_{30}H_{26}O_{13}$	577.00	0.353	+	+
Quercetin	$C_{15}H_{10}O_7$	301.00	0.331	++	+
Quercetin-3-*O*-glucoside	$C_{21}H_{20}O_{12}$	463.10	0.339	++	+
Rutin	$C_{27}H_{30}O_{16}$	609.00	0.328	+	+

+++: High abundance, ++: abundant, +: low abundance, –: not detectable in the extract.
MES: Methanolic stems extract; MLE: Methanolic leaves extract

stems. Catechin and Myricetin are present only in the methanolic extract of the leaves. However, phytochemical analysis by UHPLC revealed that the methanolic extracts of the stems include 17 compounds including Gallic acid, luteolin and kaempferol are the main compounds. Gentisic acid, hydroxytyrosol, and oleocanthal are exclusively found in the methanolic extract of the stems. Moreover, a recent study by Al-zaben et al. examined the chemical composition of *Pistacia lentiscus* from Saudi Arabia using high-performance liquid chromatography (HPLC), identifying only 13 compounds in the methanolic leaf extract, while only 10 compounds were detected in the stems.[44] This underscores the importance of utilizing ultra-high-performance liquid chromatography (UHPLC) for enhanced precision in identifying additional molecules. Furthermore, these discrepancies may be attributed to geographical variations, including climate, soil composition, altitude, and sunlight exposure, which can significantly impact the chemical composition of plant species like *Pistacia lentiscus* L. Regarding the chemical composition, the extracts from leaves and stems are rich in flavonoids, such as catechin and its derivatives, hesperetin, kaempferol and its derivatives, luteolin and its derivatives, myricetin, quercetin, and rutin. Additionally, there is a notable presence of phenolic acids and their derivatives such as gallic acid, gentisic acid, hydroxytyrosol, and oleocanthal. These findings are consistent with previous research that has revealed flavonoids, phenolic acids, and their derivatives as the most abundant compounds in the leaves and stems of this plant.[46,47]

Quantification of overall polyphenolic and flavonoid content

Total polyphenols and flavonoids were reported as mg gallic acid and Rutin/g dry sample, respectively. Figure 2 shows TPC and TFC in the methanolic extract of stems and leaves. The total Polyphenols and flavonoids concentration found by Zitouni et al. are very similar to our results.[11] However, the methanolic extract of leaves is higher in polyphenols and flavonoids at 524.87 ± 1.17 mg EAG/g DM and 147.91 ± 0.04 mg ER/g DM, respectively. The polyphenols and flavonoids for the methanolic extract of stems are lower than those corresponding to the methanolic extract of leaves with 85.27 ± 3.01 mg EAG/g DM and 25.87 ± 0.50 mg ER/g DM, respectively. This is consistent with a recent study by Mechqoq et al.[48] that found *Pistacia lentiscus* L. to have the highest polyphenol content (396.64 ± 30.79mgGAE/gDW) among four different species.

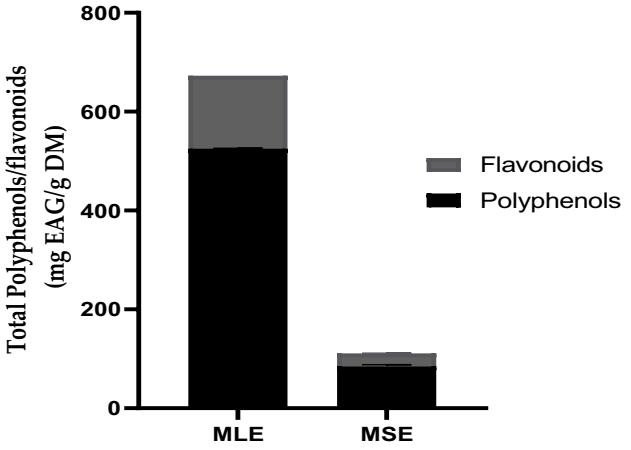

Figure 2. TPC and TFC in *P. lentiscus* L. leaves and Stems methanolic extract. Data were presented as Mean ± SEM, and experiments were done in triplicates.MES: Methanolic stems extract; MLE: Methanolic leaves extract.

