Trop J Nat Prod Res, April 2024; 8(4):6891-6900 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Exploring Phytochemical Composition, Antioxidant, Antibacterial Properties, and *in Silico* Study of Aqueous Leaf Extract of *Pistacia lentiscus* L. from the Eastern Region of Morocco

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ARTICLE INFO

Article history:
Received 06 February 2024
Revised 17 April 2024
Accepted 19 April 2024
Published online 01 May 2024

ABSTRACT

Pistacia lentiscus L., commonly called lentisk, is a Mediterranean tree with numerous biological properties. This study aims to explore the phytochemical composition, antioxidant and antibacterial activities, as well as the molecular docking simulations of the aqueous leaf extract of *Pistacia lentiscus* (ALEPL). The phytochemical composition of ALEPL was determined using ultra-high performance liquid chromatography (UHPLC). The antioxidant activity was assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. Antibacterial activity was evaluated using the agar well diffusion method. Molecular docking simulations of the major compounds against target proteins were conducted using AutoDock software. In addition, the Swiss ADMET and pk CSM softwares were used to predict the pharmacokinetic and toxicity profile, and physicochemical properties of the identified compounds in ALEPL. UHPLC analysis identified 19 compounds in ALEPL with catechingallate and epigallocatechin gallateas the predominant compounds. ALEPL demonstrated strong antioxidant activity with IC_{50} of 64.06 ± 1.09 µg/mLand 88.76 ± 0.40 µg/mL for DPPH radical scavenging activity and FRAP, respectively. The extract demonstrated a significant antibacterial effect with MIC of 1.56mg/mL, and 3.125 mg/mL against *Bacillus cereus* and *Staphylococcus aureus*, respectively. However, *Escherichia coli* were resistant with MIC of 6.25 mg/mL. Molecular docking simulations revealed strong interactions between the compounds and specific amino acid residues of the target proteins. Some of the compounds had good pharmacokinetic profile and physicochemical properties that suggest potential usefulness as drug candidates. Therefore, ALEPL shows promise as source of new candidate molecule(s) for drug discovery.

***Keywords*:** *Pistacia lentiscus* L., Phytochemical composition, Antioxidant, Antibacterial, *In Silico*

Introduction

Pistacia lentiscus L. is a member of the Anacardiaceae family that grows throughout the Mediterranean region. The plant has a rich history of traditional use, primarily for medicinal purposes.[1]The plant is found in the eastern region of Morocco, where it is known for its medicinal properties and use in traditional medicine.[2,3]Numerous scientific studies have been conducted on the plant, demonstrating its potential as a source of bioactive compounds with medicinal properties.[4,5]

Phytochemical analysis of the plant have shown the presence of flavonoids, terpenoids, and phenolic acids.[6]the plant has been reported to have anti-inflammatory, analgesic, and antimicrobial properties.[7]Furthermore, recent research has shown that aqueous extracts of *Pistacia lentiscus* have anti-cancer, anti-diabetic, anti-inflammatory, antioxidant, and analgesic effects.[8–10]

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Citation: Seddoqi S, Aouinti F, El fadili M, Conte R, Elhachlafi N, Gseyra N. Phytochemical Composition, Antioxidant, Antibacterial Activities and *In Silico* Study of Aqueous Leaf Extract of *Pistacia lentiscus* L. from the Eastern Region of Morocco. Trop J Nat Prod Res. 2024; 8(4):6891-6900. https://doi.org/10.26538/tjnpr/v8i4.20

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Bacterial infections are a major public health issue that affects millions of people each year. Despite the development of numerous antibiotics, the emergence of antibiotic-resistant bacteria strains is increasing, making bacterial infection treatment more difficult.[11] As a result, alternative agents to combat these infections are required. Plant-based products, which have been used in traditional medicine for centuries, are a potential source of such agents.[12]Plant-based products are becoming more popular in modern medicine due to their potential therapeutic benefits and relative safety compared to synthetic drugs.[13]

Recent investigations have concentrated on discovering the active constituents in plants that have antibacterial properties. Among these compounds are essential oils, tannins, and flavonoids.[14–18]Plant-based products have shown promising results in the treatment of various bacterial infections, including multidrug-resistant bacteria.[19–22] Further investigation is required to establish the effectiveness and safety of these products in humans.

The main purpose of this study is to gain a deeper understanding of the pharmacological properties of the aqueous extract of *Pistacia lentiscus* L. leaves from the oriental region of Morocco. This will be achieved by exploring its chemical composition through ultra-high performance liquid chromatography (UHPLC) analysis and assessing its antioxidant activity through its ability to neutralize free radicals. In addition, antibacterial activity study aims to determine the extract's antimicrobial properties, paving the way for potential medicinal applications. Finally, the *in silico* study will enable virtual screening of the compounds present in the extract against selected protein targets, providing insight on the possible mechanism(s) of action. These investigations will

contribute to the potential use of the extract in medicine and pharmaceutical research.

Materials and Methods

Chemicals and Reagents
Folin-Ciocalteu (Sigma-Aldrich, Switzerland), ferrous chloride ($FeCl_2$), sodium carbonate and aluminium chloride (Sigma-Aldrich, Germany), gallic acid (Sigma-Aldrich, Dorset, UK), potassium ferricyanide (Sigma-Aldrich, USA), 1,1-diphenyl-2-picryl-hydrazyl radical (Sigma-Aldrich, China), Resazurin AR (Oxford Labe Pine ChemLLP India). The solvents used were methanol, chloroform, and sulfuric acid (Riedel-de-Haën, Poland). All chemicals used were of analytical grades.

