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Phytochemical study and evaluation of the antioxidant and anti-inflammatory activity of the phenolic extract of *Pistacia lentiscus L*

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Abstract

In our days, the use of medicinal plants in phytotherapy has received great interest in biomedical research and is becoming as important as chemotherapy. This study is part of a contribution to the valorization of the waste from the fruit of this medicinal plant and to discover some constituents of this plant that possess biological activities.

The phytochemical study is carried out by spectrophotometric determination of phenolic compounds . However, the evaluation of antioxidant activity is estimated by the DPPH test. We also evaluated the anti-inflammatory activity using the protein denaturation inhibition method.

The results of the phytochemical assay show that this plant is very rich in total polyphenols, with a value of 120.86 ug GAE/mg of extract. However, the flavonoid content is 71.25 ug CE/mg of extract.

The expression of the results of antioxidant activity showed that the methanolic extracts of *Pistacia lentiscus L* were more active as an antioxidant agent with an IC50 of 0.20mg/ml for the DPPH test. While the anti-inflammatory activity was evaluated by the bovine serum albumin (BSA) denaturation method and it showed that the methanolic extract of Pistacia lentiscus L can act as an anti-inflammatory and seems to be a good anti-inflammatory (IC50 = 0.16 mg/ml).

The phenolic extracts of Pistacia lentiscus L are considered antioxidant and anti-inflammatory agents and can be used for therapeutic applications, which contribute very effectively to the

prevention of diseases such as inflammations, cancer, and cardiovascular diseases.
Keywords: *Pistacia lentiscus L*, phenolic extract, DPPH test, antioxidant activity, anti-inflammatory activity

1.Introduction

The therapeutic properties of aromatic and medicinal plants have been known since antiquity. However, it was not until the beginning of the 20th century that scientists began to take an interest in them **(Yano et *al*., 2006)**. Recently, attention has been focused on herbs and spices as a source of antioxidants, which can be used to protect against the effects of oxidative stress **(Mata et *al*., 2007).**

Pistacia lentiscus (the mastic tree) is known for its long cultivation in traditional medicine since the ancient Greeks. It is very common in the Mediterranean basin, it can be found in the wild, in scrubland and garrigue on all types of soil. although it prefers siliceous soils **(More and White, 2005).**

Pistacia lentiscus is a shrub belonging to the Anacardiaceae family and is one of the plants rich in phenolic compounds. **(Brahmi et *al.*, 2020)** this plant is widely used by the local population in traditional medicine for various purposes such as tonic, aphrodisiac, antiseptic, antihypertensive, gastrointestinal, hepatic, and urinary. **(Boutemine et al., 2018; Pachi et *al*., 2020),** and possess several pharmacological activities, notably hypoglycemic, antioxidant, anti-inflammatory, and anticancer **(Bouyahya et *al.*, 2019).**

Our work is part of a contribution to the enhancement of this medicinal plant and to discover some of its constituents that possess biological activities. For this, our study encompasses two aspects, the first of which is phytochemical, primarily based on the extraction and quantification of phenolic compounds. The second aspect is dedicated to an in vitro evaluation of the antioxidant and anti-inflammatory activity of the methanolic extract of *Pistacia lentiscus L*.

2.Materials and Methods

2.1.Plant material

The leaves of *Pistacia lentiscus L* are harvested during the month of October 2024, in the Mascara region. these leaves are then dried in the open air and away from light, then ground using a mortar until a fine powder necessary for the preparation of the phenolic extract is obtained.

2.2.Preparation of the methanolic extract

20g of *Pistacia lentiscus L* plant powder are macerated for 24 hours at room temperature in a methanol-water mixture (7:3 V/V), after which the mixture is filtered thru Whatman paper, and the

extraction is repeated several times with solvent renewal. The solvent is removed from the filtrate by rotary evaporation in a rotavapor (BÜCHI).The extract is dried and stored until further use.

2.3.Phytochemical analyzes of the extract

2.3.1.Dosage of total phenolic compounds

The concentration of total polyphenols in the Pistacia lentiscus L extract was measured by UV spectrophotometry based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was the Folin-Ciocalteu reagent. **(Singleton et *al*., 1999)**.
The results obtained from the assay are expressed in micrograms of gallic acid equivalent per milligram of extract (µg GAE/mg extract) using the linear regression equation of the calibration curve plotted with gallic acid.

2.3.2.Dosage of flavonoids

The estimation of the flavonoid content in the phenolic extract of Pistacia lentiscus L is determined by spectrophotometry, using a method based on the formation of a very stable complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of the flavonoids **(Bahorun et al., 1996).**

The total flavonoid content of the extract from this medicinal plant is expressed in micrograms of catechin equivalents per milligram of extract (µg EC/mg of extract).

2.3.3.Dosage of flavones and flavonols

The method used for the estimation of flavonol levels is that described by **(Kostova, 2005).** The concentration of flavonoids in the Pistacia lentiscus L extract is calculated from the calibration curve established with quercetin (2-14 µg/ml) and expressed in micrograms of quercetin equivalents per milligram of extract (µg EQ/mg of extract).

2.3.4.Dosage of condensed tannins

The measurement of condensed tannins is carried out using the vanillin-HCl method. Indeed, vanillin reacts with free flavan 3-ols and the terminal units of proanthocyanidins, resulting in a red coloration whose intensity is proportional to the levels of flavanols present in the medium and whose absorbance is measured by spectrophotometry **(Julkunen-Titto, 1985).**

The results obtained are expressed in micrograms of catechin equivalent (µg EC/mg of extract) using the linear regression equation of the calibration curve plotted with catechin.

2.4.Evaluation of antioxidant activity by the DPPH Test

The scavenging effect of the methanolic extract of *Pistacia lentiscus L* against the DPPH radical is evaluated according to the method described by **Que et *al*., 2006.** A volume of 2 ml of the DPPH

solution (0.1mM) is mixed with 2 ml of the extract solutions or standard antioxidants (ascorbic acid) at different concentrations. After 30 minutes of incubation in the dark and at room temperature, the absorbance is read at 517 nm.