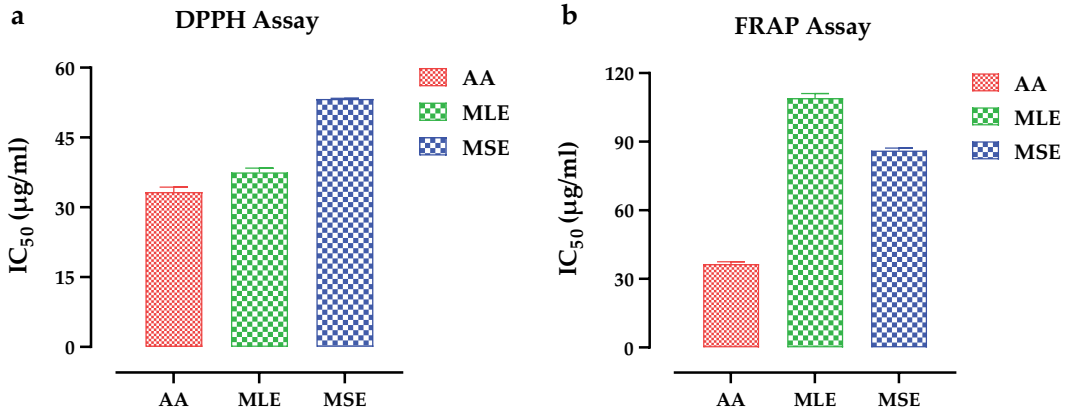

Figure 3. Antioxidant activity of methanolic leaves and stems extracts of *Pistacia lentiscus* L. using DPPH radical scavenging assay (A), and FRAP iron-reducing activity (B). Data are presented as Mean ± SEM, with *n*=4. AA: Ascorbic acid; MES: Methanolic stems extract; MLE: Methanolic leaves extract.

Antioxidant capacity of MLE and MSE

The IC_{50} values of DPPH free radical capture for MLE and MSE of *P. lentiscus* L. are 37.5 ± 0.9 mg/L and 53.23 ± 0.14 mg/L, respectively (Figure 3). These findings demonstrate that the DPPH free radical abatement activity of methanolic leaf extract is closer to that of the ascorbic acid. However, MSE exhibits lower DPPH free radical abatement potential compared to the other samples. The DPPH results obtained by the methanolic extract from the stems are close to the results obtained by the methanolic extract from the leaves of *Pistacia lentiscus* L. collected in Algeria, with an IC_{50} of 60 mg/L.[49] The ferric reducing power test shows that the MSE has a higher ferric reducing power with an IC_{50} of 86.14 ± 1.04 mg/L,followed by MLE with a value of 109.00 ± 2.00 mg/L. These findings are consistent with another study[48] that investigated the antioxidant potential (FRAP) of a methanolic extract derived from *Pistacia lentiscus* L. sourced from the Agadir region. This study revealed an IC50 value of 65.63 ± 1.41 mg/L.[48] Other studies have also shown the significant antioxidant potential of *Pistacia lentiscus* L.[5,6,50] Celik et al. (2010) showed that there is a strong correlation between total antioxidant capacity and the concentration of phenolic compounds in a sample, as these compounds play an important role in neutralizing free radicals.[51] This suggests that the antioxidant activity found