Equipment
The equipment used comprised a Spectrophotometer (UV-VIS-7220G Ray LEIGH-China), UHPLC Chromatograph (Shimadzu Nexera XR LC 40, Italy), and a Microbiological Safety Cabinet (Telstars bio-ii-A/M; G.BOYER Casablanca).

Plant collection and identification
Pistacia lentiscus L. aerial parts were collected in March 2021, from fields in Jerrada (34°22'07.9" N2°03'15.8" W), Morocco. The plant material was identified by a taxonomist and the voucher number HUMPOM080 was provided.

Preparation of extract
The leaves of *Pistacia lentiscus* L.were dried in the shade at ambient temperature (25°C). The dried plant material was ground into a fine powder, and 100g of the powdered material was macerated in 1000 mL of distilled water at ambient temperature (25°C) for 48 h with occasional agitation. The extract was filtered and there after concentrated using a rotary evaporator at reduced pressure. The concentrated extract was kept at 4°C until futher use. The percentage yield of the extract was calculated using the following equation.[23]

$$\text{PercentageYield}(\%) = \left(\frac{\text{Mextract}}{\text{Msample}}\right) \times 100$$

Where;
M extract = Extract weight in grams.
M sample = Sample weight (powdered plant material) in grams.

Phytochemical screening of aqueous leaves extract
The phytochemical screening of the extract was carried out using previously reported protocols. The phytochemicals tested were alkaloids, flavonoids, tannins, steroids, phenols, coumarins, and anthocyanins (Table 1).

LC-MS/MS analysis
The extract (80 mg) was dissolved in 1mL of ethanol. The mixture was vortexed and incubated for 60 min in a sonicator at 45ºC. Shimadzu UHPLC (Nexera XR LC 40) equipped with an MS/MS detector was employed for qualitative analysis. The MS/MS was operated by Lab Solution software and used electrospray ionization, allowing for quick transitions between low-energy scans at 4V and high-energy scans between 10-60V throughout a single LC run. The nebulizing gas flow rate was set to 2.9 L/min, the heating gas flow rate to 10 L/min, the interface temperature to 300ºC, the DL temperature to 250ºC, the heat block temperature to 400ºC, and the drying gas flow rate to 10 L/min. Analysis was conducted using flow injection without chromatographic separation, employing acetonitrile: water, fortified with 0.01% formic acid, as the mobile phase. The instrument was configured in the positive mode for a selected-ion-monitoring (SIM) experiment. To determine if a sample was positive, the area under the curve was compared to a blank.[24,25]

Determination of total polyphenol content
The total polyphenol content of the aqueous leaf extract of *Pistacia lentiscus* L.was determined using the Folin-Ciocalteu method. Specifically, 0.2 mL of the extract was combined with 1 mL of Folin-Ciocalteu's reagent and 0.8 mL of a 2%Na_2CO_3. The mixture was incubated for 90 min, and the absorbance was measured at 750 nm using a spectrophotometer (UV-VIS-7220G Ray LEIGH-China). Gallic acid was used to prepare a standard calibration curve. The total polyphenol content was calculated from the equation of the calibration curve, was expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g extract).[26,27]

Table 1: Phytochemical screening protocols of *P. lentiscus L.* leaves extract

Test	Protocol	Reference
Alkaloids	Dragendorff test: 1 mL of each extract or fraction was mixed with 1 mL of Dragendorff reagent (potassium iodide 0.11 M, bismuthnitrate 0.6 M and acetic acid 3.5 M). The formation of a cloudy orange color indicates the presence of alkaloids.	[53]
Flavonoids	Alkaline reactivity test: the extract was treated with a 10% NaOH solution; the formation of a pronounced yellow colour indicates the presence of flavonoid.	[54,55]
Tannins	2 mL of the extract was mixed with 1% lead acetate. A yellowish precipitate signifies the presence of tannins.	[56]
Steroids	1 mL of the extract was dissolved in chloroform (10 mL) and, from the side of the test tube, was added an equal volume of concentrated sulfuric acid (H_2SO_4) 37%. The presence of red colour top layer, and a greenish H_2SO_4 layer indicates the presence of steroid.	[53]
Anthocyanins	1 mL plant extract and 1 mL 2N HCl were mixed, followed by addition of ammonia. Formation of a pink-red colourthat turns blue-violet after the addition of ammonia indicates the presence of anthocyanins.	[57]
Phenol	Ferric chloride test: 4 drops of alcoholic solution of $FeCl_3$ were used to treat the test extracts. The formation of a bluish-black colour suggests the presence of phenol.	[53]
Coumarin	3 mL of 10% NaOH was added to 2 mL of the aqueous extract. Coumarins are indicated by the development of a yellow colour.	[54,55]

Determination of total flavonoid content
The total flavonoid content of the aqueous leaf extract of *Pistacia lentiscus* L. was determined by a modified aluminum chloride calorimetric method.[28]A combination of the aqueous extract (0.5 mL), sodium nitrate (0.5 mL), and aluminum chloride (0.120 mL) was added to distilled water (0.8 mL) and the absorbance of the mixture was measured at 415 nmusing a spectrophotometer (UV-VIS-7220G RayLEIGH-China). Quercetin was used as a standard. Total flavonoid was quantified from the equation of the quercetin calibration curve, and was expressed as mg quercetin equivalent per gram of dry extract (mg QE/g extract). The tests were done in triplicate.