To the naked eye, the presence of antioxidant activity against DPPH was evidenced by the change in color of the latter from the initial purple to yellow. But, the exact value of this activity was calculated according to the following equation:

Antiradical activity (%) = [(absorbance of the control - Absorbance of the test)/ absorbance of the control] x100

The effective concentration (EC50) defined as the concentration of the sample that produces 50% of the DPPH radical scavenging effect is determined.

2.5.Evaluation of in-vitro anti-inflammatory activity

We applied the BSA denaturation inhibition test caused by heat (72°C). according to **(Karthik et *al*., 2013)** 1 ml of each concentration of extract or standard was added to 1 ml of 0.2% BSA solution prepared in Tris HCL pH: 6.6 The mixture is then incubated at 37°C for 15 minutes. Then in a water bath at 72 °C for 5 minutes. At the end of the incubation, the mixture is quickly cooled, then its absorbance is measured at 660 nm in a cuvette spectrophotometer. In this test, Diclofenac sodium was used as the reference anti-inflammatory. Each experiment was conducted in triplicate, and the percentage of denaturation inhibition was determined according to the following formula: Inhibition of denaturation (%) = [A control - A sample / A control] × 100

A control: Absorbance of the control

A sample: Absorbance in the presence of the extract or the standard.

2.6. Molecular Docking Simulations

This research utilized molecular docking simulations as a computational approach to comprehensively evaluate how the selected phytochemicals interact with, and bind to, two critical anti-inflammatory therapeutic targets: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). These enzymes were specifically chosen for their established roles in the inflammatory pathway.

Ligand preparation involved converting all compounds, including the native co-crystallized ligands, from SMILES to PDBQT format using OpenBabel **(O'Boyle and *al*.,2011)**. Energy minimization followed, employing the steepest descent algorithm under the MMFF94s force field. The docking

simulations were then executed using the Uni-Dock code **(Yu and *al*.,2023)**, set to the recommended "Fast" search mode, with binding affinities determined by the Vina scoring function.

The target proteins, COX-1 (PDB ID: 1CQE) and COX-2 (PDB ID: 1CX2), were retrieved from the Protein Data Bank (PDB) (**Gasparini. and Feldmann ,2012** ; **Krakauer , 2004)** for the anti-inflammatory assessment. Protein preparation was performed using AutoDockTools 1.5.2 **(Morris and *al*.,2008)**, which included removing water molecules and co-crystallized ligands, adding polar hydrogens, and assigning Kollman charges. Any missing atoms or residues were corrected with Swiss-PdbViewer **(Guex and Peitsch,1997).** The resulting molecular docking output was visualized using Discovery Studio Visualizer **(Biovia, 2019).** The specific docking grid boxes were defined as follows: for 1CQE, the Center was (x = 28.32, y = 39.12, z = 200.7) and Size was (x = 25, y = 25, z = 25 Å); for 1CX2, the Center was (x = 25.98, y = 28.27, z = 10.94) and Size was (x = 25.0, y = 32.10, z = 36.27 Å).

2.7.Statistical study

All tests were performed in duplicate or triplicate. Results are presented as mean± standard deviation of two or three independent determinations. All statistical analyses were carried out by Graphpad prism 5 using analysis of variance (ANOVA) and differences among the means were determined for significance at $p \leq 0.05$ using least significant.

3.Results and Discussion

3.1.Extraction yields

The results reveal that the methanolic extract from *Pistacia lentiscus L* shows a high yield estimated at 32.7%. These results are consistent with those obtained by **Shiban et *al*., (2012)**, who reported an extraction yield of around 45%. Other studies indicate that methanol extraction yields better results, ranging from 31.5 to 48.2% **(Li et *al*., 2006; Zaki et *al*., 2015).** The observed variations in yields depend on the type of solvent; indeed, it affects the extraction, and it is quite evident that there is an affinity between the extraction solvent and the extracted compounds, as well as its biological **activity (Lee et *al*., 2003; Ghasemzadeh et *al*., 2011)**. Also, it is widely accepted that variations in extraction yields could be attributed not only to the difference in polarity of the solvent used, which plays a key role in increasing the solubility of phenolic compounds, but also to the polarity of the phenolic compounds that make up the extract **(Felhi et *al*., 2017)**. Therefore, this result confirmed the effect of the solvent on the extraction yield. this confirms that Pistacia lentiscus L is rich in polar substances, and that methanol is the best and most commonly used solvent to obtain a high yield of polyphenols **(Escribano Bailôn and Santos-Buelga, 2003)**. The improvement in the extraction yield of phenolic

compounds thru the use of methanol can be explained by the simple fact that this alcohol is more polar than ethanol. That is why it is known for its greater efficiency in extracting maximum amounts of components of interest **(Bourgou et *al.*, 2016)**. Methanol is capable of increasing the permeability of cell walls and facilitating the extraction of a large number of polar compounds as well as compounds of medium and low polarity **(Seidel, 2005).**

3.2. Phytochemical dosages

Total phenolic compound contents, flavonoids, flavones, and condensed tannins

The quantitative analysis of the total phenolic compound, flavonoid, flavone, and condensed tannin contents of the methanolic extract of Pistacia lentiscus L is determined from the linear regression equations of each calibration curve, successively expressed in micrograms of gallic acid equivalent, micrograms of quercetin equivalent, and micrograms of catechin equivalent per milligram of extract. (Figure 1,2,3 and 4).