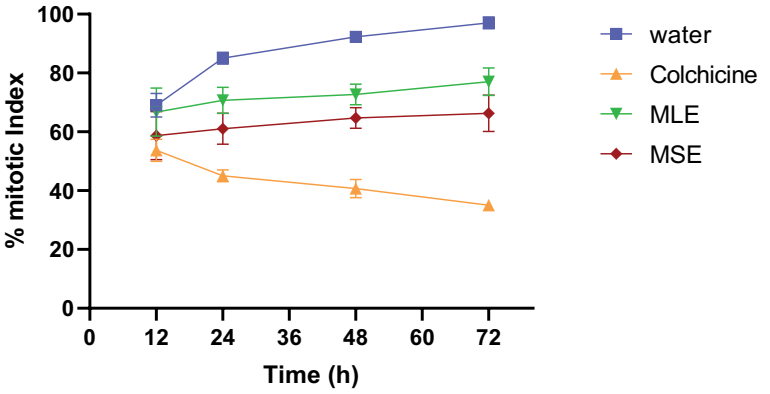

Figure 4. Antimitotic Activity of Methanolic Leaves and stems Extracts from *Pistacia lentiscus* L. at 2 mg/mL Concentration over Different Time Intervals, MES: Methanolic stems extract; MLE: Methanolic leaves extract.

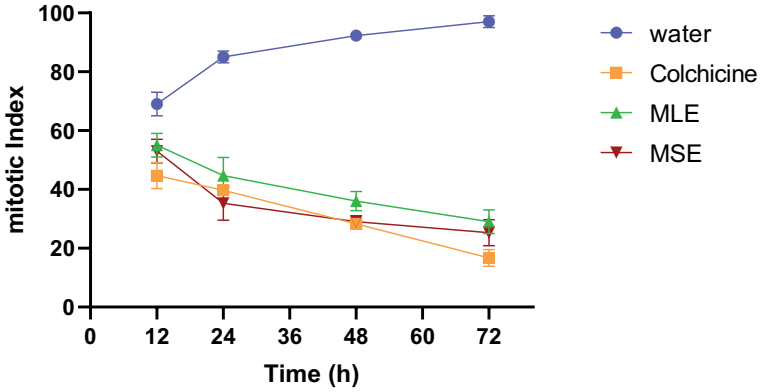

Figure 5. Antimitotic Activity of Methanolic Leaves and stems Extracts from *Pistacia lentiscus* L. at 6 mg/mL Concentration over Different Time Intervals, MES: Methanolic stems extract; MLE: Methanolic leaves extract.

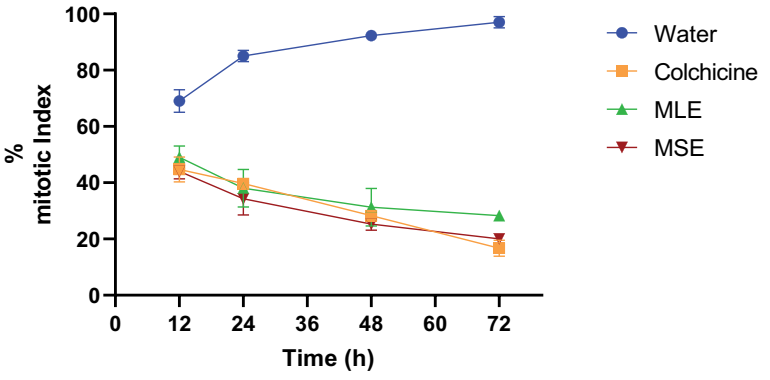

Figure 6. Antimitotic Activity of Methanolic Leaves and stems Extracts from *Pistacia lentiscus* L. at 6 mg/mL Concentration over Different Time Intervals, MES: Methanolic stems extract; MLE: Methanolic leaves extract.