Determination of antioxidant activity
Two methods were employed to assess the antioxidant activity of the aqueous leaf extract of *Pistacia lentiscus* L.
1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavengingassay
Methanol solution (100 mL) containing 4mg of DPPH was prepared. To 0.5 mL of the extract at different concentrations was added 1 mL of the DPPH solution. The mixture was incubated at room temperature (25°C) for 30 min in the dark. The absorbance was measured at 517 nm using a spectrophotometer with methanol serving as the blank. Ascorbic acid was used as the standard followed the same procedure. The measurements were repeated three times for each extract concentration and standard.[27,29] The percentage radical scavenging activity was calculated using the formula below.

$$Radical\ Scavenging\ Activity\ (\%) = [(\text{A control} - \text{A sample}) / \text{A control}] \times 100$$

The half maximal inhibitory concentration (IC_{50}) was calculated automatically using statistical software.

Ferric Reducing Antioxidant Power (FRAP) assay
The FRAP assay was conducted according to the methods of Kandsi *et al*. (2021)[23]and Laftouhi *et al*. (2024).[30] Briefly, the extract at different concentration was mixed with 1.25 mL of phosphate buffer and 1.25mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, followed by the addition of 1.25 mL of 10% trichloroacetic acid to stop the reaction. All mixtures were centrifuged at 3000 rpm for 10 min. The supernatant obtained was combined with 1.25 mL of distilled water and 0.25 mL of 0.1% ferric chloride solution, and thereafter, the mixture was stirred gently. The absorbance of the solution was measured at 700 nm using a spectrophotometer (UV-VIS-7220G Ray LEIGH-China).Ascorbic acid was used as the standard. All experiments were done in triplicates.

Determination of antibacterial activity
Organisms
The test bacteria; *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633 were obtained from food, while *Candida albicans* was obtained as clinical isolate from the microbiology laboratory of the Mohammed VI University Hospital, Fez, Morocco. Bacteria were stocked on inclined Luria-Bertani (LB) agar medium at 4°C. Before the test, bacteria were revived by subculturing in a suitable culture medium (LB) at 35°C for 20–24 h. The final bacterial concentrations of 10^6 CFU/mL was used for the screening according to the National Committee for Clinical Laboratory guidelines.[31]
Determination of Minimum Inhibitory Concentration (MIC)
Minimum Inhibitory Concentration (MIC) of the aqueous leaf extract of *Pistacia lentiscus* L. was determined using a microdilution assay in a sterile 96-well microplate.[32] Bacterial agar was utilized as an emulsifier for testing extract that had previously been dissolved in DMSO at concentration of 0.15% w/v. First, all test wells received 50 µL of Muller Hinton broth, except the first well, which received only 100 µL of extract (25 mg/mL). Extract was serially diluted by transferring 50 µL from the first to the tenth well (concentrations ranging from 0.097 to 50 mg/mL). The 11thwell served as a growth control, while the 12th well served as a sterility control. Then, in each (from first to eleventh well), 50 µL of bacterial inoculum, pre-adjusted to 0.5 McFarland (10^6 CFU/mL) was added. After 20–24 h of incubation at 37°C, 15µL of a bacterial growth indicator (resazurin) was added to each well. MIC was calculated using the earlier method described by Zhang *et al*. (2008),[33]with slight modifications. Initially, the extract was serially diluted in broth (YPG) and mixed with 0.15% (w/v) agar, resulting in final concentrations ranging from 0.097 to 50 mg/mL. Subsequently, 50 µL of inoculum at a final concentration of 10^3 CFU/mL was dispensed into each well. Finally, the microplate was placed in an incubator at 30ºC for 48 h.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)
MBC and MFC were determined by inoculating 6 µL from individual negative wells onto LB plates and incubating at 37°C for 20-24 h for bacteria and on YPG plates at 30°C for 48 h for yeast. The experiments were conducted in triplicates.

In silico study
ADMET prediction
The prediction of pharmacokinetic properties of absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) was done with the help of Swiss ADMET and pkCSM servers.[34]The BOILED-Egg model was utilized to identify potential central nervous system (CNS) agents with the greatest likelihood of crossing the blood-brain barrier (BBB).[35,36] This model is based on lipophilicity, determined by the logarithm of the partition coefficient between n-octanol and water (Log P O/W), and polarity, assessed by the topological polar surface area (TPSA) of small molecules.[37,38]

Molecular docking simulation
The oxido-reductase protein, specifically 2CDU.pdb was extracted from the Protein Data Bank (PDB) using the X-ray diffraction method with a resolution of 1.80. The docking calculation was performed in AutoDock 4.2 software using the AUTOGRID algorithm to centralize the grid box in its three-dimensional structure with dimensions of 100, 120, and 110 and a spacing of 0.375. Ten genetic algorithms were run, totaling 25 million evals. The most powerful complex with the lowest binding energy was chosen. The 2D and 3D visualizations of the intermolecular interactions of the protein-ligand complex was done using Discovery Studio 2021.[39]

Results and Discussion

Extraction yield
The percentage yield of the aqueous leaf extract of *Pistacia lentiscus* L. was 31%. This finding is consistent with the results of the study by Barbouchi *et al*, who reported a percentage yield of 27.80% for aqueous extract of *Pistacia lentiscus* L. The results suggest that the water extraction method enables efficient recovery of the bioactive components present in the leaves.[40]