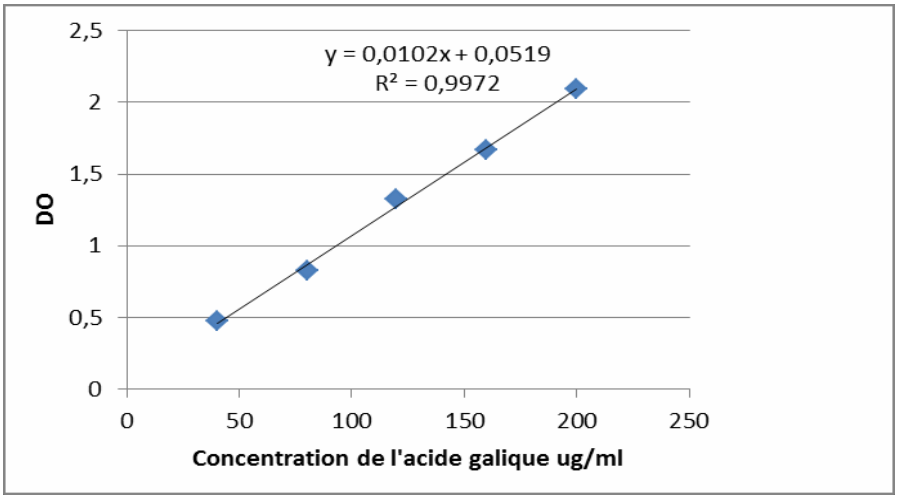

Figure 01. Calibration curve of gallic acid for the determination of total polyphenols

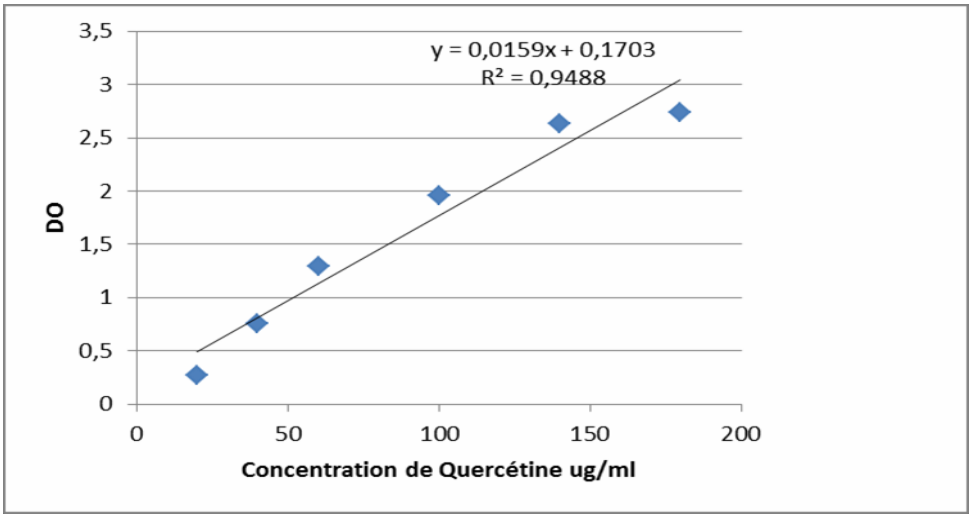

Figure 02. Calibration curve of quercetin for the determination of flavonoids

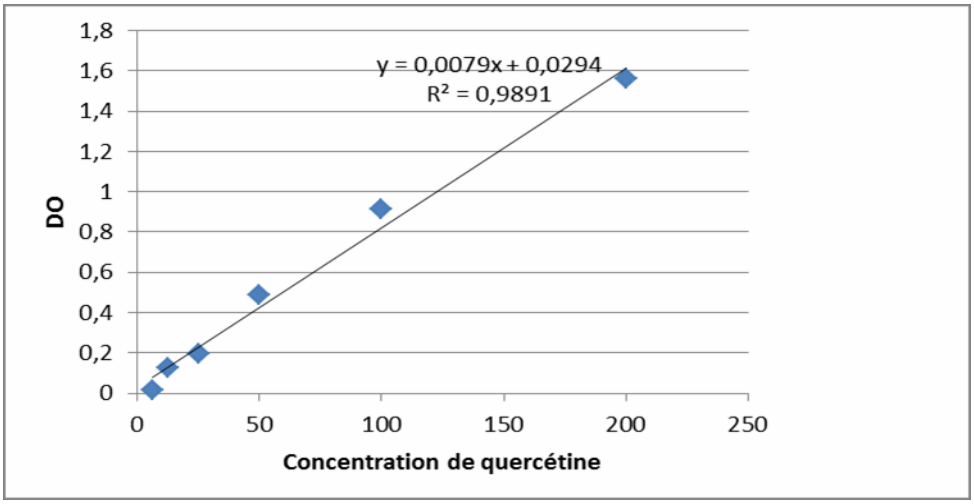

Figure 03. Calibration curve of quercetin for the determination of flavones

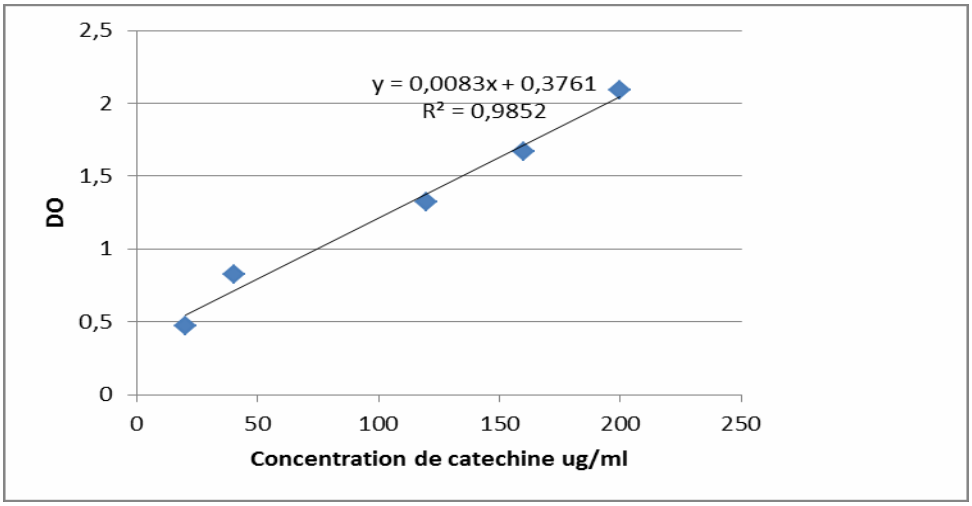

Figure 04. Calibration curve of catechin for the determination of condensed tannins