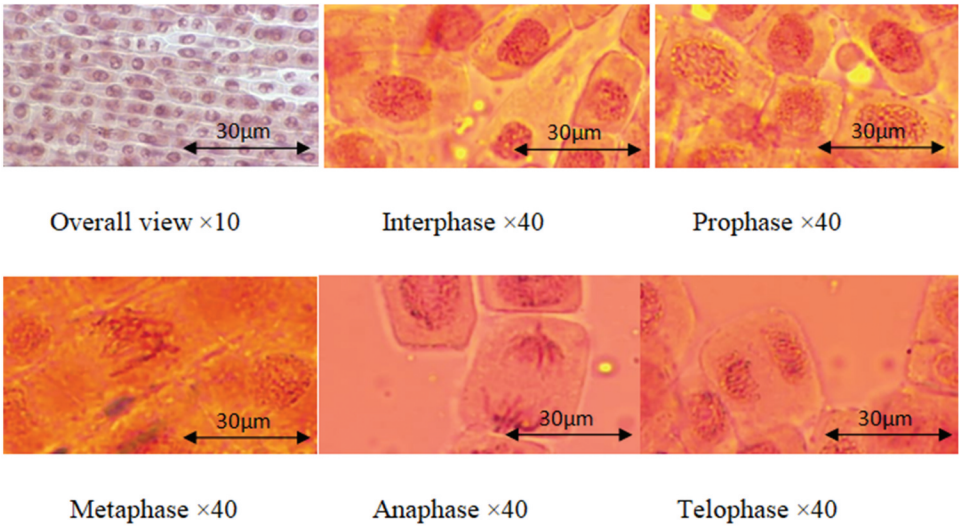

Figure 7. Meristematic cells of Allium cepa depicting the various phases of mitosis.

in *Pistacia lentiscus* L could be due to its high polyphenol content. Other research has shown that aromatic and medicinal plants, such as those studied by Idriss etal.[52] possess strong antioxidant properties. Specifically, their study revealed that the methanolic extract of Salvia cedronella and Salvia fruticosa has IC50 values of 10.23 mg/L and 12.63 mg/L, respectively.

Antimitotic activity of methanolic leaves and stems extracts

Determination of the Inhibition Rate: The inhibition rate was determined by examining the effect of extracts from sorghum seeds' leaves and stems, obtained through methanolic extraction, on the germination process at varying concentrations (2 mg/mL, 3 mg/mL, and 6 mg/mL). This investigation also included a control group treated with water and a positive control group treated with colchicine at a concentration of 2 mg/mL. The results of this study are presented in Table 3, which highlights the impact of methanolic leaf and stem extracts on sorghum seed germination at the specified concentrations.

Table 3 reveals the antigerminative activity of methanolic extracts from the leaves (MLE) and stems (MSE) of *Pistacia lentiscus* L. on sorghum seeds at different concentrations (2 mg/mL, 3 mg/mL, and 6 mg/mL), compared to both a negative control (water) and the positive control (colchicines at 2 mg/mL). At 2 mg/ml, both MLE and MSE exhibit significant inhibition rates of 33.33% and 35.66%, respectively, indicating their potency in suppressing sorghum seed germination compared to the negative control of 14.53%. As the concentration increases to 3 mg/ml, MLE, and MSE intensify their inhibitory impact, reaching 44.67% and 52.00%, respectively, further demonstrating their dose-dependent anti-germinative effects. Notably, at 6 mg/ml, MLE and MSE maintain their inhibitory

Table 3. Effect of Methanolic Leaves and Stems Extracts from *Pistacia lentiscus* L. The rate inhibition of Sorghum sp. seed germination.

	MLE	MSE	CONTROL	COLCHICINE
2mg/ml	$33{,}33 \pm 2{,}40^{ab}$	$35{,}66 \pm 2{,}40^{ab}$	$14{,}53 \pm 2{,}40^{a}$	$76{,}66 \pm 2{,}40^{c}$
3mg/ml	$44{,}67 \pm 2{,}45^{ab}$	$52{,}00 \pm 2{,}45^{ab}$		
6mg/ml	$61{,}10 \pm 2{,}13^{ab}$	$57{,}80 \pm 2{,}23^{ab}$		

MES: Methanolic stems extract; MLE: Methanolic leaves extract; a,b, c and indicate statistical significance between different measurements. There is no significant difference between samples that share the same letter; (b' and 'ab' are used for intermediate groups or values situated between the two controls)

potential, with rates of 61.10% and 57.80%, respectively. While colchicine consistently exhibits the highest inhibition rate (76.66% at 2 mg/mL), it is noteworthy that both MLE and MSE show considerable inhibitory effects, particularly at higher concentrations.

Determination of mitotic index

Our investigation into the antimitotic potential of methanolic extracts derived from *P. lentiscus* L. leaves and stems involved the use of concentrations of 2 mg/mL, 6 mg/mL, and 12 mg/mL over specific time intervals. The mitotic indices were measured and presented in Table 4, 5 and 6, and in Figures 4, 5, 6 and 7 which showcase concentration-dependent trends. These findings suggest that the extracts may have antimitotic properties that are influenced by concentration.