Phytochemical Analysis
The results of phytochemical screening of the aqueous extract of *Pistacia lentiscus* L. leaves revealed the presence of various bioactive compounds (Table 2).The extract contained considerable quantities of flavonoids and tannins, indicating that they may contribute to the antioxidant activity of the extract. In addition, the presence of phenols suggests that the extract may have antioxidant and antibacterial properties. Previous studies have identified the presence of phenolic acids, tannins and flavonoids in *P. lentiscus* L. leaves, findings that are consistent with the result obtained from the current study. Although no alkaloids were found in the extract, other chemicals such as steroids and coumarins were found in small quantities.[1,7]

Compounds identified from the LC-MS/MS analysis of Pistacia lentiscus L. aqueous leaf extract
Table 3 presents the result of the LC-MS/MS analysis of theaqueous extractof *Pistacia lentiscus* L leaves. A total of nineteen (19) compounds with varying levels of abundance were identified in the extract. Flavonoids, phenolic acids and procyanidins were among the compounds identified. Catechingallate and Epigallocatechin gallate

were the major compounds in *Pistacia lentiscus* L. leaves. Additionally, significant amounts of Epigallocatechin, Gallic acid, Quercetin-3-*O*-glucoside, Kaempferol-3-*O*-hexose deohyhexose, Isorhamnetin-7-*O*-Pentose, and Luteolin-7-*O*-glucoside were detected, while the remaining compounds were detected at lower amount. The chemical structures of some of the major compounds identified are shown in Figure 1.

Total polyphenol and flavonoid contents of the aqueous leaf extract of Pistacia lentiscus L.

The results show that the aqueous leaf extract of *P.lentiscus L.* contains a relatively high amount of polyphenols, with a total polyphenol content of 396.41 mg GAE/g of dry extract (Table 4).In addition, the extract contained a moderate amount of flavonoids, with a total flavonoid content of 56 mg QE/g of dry extract. These results are consistent with the results from previous studies on other plants, indicating that the concentration of polyphenols is generally higher than that of flavonoids.[7,8]

Antioxidant activity of aqueous leaf extract of Pistacia lentiscus L. (ALEPL)

The antioxidant activity of ALEPL is presented in Table 5. The DPPH radical-scavenging assay and the FRAP assay were used to assess the antioxidant activity of ALEPL. The results were expressed as IC_{50} values, which represent the concentration of the extract needed to scavenge 50% of the free radicals or reduce 50% of the ferric ions.

The IC_{50} value for the DPPH radical scavenging activity of ALEPL was 64.06 ± 1.09 µg/mL, indicating a high antioxidant activity. Similarly, the IC_{50} value for FRAP was found to be 88.76 ± 0.40 µg/mL, underscoring its robust antioxidant properties (Table 5).

Previous studies have reported varying IC_{50} values for the antioxidant activity of *P. lentiscus* leaf extract. One study reported a significantly higher IC_{50} value of 363.07 µg/mL for the DPPH radical scavenging activity,[41]while another study found a significantly lower IC_{50} value of 9.89±0.70 µg/mL and a ferric-reducing power of 54.06±12.66 µg/mL.[42]

Antimicrobial activity of ALEPL

MIC, MBC, and MFC of ALEPL were determined through the broth microdilution assay. As shown in Table 6, ALEPL exhibited significant antibacterial activity against both Gram (-) (*E. coli*) and Gram (+) bacteria (*B. cereus* and *S. aureus*). Gram (+) bacteria, including *B. cereus*, and *S. aureus*, appeared to be more sensitive to the extract, with MIC values of 1.560 and 3.125 mg/mL against *B. cereus*, and *S. aureus*, respectively. *E. coli* appeared to be resistant to the extract, with a MIC value of 6.25 mg/mL. These findings confirmed the common observation that some bioactive natural products tend to exhibit greater efficacy against Gram(+) bacteria than Gram(-) bacteria.[43–45]The MIC, MBC, and MFC results were generally efficient and comparable to the referenced antibacterial agent, chloramphenicol.

Our findings are consistent with the findings from previous studies that investigated the antimicrobial activity of *Pistacia lentiscus* L. aerial parts, such as leaves and stems, against a variety of bacteria. These results showed that the extracts from the aerial parts of *Pistacia lentiscus* L. exhibited antimicrobial properties by inhibiting the growth of various bacterial strains. Gram (+) bacteria were found to be more sensitive to the plant extracts than Gram (-) bacteria.[41,46,47]

Molecular docking results

The computational method of molecular docking is used to predict the binding modes of small molecules to a target protein or receptor. This method is extensively employed in the field of drug discovery and design to identify and optimize potential drug candidates' binding affinity to the target.[48,49]

The first complex C1 (Figure 2A)consists of a catechingallate ligand bound to the protein via Pi-Sigma ALA295 and Pi-Alkyl ILE297 bonds, as well as conventional hydrogen bonds with THR291, TYR188, and PHE245. The epigallocatechin ligand forms five conventional hydrogen bonds with CSX42, ASP 282, SER115, THR 113, and GLU 32 in the second complex C2 (Figure 2B).In the third complex C3, gallic acid

Firstly, the major compound formed a hydrogen bond with the Leu83 amino acid residue in the A-chain of the 6GUE.pdb protein and Pi-ligand forms a Pi-Alkyl bond with VAL6 and three conventional hydrogen bonds with GLU32, HIS79, and VAL81 (Figure 2C). The ligand Luteolin-7-*O*-glucoside forms a Pi-alkyl bond with the Met33 residue, resulting in a stacking-type contact between the methionine residue and the ligand C4 (Figure 2D). A Pi anion bond also exists with the GLU32 residue, involving a stacking-type contact between the glutamate residue and the ligand. Furthermore, conventional hydrogen bonds were observed between the ASN135, LYS116, THR 9, CYS8, and THR112 residues and the ligand.

