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In vitro Antioxidant and *In Silico* Wound Healing Activity of Quercus Infectoria Dry Extract

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Abstract

In the present study we were interested in the healing activity of Quercus infectoria gall extract. We started by preparing the extract by two methods: maceration and digestion. Then, polyphenol and tannin contents were determined. The antioxidant activity of the extract was evaluated *in vitro* using DPPH radical scavenging assay. The last part of this study concerns the study of the healing activity in silico by molecular docking assisted by the Shrödinger program and this via the inhibition of GSK3-β, an enzyme involved among others in the healing process. The extract obtained shows a high tannin content, which explains most of its antioxidant activity. The in-silico study, revealed that ellagic acid, isocryptomerin, propyl gallate, ethyl gallate, and methyl gallate are likely inhibitors.

Key Words: Quercus infectoria, tannins, healing activity, antioxidant activity, molecular docking, GSK3-β

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1. Introduction

The gall oak or Aleppo oak (Quercus infectoria), is a shrub of the genus Quercus belonging to the family of Fagaceae (Shrestha et al., 2014). It is a common shrub in Iran, Syria and Turkey and is also found in Greece (Fabre et al., 1992). Phytochemical analysis of the aqueous extract of galls indicates the presence of polyphenolic compounds (gallic acid, methyl gallate, ethyl gallate, propyl gallate, syringic acid and ellagic acid), flavonoids (amentoflavone and isocryptomerin), triterpenoids (roburic acid, methylbetulate, methyloleanolate and nyctanthic acid) and sterols (β-sitosterol). Tannins are the main constituents of the dry extract. In the last decades, Quercus infectoria galls have been increasingly studied due to their wide spectrum of physiological and pharmacological properties. Several biological activities have been reported, such as wound healing (Elham et al., 2021), anti-inflammatory (Kaur et al., 2004), and anti-tumor activities (Kuo et al., 2014). It has been reported that the inhibition of glycogen synthase kinase-3β (GSK3-β) promotes wound healing (Harish et al., 2008). This study aims to investigate both the *in vitro* antioxidant activity and the *in silico* wound healing activity of the extract by docking its constituent molecules to GSK3-β.

2. Material and Methods

2.1. Plant material

The galls of Quercus infectoria were obtained from a local herbal shop in the city of Tlemcen (Algeria). They were ground with an electric coffee grinder. The powder obtained is kept in a dark place until it is used. The plant material was identified on the basis of its macroscopic characteristics.

2.2. Extraction of plant material

The extraction of compounds was carried out by two methods:

2.2.1. Extraction by maceration: 45 g of gallnut powder was introduced into an Erlenmeyer flask. 400 mL of a hydroacetone solution (water-acetone 60/40 : v/v) was added. Magnetic stirring was maintained for 24 hours at room temperature.

2.2.2. Extraction by digestion: 45 g of gallnut powder was introduced into 400 mL of distilled water. The whole was heated for 2 hours at 50°C and then left to macerate for 24 hours while maintaining magnetic stirring.

The filtrates were concentrated until a dry extract was obtained, this was then reduced to powder.

2.3. Quantification of tannins

This determination was carried out according to the indirect method described by Lastra et al. (2000). This method consists of two parts: a determination of total polyphenols (TPP), followed by residual polyphenols (RPP), after precipitation of the tannins by 20% gelatin in a NaCl-saturated solution.

The total polyphenol content is given by the following formula:

Tanin content = TPP content- RPP content

The analysed solution was prepared by dissolving 0.1 g of dry extract in 100 mL of distilled water. The total phenolic compounds were quantified by spectrophotometry using the Folin–Ciocalteu reagent. 10 mL of distilled water was added to 0.5 mL of the prepared solution. 0.5 mL of Folin–Ciocalteu's reagent and 1 mL of sodium carbonate (10%) were added to the previous mixture. Absorbance was measured at 750 nm after 1 hour against a standard curve of gallic acid. The residual polyphenols were then quantified after the precipitation of tannins. 10 mL of the prepared solution, 15 mL of 20% salted gelatin and 1 g of kaolin were mixed. The previous mixture was kept under magnetic stirring for 30 min in order to allow a better precipitation of tannins. The mixture was separated by centrifugation (10 min 4000 rpm). The surnageant containing the residual polyphenols was then analysed following the same protocol as previously described.