The results obtained from the tables, which highlight the antimitotic activity of *P. lentiscus* L. methanolic extracts at different concentrations and time intervals, offer important insights into the effect of these extracts on the process of cellular division Figure 7. This investigation suggests that the extracts may have the ability to inhibit or alter the normal course of cell division, which could have significant implications for their potential use in various applications.

In Table 4 and Figure 4, where the concentration is 2 mg/ml, colchicine consistently demonstrates superior antimitotic activity with the lowest mitotic indices. Both MSE and MLE exhibit significant antimitotic effects at 24 h, 48 h, and 72 h compared to the control. Transitioning to Table 5 and Figure 5, with an increased concentration of 6 mg/ml, colchicine maintains its superiority, showing consistently lower mitotic indices 44.67 ± 2.95. Notably, MSE demonstrates values closer to colchicine, particularly at 12 (53.00 ± 1.89) and 24 hours 42.33 ± 2.34, suggesting a potential concentration-dependent enhancement of antimitotic properties. MLE also exhibits significant antimitotic potential,

Table 4. Antimitotic Activity of Methanolic Leaves and stems Extracts from *Pistacia lentiscus* L. at 2 mg/mL Concentration over Different Time Intervals.

Extract	12h	24h	48h	72h
Colchicine	53.66 ± 2.75^{a}	45.00 ± 2.07^{a}	40.67 ± 3.01^{a}	35.00 ± 2.32^{a}
MSE	58.67 ± 2.17^{a}	61.00 ± 1.98^{b}	64.67 ± 2.13^{b}	66.33 ± 3.01^{b}
MLE	66.67 ± 2.38^{a}	70.67 ± 2.56^{b}	72.67 ± 2.23^{b}	77.00 ± 2.65^{b}
Water	69.00 ± 4.55^{a}	85.00 ± 3.12^{c}	92.33 ± 32.05^{c}	97.00 ± 2.42^{c}

MES: Methanolic stems extract; MLE: Methanolic leaves extract; a,b, c and indicate statistical significance between different measurements. There is no significant difference between samples that share the same letter;

Table 5. Antimitotic Activity of Methanolic Leaves and stems Extracts from *Pistacia lentiscus* L. at 6 mg/mL Concentration over Different Time Intervals.

Extract	12 h	24 h	48 h	72 h
Colchicine	44.67 ± 2.95^{a}	39.67 ± 2.45^{a}	28.33 ± 2.03^{a}	16.67 ± 3.00^{a}
MES	53.00 ± 1.89^{a}	42.33 ± 2.34^{a}	29.00 ± 2.83^{ab}	25.33 ± 2.17^{ab}
MEL	55.00 ± 2.77^{ab}	44.67 ± 2.54^{a}	36.00 ± 1.87^{b}	32.00 ± 2.37^{b}
Water	69.00 ± 4.55^{b}	85.00 ± 3.12^{b}	92.30 ± 32.05^{c}	97.00 ± 2.42^{c}

MES: Methanolic stems extract; MLE: Methanolic leaves extract; ; a,b, c and indicate statistical significance between different measurements. There is no significant difference between samples that share the same letter; (b' and "ab" are used for intermediate groups or values situated between the two controls)

Table 6. Antimitotic Activity of Methanolic Leaves and stems extracts from *Pistacia lentiscus* L. at 12 mg/mL Concentration and Colchicine at 6 mg/mL Over Various Time Intervals.