The total phenolic content, flavonoids, flavones, and condensed tannins of the methanolic extract of Pistacia lentiscus L is represented in the following figure:

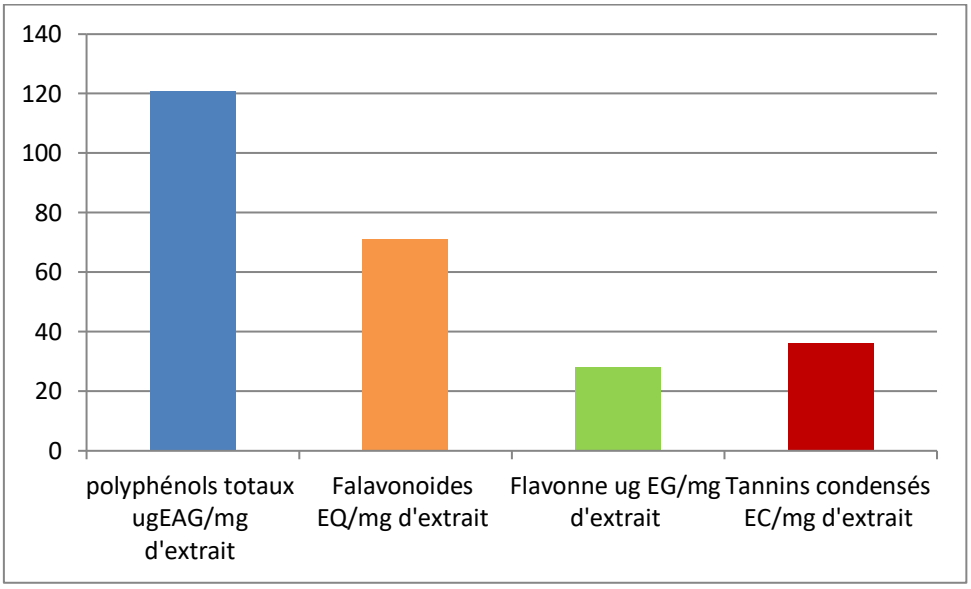

Figure 05: the content of total polyphenols, flavonoids, flavones, and condensed tannins in the methanolic extract of *Pistacia lentiscus L*.

The results obtained show that the methanolic extract of Pistacia lentiscus L is rich in polyphenols, with a total polyphenol value of 120.86 ug GAE/mg, while the flavonoid content is 71.25 ug CE/mg, whereas the values for flavones and condensed tannins are 27.31 ug QE/mg and 38.71 ug CE/mg respectively.

According to our results, it appears that the leaves of Pistacia lentiscus L are richer in polyphenols. According to the results of **Zitouni et *al*., (2016)**, the total polyphenol content of methanolic extracts from *Pistacia lentiscus* leaves was 216 ±20.6 mg GAE/g DW. While the total flavonoids were 19 ± 0.4 mg CE/g. On the other hand, **Azib et *al*., (2019)** found that the ethanolic extract of the leaves contains 95.89 ± 1.88 g GAE / kg of dry weight of total polyphenols, and 5 ± 0.05 g QE / kg of dry weight of total flavonoids. These results are lower than those of **Zitouni et *al*., (2016)** in the same study. In another study in Tunisia conducted by **Yemmen et *al*., (2017)**, the hydromethanolic (80%) extracts from Pistacia lentiscus leaves showed a total polyphenol content similar to that reported by **Zitouni et *al*., (2016)** for the methanolic extract with a flavonoid content lower than that of **Zitouni et *al*.,**

(2016). In addition to being affected by the geographical origin of the plant Algeria **(Azib et *al*., 2019)**, Morocco **(Zitouni et *al*., 2016)**, and Tunisia **(Yemmen et *al*., 2017)**, the content of polyphenols and flavonoids is strongly influenced by the solvent used for extraction and the extraction method.

Also, the comparison of the extracts reveals a very abundant presence of polyphenols and flavonoids in the methanolic extracts compared to the ethanolic extracts. This suggests that maceration extraction using methanol yields higher levels of phenolic compounds, primarily flavonoids, compared to ethanol.

However, a divergence in the levels of phenolic compounds was observed between our results and the results of other studies. Indeed, several factors can influence the content of phenolic compounds and flavonoids. Studies have shown that extrinsic factors such as geographical, climatic, and genetic factors, as well as the degree of plant maturation and storage duration, have a strong influence on polyphenol content **(Fiorucci, 2006).**

3.3.Results of antioxidant activity by the DPPH test

The DPPH radical is a stable organic free radical, with a maximum absorption band between 515-528 nm. In this assay, antioxidants reduce and decolorize the DPPH radical to a yellow compound, diphenyl picryl hydrazine, the extent of the reaction will depend on the ability of the antioxidants to donate hydrogens.

The results of the antiradical activity by the DPPH test are presented in the following figures:

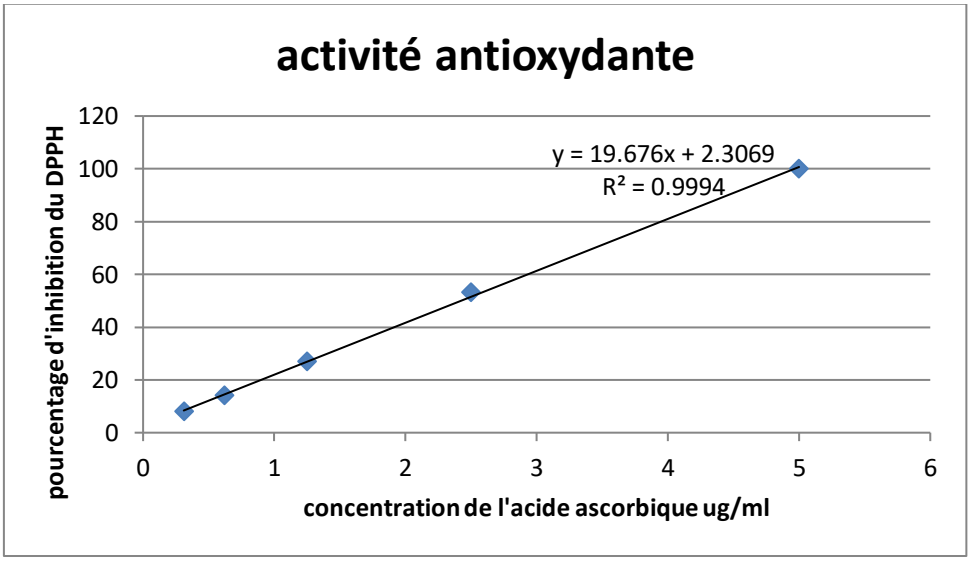

Figure 6: Anti-radical activity of ascorbic acid by the DPPH test

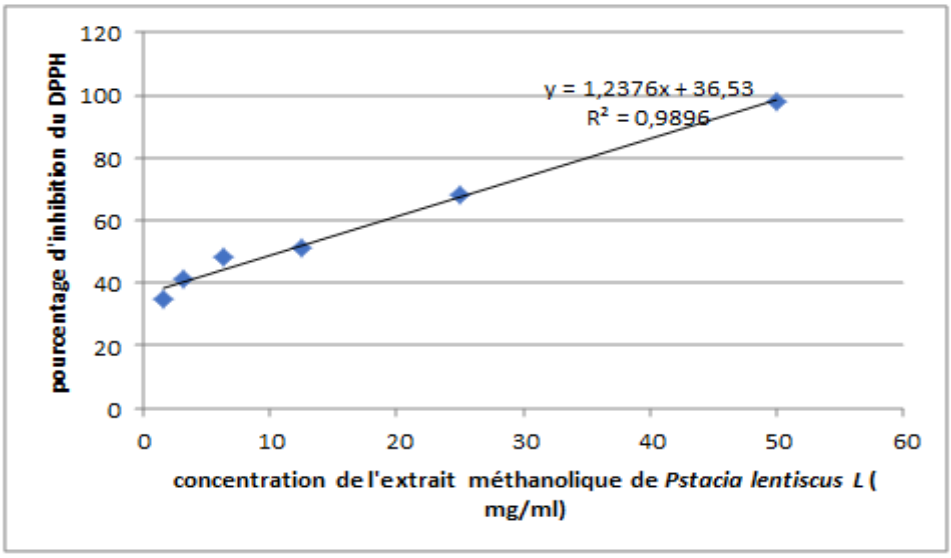

Figure 07: Anti-radical activity of the methanolic extract of *Pistacia lentiscus L* by the DPPH test

The anti-radical activity is carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method, which is a frequently used method due to its simplicity. This method is based on the reduction of an alcoholic solution of DPPH in the presence of an antioxidant that donates a hydrogen or an electron, the non-radical form DPPH-H is formed **(Bortolomeazzi et *al*., 2007)**. The inhibition of DPPH radical discoloration depends on the concentration of the different extracts used and the control.

The antioxidant activity of the extracts is expressed in IC50, this parameter has been used by several research groups to present their results **(Abdulmajed et *al*., 2005; Ahmad et *al*., 2012; Ranga et *al*., 2009)**, it defines the effective concentration of the substrate that causes a 50% loss of DPPH radical activity. the IC50 of the methanolic extract is 4.18 mg/ml. These results are close to those of **Ghenima et *al*., (2015)**, who showed that the aqueous extract of the leaves gave an IC50 of 9 ±0.37 µg/mL.

The protective role of plants is generally attributed to carotenoids and antioxidant vitamins; however, growing evidence indicates that there are other compounds that contribute to varying degrees to the antioxidant activity of fruits; in this regard, attention is focused on phenolic acids, flavonoids, and particularly anthocyanins **(Naczk and Shahidi, 2004, Rice-Evans, 2001).**

Kaempferol and quercetin are the main compounds isolated in the methanolic extract of the aerial part. These two constituents are endowed with strong antioxidant activity **(Cai et *al*., 2004)**. These properties are probably due to these two flavonols. For the extracts from other plants, their

behaviors in donating the proton or electron to express antioxidant properties are different. This result was corroborated by **Miliauskas et *al* (2004)** who showed that an antioxidant effective in one test is not necessarily effective in another on the one hand. On the other hand, antioxidant activities are more variable among plant species (inter-species) than within the same species (intra-species) **(Ksouri et *al*., 2008).**

Previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity **(Jeong et *al*., 2007)**. According to the results obtained, we observe that the reducing capacity of the methanolic extract is proportional to the increase in the extract's concentration (dose-dependent). This is explained by the contribution of electron-donating antioxidants present in the extract, as well as their chemical profile rich in flavonoids, procyanidins, and total polyphenols.
The variability of antioxidant power among the tested extracts may be related to the physicochemical nature of the molecules present in the extracts and by the difference in solvent selectivity for extracting certain groups of antioxidants. According to **Cowan (1999),** during extraction, bioactive molecules are distributed between solvents based on their polarity and solubility. Therefore, changing the nature of the solvent modifies its effectiveness in extracting a specific group of antioxidants, which influences the antioxidant activities of the extracts.

According to **Namiki (1999),** antioxidants can be classified into two groups based on their solubilities: water-soluble antioxidants such as the majority of phenolic compounds and fat-soluble antioxidants such as vitamin E and carotenoids, and according **to (Turkmen et *al*. 2007),** polyphenols seem to be effective hydrogen donors to the DPPH radical due to their ideal structural chemistry.

3.4.Anti-inflammatory activity

It is very important to emphasize that there are numerous reports indicating the importance of protein denaturation as a cause of inflammation **(Sharma et *al*., 2021)**. The production of auto-antigens in inflammatory diseases can be due to the *in vivo* denaturation of proteins.
For the study of the anti-inflammatory power of polyphenols from the aerial parts of Pistacia lentiscus, we chose the method of denaturation of bovine serum albumin (BSA).
The anti-inflammatory activity of the extracts is expressed in IC50; we studied the anti-inflammatory activity of the extracts from our plant *Pistacia lentiscus L*, IC50 was determined in order to compare this capacity.