Finally, in the complex C5 (Figure 2E), Quercetin-3-*O*-glucoside forms a Pi-anion connection with CSX 42, a Pi-Pi-stacked association with SER328, and a Pi-alkyl bond with LEU299. It also forms four standard hydrogen bonds with GLN400, ASN403, SER399, and ARG305.

In the present study, the primary constituents of *Pistacia lentiscus* L, gallic acid with a percentage peak area of 61.23%, and with a good ADME profile without any toxic effect, was selected for the molecular docking simulation with the target protein to explore the intermolecular interactions that could be produced. The molecular docking results shown in Figure 2 revealed that:

Table 2: Phytochemical constituents of the aqueous leaf extract of *P. Lentiscus L*

Phytochemical	Inference
Flavonoids	+
Tannins	+
Anthocyanins	+
Steroids	+
Coumarin	+
Phenol	+
Alkaloids	-

+: present; -: absent

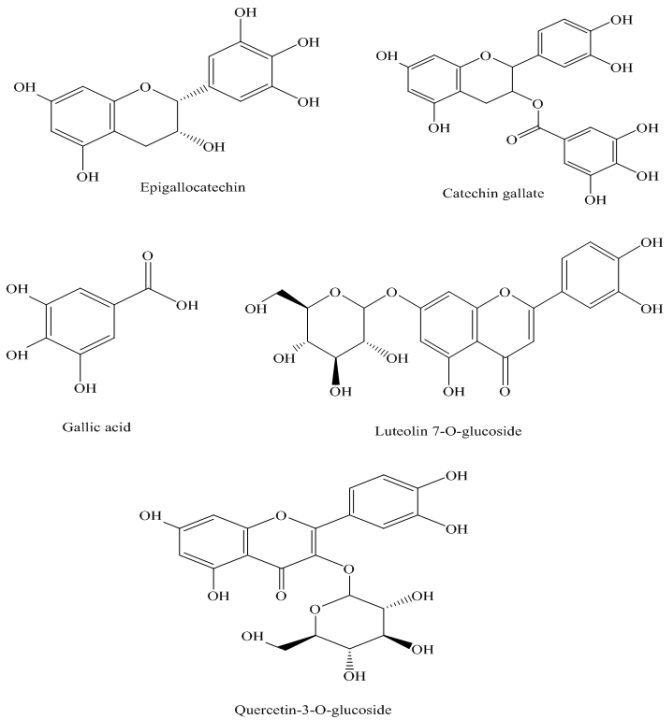

Figure 1: Chemical structures of the major compounds found in the aqueous leaf extract of *Pistacia lentiscus* L.

Sigma bond with the Leu134 and Phe80 amino acid residues, then produced various alkyl and Pi-alkyl interactions with Ala31, Val18, Val64, and Ile10 amino acid residues.
Secondly, the Quercetin-3-*O*-glucosidebind with 2CDU.pdb protein, through three H-bond with Ser115, Lys134, and Asp282 amino acid residues and different alkyl and Pi-alkyl interaction with Ala11 and Ala333 amino acid residues.
Thirdly, this compound interacted with 1OG5.pdb protein, forming 2H-bonds with Arg124 and Arg433 amino acid residues, one Pi-Pi-stacked bond with Ile434 amino acid residue, one Pi-Alkyl connection with Cys435 amino acid residue, two alkyl bonds with Val436, and Ile 112 amino acid residues.
Finally, the Quercetin-3-*O*-glucoside-4JK4.pdb protein complex reveals 1H-bond interaction with Tyr149 amino acid residue, 1 Pi-anion interaction with Arg256 amino acid residue, 2 Pi-alkyl bonds with Leu237, and Ala290 amino acid residues and 4 alkyl bonds with Ala290, Ala260, Leu259, and Il289 amino acid residues.

Docking validation result
The molecular docking procedure was successfully validated, and all five molecules were docked into the active sites of the target protein (Val81, Met33, Glu32, Thr9, Ser115, Lys134, Ala11, Asp282, Ala300, and Phe245 amino acid residues), which were obtained experimentally using the X-ray diffraction method (Figure 3). In addition, the result of the superimposition of docked and co-crystallized ligands with the target protein (2CDU.pdb) was good, as the root mean square deviation (RMSD) was less than 2 Å.[37,38] The RMSD was 1.756 Å.

ADME and toxicity prediction result
The result of the prediction based on the BOILED-Egg model, which is extremely useful in the fields of drug discovery and medicinal chemistry,[50]demonstrated that the chemical compound marked as C5 was the only compound that was part of the yellow Egan egg, and the compounds 2, 4, 6, 7, and 12 were part of the white Egan egg, while the compounds 1, 3, 8, 9, 10, 11, 13, 14, 15, and 16 were out of the Egan egg (Figure 4). Therefore, the compound C5 is expected to cross the blood-brain barrier (BBB) passively, and the compounds 2, 4, 6, 7, and 12 were predicted to be passively absorbed by the gastrointestinal tract. Compounds 8 and 9 marked in blue were predicted to be excreted from the central nervous system via the P-glycoprotein.
Additionally, the prediction of physicochemical properties for sixteen of the compounds demonstrated that half of the compounds tested (1, 2, 4, 5, 6, 7, 10, and 12) followed the Lipinski rule of five (MW ≤ 500 g/mol, 40 ≤ MR ≤ 130, HBD < 5, Log P (Octanol/Water) < 5, HBA ≤ 10), while the remaining compounds (3, 8, 9, 11, 13, 14, 15, and 16) did not obey the Lipinski rule of five (Table 7).