Extract	12 h	24 h	48 h	72 h
colchicine	44.67 ± 2.95^{a}	39.67 ± 2.45^{a}	28.33 ± 2.03^{a}	16.67 ± 3.00^{a}
MES	49.67 ± 3.67^{a}	34.33 ± 2.77^{a}	25.33 ± 2.09^{ab}	20.01 ± 2.73^{ab}
MEL	50.00 ± 2.23^{a}	38.00 ± 3.04^{a}	31.33 ± 3.33^{b}	28.33 ± 2.98^{b}
Water	69.00 ± 4.55^{b}	85.00 ± 3.12^{b}	92.33 ± 2.05^{c}	97.00 ± 2.42^{c}

with values closer to colchicine, particularly at early time points. In Table 6 and Figure 6, where the concentration further increases to 12 mg/mL for the extracts and 6 mg/ml for colchicine, the mitotic index of both MSE and MLE experienced a significant decrease, with certain time intervals exhibiting effects comparable to colchicine, emphasizing a concentration-related convergence in antimitotic effects. This intriguing similarity suggests that at higher concentrations, MSE and MLE may exhibit antimitotic properties comparable to the medicament. Overall, the tables underscore the concentration-dependent nature of antimitotic activity, with MSE showing proximity to colchicine, especially at increased concentrations, warranting further exploration into the potential of MSE as a potent antimitotic agent. The encouraging results motivate further exploration of the effects of these extracts on antiproliferative activity in animal cells, paving the way for more in-depth investigations into their potential anticancer properties. The research conducted by Francesco Siano et al. on Lentisk berry oil (LBO)[8] has shown promising dose-dependent effects on the growth of HT-29 cells, which are derived from human colorectal adenocarcinoma. This was demonstrated by the observed cell cycle arrest, highlighting the potential anti-cancer properties of LBO. In a different study, Yemmen et al.[42] investigated the extracts of *Pistacia lentiscus* L. and found that the methanol/water (8:2) leaf extract had significant antiproliferative activity. The calculated IC50 values of 135.67 ± 2.5 mg/L in CaCo2 cells and 250.45 ± 1.96 mg/L in AGS cells indicate a strong inhibitory effect on cell growth, suggesting the potential of *Pistacia lentiscus* L. extracts as a therapeutic option for colorectal adenocarcinoma. Remila et al.[14] also conducted a study on the anticancer activity of *Pistacia lentiscus* L. extracts on human non-tumor and tumor cell lines. Their research revealed that *P. lentiscus* fruit extracts did not have cytotoxic effects on the THP-1 cell line. However, a significant reduction in cell viability was observed with leaf extracts, particularly at higher concentrations (75, 100 mg/L), suggesting a selective impact on cancer cells.[14] It should also be noted that certain plant compounds can have a genotoxic effect and induce DNA damage.[53]

Given these findings, we are inspired to further investigate the potential antimitotic properties of methanolic extracts from both the leaves and stems of *Pistacia lentiscus* L. in the context of human cancer cells. Our research represents the first exploration into this area, and we are eager to build upon the discoveries of these researchers to explore the potential therapeutic applications of *Pistacia lentiscus* L. compounds in cancer treatment.

In vitro assessment of anti-inflammatory activity: exploring protein denaturation and membrane stabilization methods

The Figure 8 illustrates the in vitro anti-inflammatory activity of different extracts using two distinct methods: protein denaturation and membrane stabilization. In the protein denaturation method, both indomethacin and MSE extracts exhibit a similar inhibitory effect. This implies that MSE, akin to the reference drug indomethacin, effectively prevents the denaturation of proteins, a key indicator of anti-inflammatory potential. Shifting to the membrane stabilization method, both indomethacin and MSE extracts again demonstrate a comparable ability to stabilize cell membranes and protect against damage. Conversely, the MLE extract displays a statistically significant difference in its membrane stabilization potential when compared to indomethacin and MSE. These results suggest that MSE shares a noteworthy anti-inflammatory activity profile with indomethacin, evident in both protein denaturation and membrane stabilization methods. On the other hand, MLE exhibits distinct characteristics, signifying a significant difference in its anti-inflammatory effects, particularly in membrane stabilization. These findings underscore the potential of MEL for further investigation as a promising candidate in anti-inflammatory research.

In vivo assessment of anti-inflammatory efficacy

In the carrageenan-induced rat model, three hours post-injection, the control group treated with NaCl 0.9% exhibited a significant increase in paw diameter to 3.4 cm, indicating inflammation (Table 7).