The results of the evaluation of the anti-inflammatory activity of pistacia lentiscus polyphenols show moderate to potent efficacy in preventing protein denaturation *(***Rios, J. L et *al*., 2005)**. The inhibition of protein denaturation depends on the concentration of the different extracts used and the control (reference anti-inflammatory) (µg/ml). The results obtained are reported in the figure below:

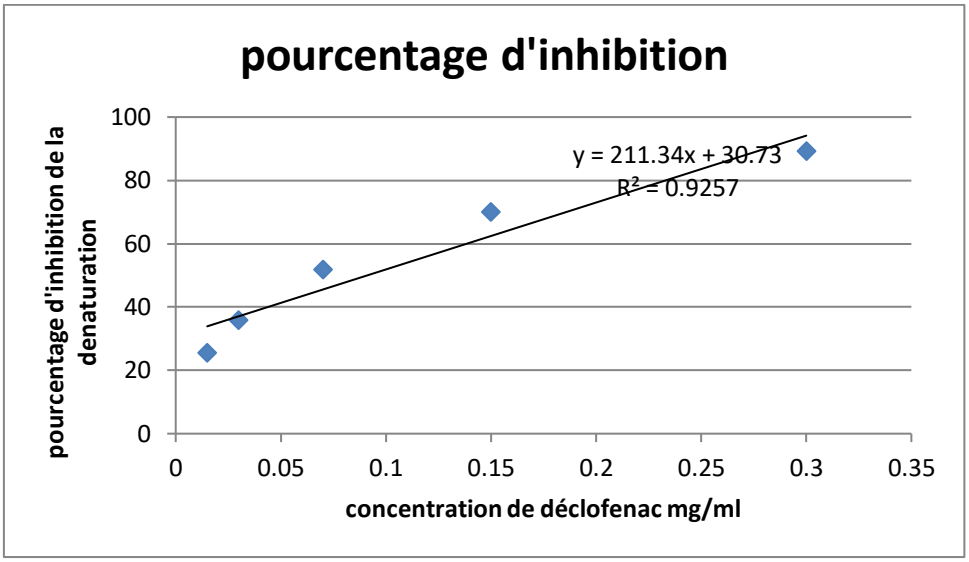

Figure 08: Anti-inflammatory activity of the standard (diclofenac) by the denaturation inhibition method (IC50 = 0.091 mg/ml)

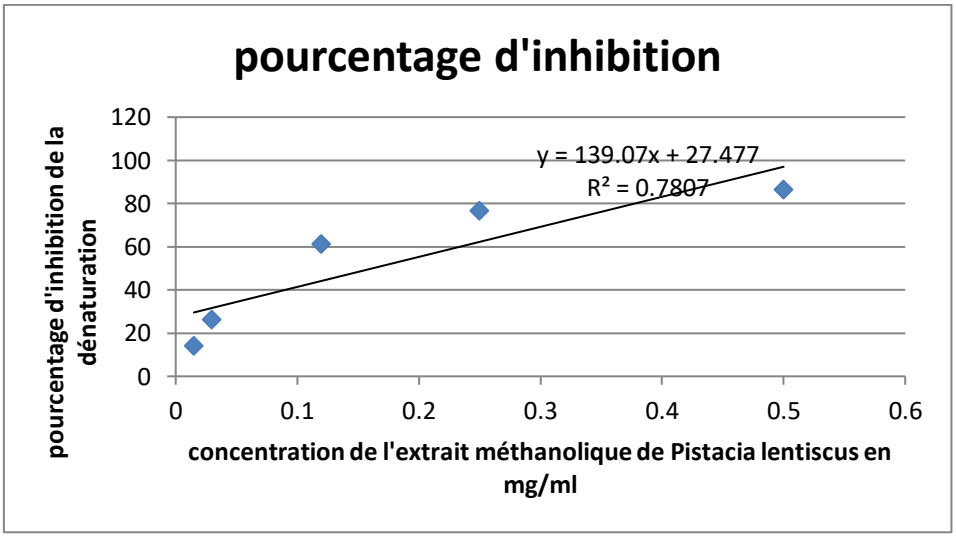

Figure 09: Anti-inflammatory activity of the methanolic extract of *Pistacia lentiscus L* by the denaturation inhibition method (IC50 = 0.16 mg/ml).

After the results of the bovine serum albumin (BSA) denaturation technique. The results obtained show that *P. lentiscus* extracts inhibit the heat-induced denaturation of bovine albumin. This

inhibition is statistically superior to that of the reference anti-inflammatory (Diclofenac). Our test showed that there is strong anti-inflammatory activity (IC50 = 0.16 mg/ml). In light of these results, we note that our extract has high anti-inflammatory efficacy comparable to that of the anti-inflammatory activity of the drug "diclofenac" (IC50 = 0.091 mg/ml).

In order to evaluate the anti-inflammatory potential of the bioactive substances in the studied extract, an albumin denaturation inhibition test was conducted. Indeed, the conformation of a protein is linked to the secondary and tertiary structure, it is achieved thru low-energy bonds (hydrogen bonds, electrostatic, hydrophobic, and disulfide bridges) and therefore fragile. Denaturation results from a modification of quaternary, tertiary, and secondary structures without fragmentation of the peptide chain under the influence of various chemical agents (acid, base, detergent) or physical agents (heat, pH) **(Karthik et *al.,* 2013).** The increase in temperature causes thermal agitation of the atoms of the molecule, so protein denaturation goes thru ephemeral structures that can lead to a total unfolding of the molecule, but it is also considered that denaturation can result from an increase in structure beyond the native form. The unfolding analogous to a statistical coil structure increases the stability of the molecules. This denaturation alters the properties of proteins **(Karthik et *al*., 2013)**. As part of the investigation into the mechanisms of anti-inflammatory activity, the extract's ability to inhibit protein denaturation was studied. Indeed, the tested nigella seed extract exhibits an inhibitory efficacy against thermal denaturation, as well as the stabilizing power of ovalbumin. The stabilization of proteins by nigella extracts likely involves polyphenols and their metabolites, which act as modulators of inflammation signaling pathways **(Sangita et *al*., 2012).**

3.5.Molecular Docking Simulations Results

To predict the binding orientation and affinity of the identified phytochemicals, molecular docking simulations were performed against the crystallographic structures of COX-1 (PDB ID: 1CQE) and COX-2 (PDB ID: 1CX2). The robustness of the docking protocol was validated by re-docking the co-crystallized inhibitors (Flurbiprofen and S-58) and the standard Diclofenac. The resulting binding energies (Vina scores) are summarized in Table 1.