Table 3: Compounds identified from LC-MS/MS analysis of *Pistacia lentiscus* L. aqueous leaves extract

N°	Compound	Molecular formula	$[M-H]^+$	RT (min)
1	Catechingallate	$C_{22}H_{18}O_{10}$	441.00	0.349
2	Epigallocatechin	$C_{15}H_{14}O_7$	305.00	0.332
3	Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	457.00	0.329
4	Gallic acid	$C_7H_6O_5$	168.90	0.330
5	Trans ferulic acid	$C_{10}H_{10}O_4$	193.00	0.330
6	Hesperetin	$C_{16}H_{14}O_6$	301.30	0.332
7	Kaempeferol	$C_{15}H_{10}O_6$	285.00	0.33
8	Kaempferol-3-*O*-pentose	$C_{20}H_{18}O_{10}$	417.1000	0.330
9	Kaempferol-3-*O*-glucose		609.1000	0.330
10	Kaempferol-3-*O*-hexose deohyhexose		593.1000	0.371
11	Isorhamnetin-7-*O*- Pentose		447.1000	0.328
12	Luteolin 7-*O*-glucoside	$C_{21}H_{20}O_{11}$	447.10	0.328
13	Myricetin	$C_{15}H_{10}O_8$	317.00	0.330
14	Procyanidin	$C_{30}H_{26}O_{13}$	577.00	0.353
15	Quercetin	$C_{15}H_{10}O_7$	301.00	0.331
16	Quercetin-3-*O*-glucoside	$C_{21}H_{20}O_{12}$	463.10	0.339
17	Quercetin-3-*O*.hexose deoxyhexose		609.1000	0.328
18	Rutin	$C_{27}H_{30}O_{16}$	609.00	0.328
19	Naringin	$C_{27}H_{32}O_{14}$	579.0000	0.329

Table 4: Polyphenol and flavonoid contents of aqueous leaf extract of *Pistacia lentiscus L;*

	Polyphenols (mgGAE/g extract)	Flavonoids (mgQE/g extract)
Aqueous leaf extract	396.41 ± 11.90	56.00 ± 0.30

All data are expressed as the mean ± SEM (n = 3).

Table 5: Antioxidant activity of aqueous leaf extract of *Pistacia lentiscus* L.

	ALEPL	Ascorbic acid
DPPH RSA(IC_{50} in µg/mL)	64.06 ± 1.09	33.70 ± 0.05
FRAP (IC_{50} in µg/mL)	88.76 ± 0.40	137.76 ± 0.05

All data are expressed as the mean ± SEM (n = 3). ALEPL = Aqueous leaf extract of *Pistacia lentiscus* L.

Additionally, the ADME-Toxicity profile analysis predicted that compounds 1, 2, 4, 5, 6, 7, 10, and 12 are well absorbed, with human intestinal absorption (HIA) exceeding 50%. In terms of metabolism, compounds C3 and C12 exhibited inhibitory effects on cytochromes 1A2 and 3A4, respectively, while compounds C1, C7, and C10 showed inhibitory effects on cytochrome 1A2. The AMES toxicity test suggests that six of the compounds (C4, C5, C6, C7, C9, and C11) do not present any toxic effect to humans, while ten of the compounds (C1, C2, C3, C8, C10, C12, C13, C14, C15, and C16) showed potential mutagenic effect, but without any hepatotoxic or skin sensitization effects(Table 8).

In conclusion, the BOILED-Egg model was shown to be a practical and accurate tool for drug discovery. The model predicted that C5 is the only compound in the yellow Egan egg and that compounds 2, 4, 6, 7, and 12 are part of the white Egan egg, while compounds 1, 3, 8, 9, 10, 11, 13, 14, 15, and 16 are outside of the Egan egg. Compound C5 was predicted to passively permeate through the blood-brain barrier, and compounds 2, 4, 6, 7, and 12 were predicted to be passively absorbed by the gastrointestinal tract. The physicochemical properties of all compounds obeyed the Lipinski's rules except for compounds 3, 8, 9, 11, 13, 14, 15, and 16. ADME-Toxicity profile analysis predicted that most of the chemical compounds of *Pistacia lentiscus* L.are well absorbed, and the AMES toxicity test showed that the compounds do not present any toxicity to the humans except for some of them which showed potential mutagenic effect. Molecular docking simulations were performed on the major compounds of *Pistacia lentiscus* L, and the results showed various intermolecular interactions with the target proteins. Overall, these findings provide valuable insights into the properties and potential applications of the compounds in this plant for drug discovery.

Over the last few decades, scientific research has shown a strong interest in plant extracts due to their rich composition of bioactive compounds, particularly polyphenols.[51]Polyphenols, such as flavonoids, are secondary metabolites present in various plant species, and their potential as natural antioxidants has attracted considerable interest.[13]Developments in analytical techniques, notably Ultra-High-Performance Liquid Chromatography (UHPLC), have enabled in-depth characterization of the polyphenol content of plant extracts. This analytical precision has opened the way to a finer understanding of the chemical profiles of these extracts, highlighting the diversity of polyphenols present.