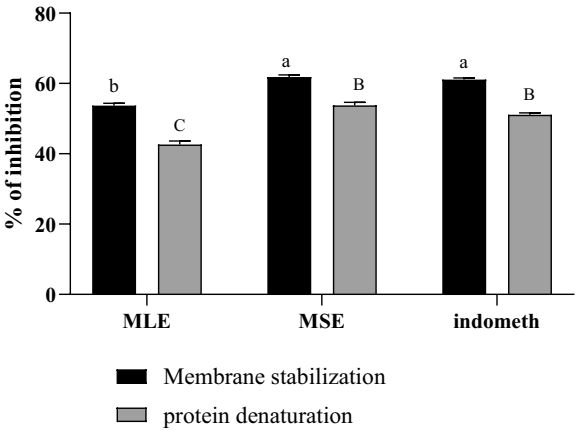

Figure 8. Anti-Inflammatory Potential of Methanolic Leaf and Stem Extracts from *Pistacia lentiscus* L. *in vitro*: Evaluating Membrane Stabilization and Protein Denaturation.MES: Methanolic stems extract; MLE: Methanolic leaves extract; indometh: indomethacine; a A, b B, and c C indicate statistical significance between different measurements. There is no significant difference between samples that share the same letter.

Table 7. Paw Diameter in Treated Groups and Control (NaCl 0.9%) Before and After Carrageenan Injection.

	0h	3h	4h	5h	6h
NaCl 0.9%	2.63 ± 0.09	3.40 ± 0.16^{a}	3.40 ± 0.05^{a}	3.37 ± 0.12^{a}	3.40 ± 0.08^{a}
Indometh	2.47 ± 0.21	3.33 ± 0.09^{a}	3.40 ± 0.05^{a}	3.10 ± 0.08^{b}	2.83 ± 0.12^{b}
MLE	2.63 ± 0.09	3.30 ± 0.14^{a}	3.10 ± 0.08^{ab}	2.97 ± 0.21^{b}	2.83 ± 0.25^{b}
MSE	2.57 ± 0.09	3.27 ± 0.05^{a}	3.20 ± 0.08^{a}	2.93 ± 0.05^{b}	2.63 ± 0.09^{b}

MES: Methanolic stems extract; MLE: Methanolic leaves extract; indometh: indomethacine

Conversely, indomethacin, MLE, and MSE extracts all demonstrated a significant increase in paw diameter, with diameters of 3.33 cm, 3.3 cm, and 3.27 cm, respectively, but with no significant difference compared to the control.

As the observation continued, at four hours post-injection, MLE showed slightly lower diameters at 3.1 cm compared to both the control and indomethacin at 3.4 cm. At five hours post-injection, indomethacin exhibited notable anti-inflammatory effects, with a significantly lower paw diameter of 3.1 cm, outperforming the control, MLE, and MSE, which showed slightly lower diameters at 2.97 cm and 2.93 cm, respectively, with no significant difference between the two extracts and indomethacin.

At the final observation point (six hours post-injection), indomethacin, MLE, and MSE continued to exhibit strong anti-inflammatory effects, with paw diameters of 2.83 cm, 2.83 cm, and 2.63 cm, respectively, significantly lower than the control at 3.4 cm. Comparing MLE and MSE, at most time points, there was no significant difference between the two.

Recent research highlights that natural products continue to be a valuable source of biologically active compounds, including those that inhibit the kinase PAK1, which is involved in cellular processes and inflammation, offering potential for developing new antiviral and anti-inflammatory therapies.[54]

Actually, in previous studies, there haven't been analyses of the anti-inflammatory activity of the methanolic extract from the leaves and stems of *Pistacia lentiscus* L., especially from Morocco. However, there have been studies showing that this plant exhibits significant anti-inflammatory activity.