As shown in Table 1, the native ligands Flurbiprofen (COX-1) and S-58 (COX-2) exhibited the strongest binding energies (-9.351 and -11.798 kcal/mol, respectively), confirming the accuracy of the grid parameters in targeting the catalytic site. Among the test compounds, the glycosylated

flavonoids showed distinct selectivity profiles (Table 1). Myricetin-3-rutinoside exhibited the strongest affinity for COX-1 (-8.726 kcal/mol), surpassing the standard Diclofenac (-7.919 kcal/mol). Conversely, Kaempferol-3-O-D-glucoside showed the most favorable interaction with COX-2 (-7.994 kcal/mol), with an affinity comparable to Diclofenac (-8.041 kcal/mol). To understand the structural basis of these binding affinities, the protein-ligand interactions of the top-performing compounds were visualized and analyzed.

Table1 Vina scores (kcal/mol) of the studied phytochemicals against COX targets.

Molecule	**1CQE (COX-1)**	**1CX2 (COX-2)**
Controls		
1cqe_M_FLP	-9.351	-9.158
1cx2_I_S58	-7.149	-11.798
Diclofenac	-7.919	-8.041
Phytochemicals		
kaempferol-3-O-D-glucoside	-7.374	**-7.994**
Myricetin-3-glucoside	-6.783	-7.357
Myricetin-3-O-galactoside	-7.858	-7.504
Myricetin-3-rutinoside	**-8.726**	-7.499
Quercetin-3-glucoside	-7.325	-7.006
Quercetin-3-O-galactoside	-7.442	-7.894
Quercetin-3-O-galactoside-7-O-glucoside	-6.022	-4.398
Quercetin-3-rutinoside	-7.497	-4.225
Vicenin-2	-8.249	-7.472

The high affinity of Myricetin-3-rutinoside for COX-1 is driven by a robust network of hydrogen bonds and hydrophobic contacts (Figure 1). The ligand established four critical hydrogen bonds with the catalytic site residues. Specifically, the hydroxyl groups of the ligand formed H-bonds with His207 (2.01 Å and 2.23 Å) and His386 (2.12 Å). Additionally, a hydrogen bond was observed with Thr212 (2.61 Å), further stabilizing the ligand within the pocket. Electrostatic interactions were observed between the ligand and His388 (3.60 Å), while hydrophobic interactions anchored the aromatic rings of the flavonoid scaffold against Gln203, Leu295, and Ile444.

Myricetin, the parent aglycone, is a well-known flavonoid with powerful antioxidant and anti-inflammatory properties, which have been shown to reduce the production of pro-inflammatory agents and downregulate COX-2 expression **(Rosas and Gutiérrez ,2019** ; **Almatroodi and Rahmani ,2025).** However, the pronounced affinity of its rutinoside conjugate for COX-1 is a novel and intriguing finding.

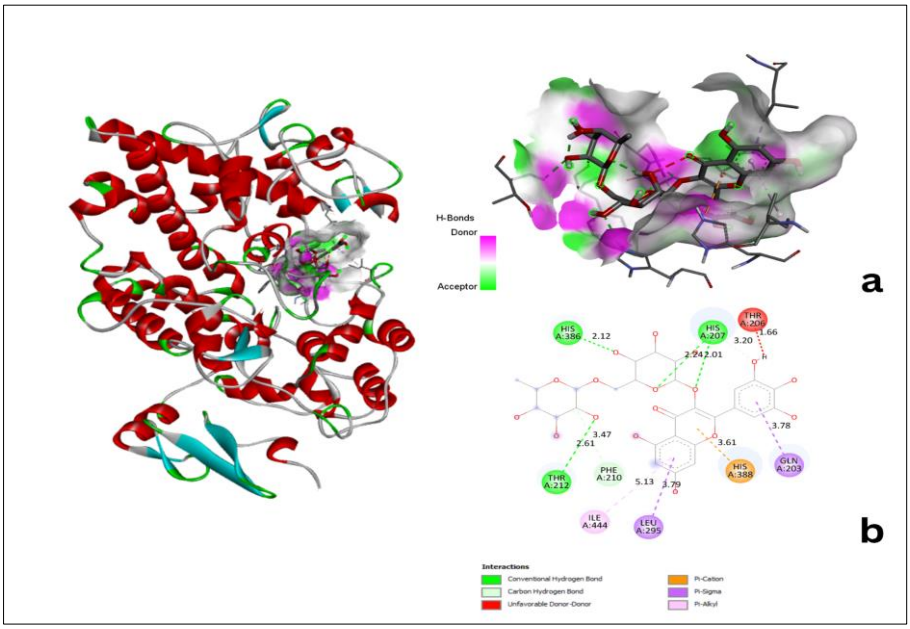

Figure 10: Schematic representation of hydrogen bonds, electrostatic, and hydrophobic interactions a) Hydrogen Bond Pocket b) 2D interactions Myricetin-3-rutinoside against COX-1 (PDB ID: 1CQE).