In the present study, we have focused on understanding the composition and functional characteristics of polyphenolic compounds in the aqueous extract of *Pistacia lentiscus* L. leaves from the eastern region of Morocco. The results of UHPLC analysis revealed a rich content of flavonoids, such as catechingallate, epigallocatechin gallate, epigallocatechin, gallic acid, quercetin-3-*O*-glucoside, kaempferol-3-*O*-hexose deohyhexose, isorhamnetin-7-*O*-Pentose, and luteolin-7-*O*-glucoside. These compounds, identified as the most abundant, demonstrate a significant diversity of polyphenols in *Pistacia lentscus* L. aqueous leaf extract. The presence of these polyphenols, particularly flavonoids, strongly suggests their potential contribution to the antioxidant activity of the extract.[52]Indeed, flavonoids are widely recognized for their antioxidant properties, acting as agents capable of neutralizing free radicals and preventing oxidative stress. DPPH and FRAP assays confirmed this hypothesis, revealing the extract's high antioxidant activity. This correlation between high flavonoid content and antioxidant activity reinforces the idea that these compounds contribute significantly to the antioxidant properties of *Pistacia lentscus* L. aqueous leaf extract. In addition, broth microdilution tests indicate significant antibacterial efficacy of the extract against Gram-negative *(E. coli)* and Gram-positive (*B. cereus and S. aureus*) bacteria. The correlation between polyphenol and flavonoid contents and antibacterial activity suggests that these compounds may play a role in the ability of the extract to inhibit bacterial growth.

Conclusion

The present investigation has identified19 compounds in the aqueous leaf extract of *Pistacia lentiscus* L. with the major compounds being catechingallate and epigallocatechin gallate. The aqueous extract also had significant quantities of polyphenols and moderate levels of flavonoids, as well as strong antioxidant activity and promising antibacterial effects against both Gram-positive and Gram-negative bacteria. These results offer encouragement for further research and a wider exploration of the potential applications of this extract in a variety of fields, including medicine, cosmetics, and the food industry. The promising properties highlighted suggest that *Pistacia lentiscus* L. leaf extract could play a significant role in the development of natural solutions to different health challenges.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

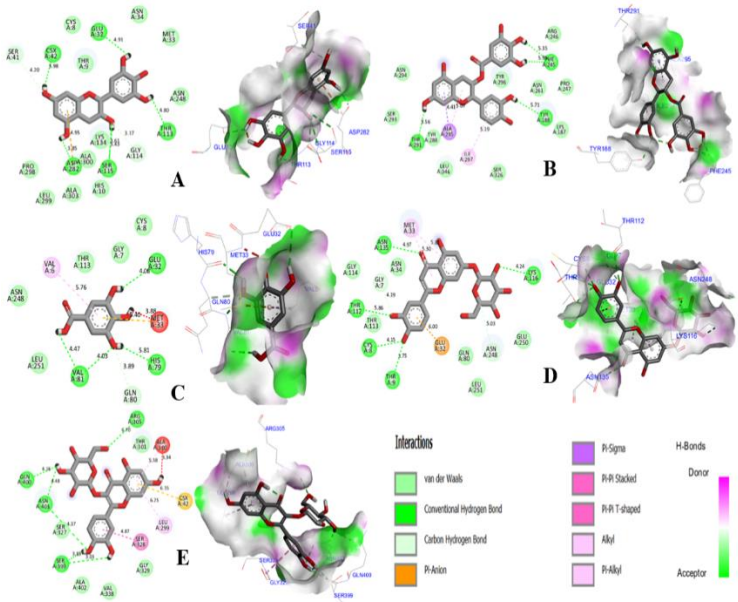

Figure 2: 2D and 3D visual representations illustrating the intermolecular interactions formed between C1, C2, C3, C4, and C5 ligands and the target protein, with binding energies of -4.39 kcal/mol, -5.49 kcal/mol, -3.51 kcal/mol, -5.74 kcal/mol and -5.32 kcal/mol, respectively.

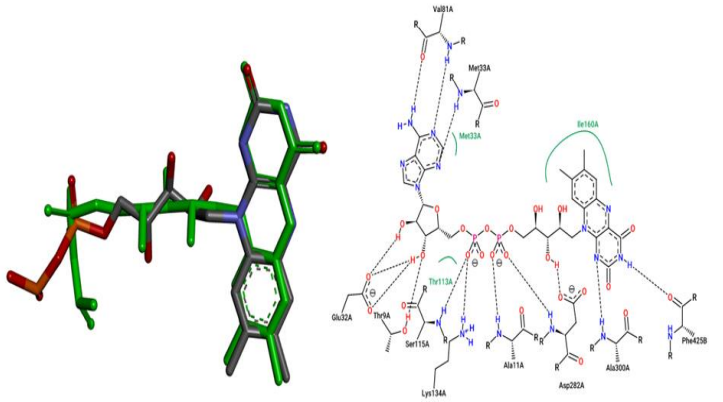

Figure 3: The superposition results of docked and co-crystallized ligands of the target protein with RMSD value of -1.756 Å, and the active sites of co-crystallized ligand complexed with 2CDU protein.

Table 6: Antimicrobial activity of aqueous leafextract of *Pistacialentiscus L.*

	ALEPL		Chloramphenicol
Organism	**MIC(mg/mL)**	**MBC/MFC(mg/mL)**	MIC(**µg/mL**)
E.coli	6.25	6.25	50
B. cereus	1.56	1.56	25
S. aureus	3.125	3.25	50
C.albicans	6.25	12.5	-

MIC =Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration; MFC = Minimum Fungicidal Concentration; ALEPL = Aqueous leaf extract of *Pistacia lentiscus* L.; - = Analysis not performed.