Dellai et al.[47] conducted an *in vivo* study to evaluate the anti-inflammatory activities of aqueous and organic extracts from *Pistacia lentiscus* L. leaves using the carrageenan-induced paw edema assay. The results showed a dose-dependent anti-inflammatory effect for aqueous, chloroformic, ethyl

acetate, and methanolic leaf extracts administered intraperitoneally at concentrations of 50, 100, and 200 mg/kg. Our research focused on the methanolic extract of the stems, an aspect not previously studied. Labhar et al. reported significant inhibitory effects (92.65 ± 0.67) for the ethanolic extract and (94 ± 0.29) for the aqueous extract at a concentration of 1000 mg/L, highlighting the diverse anti-inflammatory potentials of different extracts.[17]

Ostovan et al. investigated the anti-inflammatory effects of mastic, derived from the mastic tree of *Pistacia lentiscus*, in rats with colitis. The mastic oil, administered in enema form, significantly reduced TNF-α levels, demonstrating effects comparable to prednisolone and the control group. The total colitis index exhibited a significant decrease through oral administration, suggesting the potential use of mastic oil as a natural remedy for alleviating inflammation in ulcerative colitis.[16]

Sameh et al.[55] investigated the anti-inflammatory effect of *Pistacia lentiscus* fruit oil using the carrageenan-induced paw edema rat model. The fruit oil demonstrated substantial anti-inflammatory activity compared to Inflocine, with edema inhibition percentages of 70% and 51.5% ($p < .01$) after five hours.

All these studies provide a comprehensive perspective on the diverse anti-inflammatory properties exhibited by different extracts of *Pistacia lentiscus* L. The findings highlight the potential therapeutic applications of these natural compounds across a spectrum of inflammatory conditions.

Although our study focuses on the anti-mitotic and anti-inflammatory activity of compounds isolated by UHPLC, it is also essential to consider other pharmacological properties that may influence their overall therapeutic potential. For example, recent studies have employed virtual tools to assess the diabetes-related enzyme catalytic activity and antibacterial activity of plant-derived compounds, utilizing molecular docking analyses and Lipinski's rule of five to evaluate their drug-likenes.[56–58] These complementary approaches provide valuable insights into the multifunctional potential of these compounds, underscoring the importance of a comprehensive characterization in the development of new therapeutic agents.

Conclusion

In summary, this study examined the complex properties of methanolic extracts derived from the leaves (MLE) and stems (MSE) of *Pistacia lentiscus* L. from Morocco's eastern region. The phytochemical analysis, using Ultra-high-performance liquid chromatography (UHPLC), identified 16 and 17 components in MLE and MSE, respectively, with Epigallocatechin being the most abundant in both extracts. The study found that MLE had higher concentrations of polyphenols and flavonoids, and it exhibited potent free radical scavenging activity, like ascorbic acid. Meanwhile, MSE demonstrated superior ferric-reducing power. Both extracts inhibited sorghum seed germination in a dose-dependent manner, with MSE showing slightly higher efficacy. The investigation into antimitotic effects revealed that MSE had a concentration-dependent response, like colchicine, indicating its potential as an antimitotic agent. *In vitro* anti-inflammatory assays showed that MLE had similar properties to indomethacin in protein denaturation and membrane stabilization, while MSE exhibited unique characteristics. In a carrageenan-induced rat model, both extracts demonstrated anti-inflammatory potential, with MLE showing a trend of slightly better efficacy. The findings suggest that *Pistacia lentiscus* L. extracts have a multifaceted therapeutic profile due to their antioxidant richness, antimitotic potential, and anti-inflammatory effects. These results highlight the plant's value as a natural resource for medicinal applications and the need for further research, particularly focusing on the impact of *Pistacia lentiscus* L. compounds on human cancer cells, which could lead to novel avenues in cancer research and therapeutic development.

Acknowledgments

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Author's contributions

SS & FA: Conceptualization, writing the original draft; HF & GN: formal analysis, investigations, funding acquisition; RC & AAA: resources, project administration, reviewing and editing; OBM& FK: data validation, and data curation; MB & NG: supervision.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval

The procedures used to perform this study are in agreement with the international guidelines used for the use of laboratory animals. The Ethical Committee of Ethnopharmacology and Health, Faculty of Sciences, Mohammed First University, revised and approved this work.

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