Kaempferol-3-O-D-glucoside exhibited the most favorable binding orientation within the COX-2 active site (Figure 2). The binding was anchored by a hydrogen bond with the backbone oxygen of ALA199 (2.44 Å). The stability of the complex was further reinforced by significant hydrophobic interactions involving TRP387, ALA202, and HIS388.

Furthermore, the electrostatic interaction with HIS207 and the hydrophobic environment provided by the surrounding residues suggest that Kaempferol-3-O-D-glucoside effectively mimics the binding mode of established NSAIDs.

The identification of Kaempferol-3-O-D-glucoside as a potent and selective inhibitor of COX-2 is a significant outcome of this research. The therapeutic quest for selective COX-2 inhibitors is driven by the goal of mitigating the gastrointestinal side effects associated with traditional non-steroidal anti-inflammatory drugs (NSAIDs), which non-selectively inhibit both COX-1 and COX-2**(Vernieri and *al***

.,2013). This finding is well-supported by existing literature, which documents the anti-inflammatory properties of kaempferol and its derivatives, often linked to the downregulation of COX-2 expression and activity **(Lee and *al.,* 2010; Yoon and *al* .,2013).** .

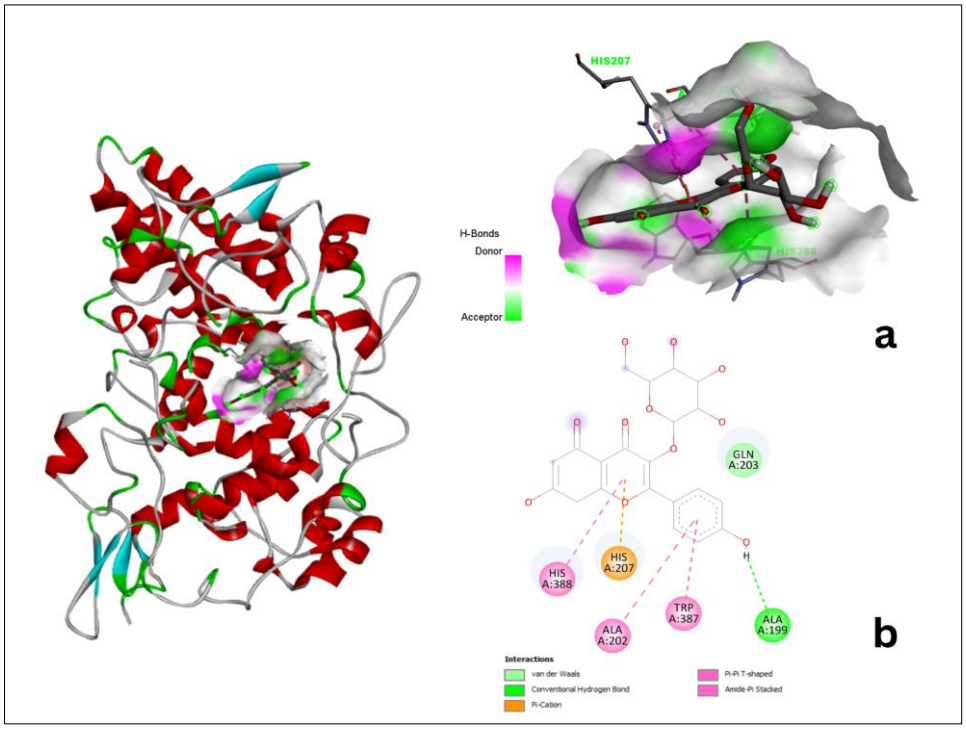

Figure 11: Schematic representation of hydrogen bonds, electrostatic, and hydrophobic interactions a) Hydrogen Bond Pocket b) 2D interactions kaempferol-3-O-D-glucoside against COX-2 (PDB ID: 1CX2).

In conclusion, this computational study has successfully identified and provided a molecular rationale for two promising phytochemical inhibitors with distinct selectivities for COX isoforms. Kaempferol-3-O-D-glucoside stands out as a potential selective COX-2 inhibitor, aligning with the modern strategy for developing safer anti-inflammatory drugs. Myricetin-3-rutinoside presents as a potent COX-1 inhibitor, a finding that merits further exploration for its therapeutic implications. While these in silico results are compelling, they represent the initial step in the drug discovery process. It is imperative that these findings are now progressed to experimental validation, beginning with in vitro enzyme inhibition assays to determine IC50 values, followed by cell-based and in vivo studies to confirm their efficacy and safety profiles. Such a path will be crucial to fully unlock the therapeutic potential of these natural compounds.

4.Conclusion

Plants remain the predominant source of medicine for the majority of the world's population, particularly in developing countries. The use of these plants is certainly linked to therapeutic virtues such as burns, but also as medical agents like antioxidants and anti-inflammatories.

Our country enjoys considerable biodiversity, possessing numerous aromatic and medicinal plants rich in secondary metabolites with therapeutic and pharmacological characteristics.

As part of the enhancement of these resources, our work has focused on a phytochemical study of a plant species, namely *Pistacia lentiscus*, by analyzing their chemical composition of polyphenols but also by evaluating their biological properties such as antioxidant and anti-inflammatory activity.

The richness of *Pistacia Lentiscus L*. leaves in phenolic compounds helps explain the use of this plant in traditional medicine for its antioxidant, antiviral, antitumor, anti-inflammatory, and antimicrobial activities.

The evaluation of the in vitro antioxidant activity of the methanolic extract of *Pistacia lentiscus L* by the DPPH test showed that the extract can act as free radical scavengers and seems to be a good free radical scavenger with an IC50 value of 4.18 mg/ml. While the anti-inflammatory activity was evaluated by the inhibition of bovine serum albumin (BSA) denaturation method and it showed that the methanolic extract of *Pistacia lentiscus L* can act as an anti-inflammatory and seems to be a good anti-inflammatory (IC50 = 0.16 mg/ml).

In perspective, to enhance these results, it is interesting to: - Conduct a more in-depth study to isolate, purify, and identify the molecules responsible for antioxidant and anti-inflammatory activity. - Study the mechanisms of action of phenolic compounds involved in the previous in vitro activities.

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