Table 7: Predicted physicochemical properties of the ligands using Lipinski, Veber, Egan, Muegge, and Ghose rules

	Physicochemicalproperties						**Lipinskirule**
Compound number	**Molecularweight (g/mol)**	**Molarrefractive index**	**Rotatable bondsNumber**	**Log P (Octanol/Water)**	**Hydrogen bond acceptors**	**Hydrogenbonddonors**	**Yes/No**
Threshold	**≤500**	**40≤ MR ≤130**	**<10**	**<5**	**≤10**	**<5**	**Yes/No**
C1	442.37	110.04	4	1.44	10	7	Yes
C2	306.27	76.36	1	0.98	7	6	Yes
C3	458.37	112.06	4	1.53	11	8	No
C4	170.12	39.47	1	0.21	5	4	Yes
C5	194.18	51.63	3	1.62	4	2	Yes
C6	302.28	77.83	1	2.51	6	3	Yes
C7	286.24	76.01	1	1.70	6	4	Yes
C8	478.40	114.63	5	1.78	12	7	No
C9	448.38	108.13	4	1.76	11	7	No
C10	318.24	80.06	1	1.08	8	6	Yes
C11	594.52	147.52	4	2.17	13	10	No
C12	302.24	78.03	1	1.63	7	5	Yes
C13	464.38	110.16	4	0.94	12	8	No
C14	610.52	141.38	6	1.95	16	10	No
C15	610.52	141.38	6	0.46	16	10	No
C16	580.53	134.91	6	1.96	14	8	No

Table 8: Predicted ADMET (Pharmacokinetic) characteristics of the ligands

N°	**Absorption**	**Distribution**		**Metabolism**							**Excretion**	**Toxicity**		
	Intestinal Absorption (Human) Numeric (% Absorbed)	BBB permeability Numeric (Log BB)	CNC Permeability Numeric (LogPS)	substrate		Inhibitor					Total Clearance Numeric (Log ml/min/kg)	AMES toxicity	Hepatotoxicity	Skin Sensitization
				Cytochromes										
				2D6	3A4	1A2	2C19	2C9	2D6	3A4				
				Categorical (Yes/No)								Categorical (Yes/No)		
C1	60.641	-1.738	-3.993	No	No	Yes	No	No	No	No	0.016	Yes	No	No
C2	65.43	-1.385	-3.721	No	No	No	No	No	No	No	0.474	Yes	No	No
C3	49.085	-2.016	-4.296	No	No	Yes	No	No	No	Yes	0.519	Yes	No	No
C4	40.154	-1.111	-4.157	No	No	No	No	No	No	No	0.625	No	No	No
C5	95.598	-0.25	-2.569	No	No	No	No	No	No	No	0.641	No	No	No
C6	78.513	-0.952	-3.356	No	No	No	No	No	No	No	0.473	No	No	No
C7	75.342	-1.234	-2.368	No	No	Yes	No	No	No	No	0.592	No	No	No
C8	38.15	-1.834	-4.667	No	No	No	No	No	No	No	0.655	Yes	No	No

C9	46.007	-1.611	-4.644	No	No	No	No	No	No	No	0.64	No	No	No
C10	65.877	-1.694	-3.729	No	No	Yes	No	No	No	No	0.556	Yes	No	No
C11	56.303	-2.276	-4.588	No	No	No	No	No	No	No	0.263	No	No	No
C12	74.994	-1.355	-3.432	No	No	Yes	No	No	No	Yes	0.578	Yes	No	No
C13	40.416	-1.858	-4.885	No	No	No	No	No	No	No	1.142	Yes	No	No
C14	28.301	-2.096	-5.751	No	No	No	No	No	No	No	-0.269	Yes	No	No
C15	28.202	-2.076	-5.742	No	No	No	No	No	No	No	-0.28	Yes	No	No
C16	22.117	-1.791	-5.058	No	No	No	No	No	No	No	0.496	Yes	No	No

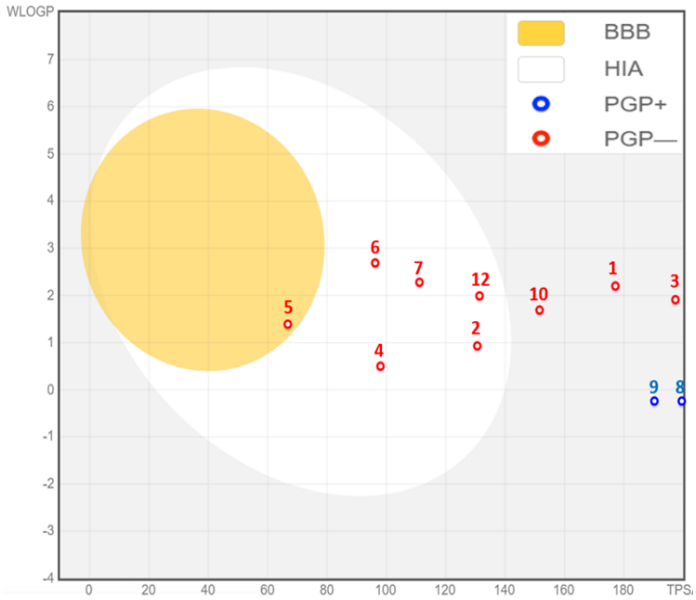

Figure 4: Predicted physicochemical properties of the ligands using Lipinski, Veber, Egan, Muegge, and Ghose rules.

Acknowledgments

Mostafa ELACHOURI and AbdelbassetBERRICHI are acknowledged for the identification of the plant.

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