2.4. Antioxidant activity determination using DPPH radical scavenging assay

The determination of the antioxidant activity of the extract was carried out using the method described by C. Sánchez-Moreno et al. (1998). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was defined as a stable free radical which forms a violet-coloured solution. In the presence of anti-radical compounds, the DPPH radical was reduced and changes the colour from violet to yellow. The absorbances measured at 515 nm were used to calculate the percentage of inhibition of the DPPH radical. 50 µl of each solution of the extracts at different concentrations (from 0.0625 to 1 mg/ml) were added to 1.95ml of the methanolic solution of DPPH (0.04 g/l).

In parallel, a negative control is prepared by mixing 50µl of methanol with 1.95ml of the methanolic solution of DPPH. The absorbances are measured at 515 nm against a blank prepared for each concentration after a 30-minute incubation in the dark at room

temperature. The positive control is represented by a solution of ascorbic acid whose absorbance was measured under the same conditions as the samples, and for each concentration, the test is repeated three times. The results were expressed as a percentage of inhibition (I%).

I% =(〖Abs〗_control- 〖Abs〗_sample)/ 〖Abs〗_control ×100

2.5. Molecular docking

Automated docking was used to determine the orientation of inhibitors bound to the active site of GSK3-β. The Schrödinger Maestro programme was employed. GSK3-β (EC: 2.7.1.37) represented by PDB code 1Q5K co-crystallised with 1-[(4-methoxyphenyl)methyl]-3-(5-nitro-1,3-thiazol-2-yl) urea was selected as the biological target for our research theme (Bhat et al., 2003). Grid generation was achieved in the presence of the co-crystallized reference inhibitor CID: 448014. Before the actual docking step, we performed a redocking using the co-crystallised reference ligand: 1-[(4-methoxyphenyl)methyl]-3-(5-nitro-1,3-thiazol-2-yl)urea (TMU). Cross-docking was also performed for a ligand already known to have inhibitory activity on GSK-3β: staurosporine (STU), obtained from the PubChem database. Redocking and cross-docking protocols were employed in order to evaluate the accuracy of docking procedures. For the screening step, we limited ourselves to molecules from the gallnut, so a small chemical library of thirteen molecules was prepared. These were downloaded from the PubChem website (www.pubchem.ncbi.nlm.nih.gov) in SDF format and prepared. The ligands were minimised using the OPLS3 force field implemented in Maestro's Ligprep application. Their ionisation and tautomerization states were predicted by Epik 2.2 at pH 7±2.

3. Results and Discussion

3.1. Extraction of plant material

These findings demonstrate that extraction using maceration in a hydroacetone solution yields a greater amount of material than digesting. This is attributed to the presence of acetone, a polar solvent that is suitable for the extraction of tannins.

Table 1. Extraction yields

	Maceration	Digestion
Yields (%)	47.69	44.16

3.2. Quantification of tannins: The tannin content was determined by indirect spectrophotometry in the visible range using gallic acid as standard. Total and residual polyphenol contents are calculated from the equation $y = 1.152x + 0.046$ ($R^2 = 0.990$) of the gallic acid calibration curve Abs=f(C).

Table 2. Quantification of tannins

TPP content (*)	594.62 ± 0.030
RPP content (*)	222.22 ± 0.002

(*): mg Gallic acid equivalent/g dry matter

Total tannin content = 62.63 % of total polyphenols.

These results show that the gallnut is an important source of tannins, which represent 62.63 % of the total polyphenols in the dry extract. This value is comparable to that reported by Zhang et al. (2023), i.e., 70% for Quercus chenii and Quercus aliena shell extract.

3.3. Antioxidant activity determination using DPPH radical scavenging assay

The antioxidant activity of the gallnut extract was determined by calculating the concentration of the extract inhibiting 50%

of the DPPH radicals (IC50). In this test, ascorbic acid was used as a standard. The results obtained are shown in Figure 1.

The IC50s of gallnut extract and ascorbic acid were calculated from the equations y=140.1x+5.900 (R2=0.984) and y=290.2x+2.540 (R2=0.978) respectively.

IC 50Extract = 0.16 ± 0.01 g/L

IC 50Ascorbic acid= 0.31 ± 0.2 g/L

From these results, gallnut extract has twice the antioxidant activity of the ascorbic acid used as a reference. This value was in accordance with that reported by Arina et al. (2019), i.e., 0.13 g/L for gallnut extract prepared by decoction at 50°C, and Kamarudin et al. (2021) i.e., 0,14 g/L for the aqueous gallnut extract. The antioxidant activity of gallnut extract was mainly due to the presence of phenolic compounds (594.62 ± 0.030 mg EAG/g dry extract), secondary metabolites with pronounced antioxidant properties.

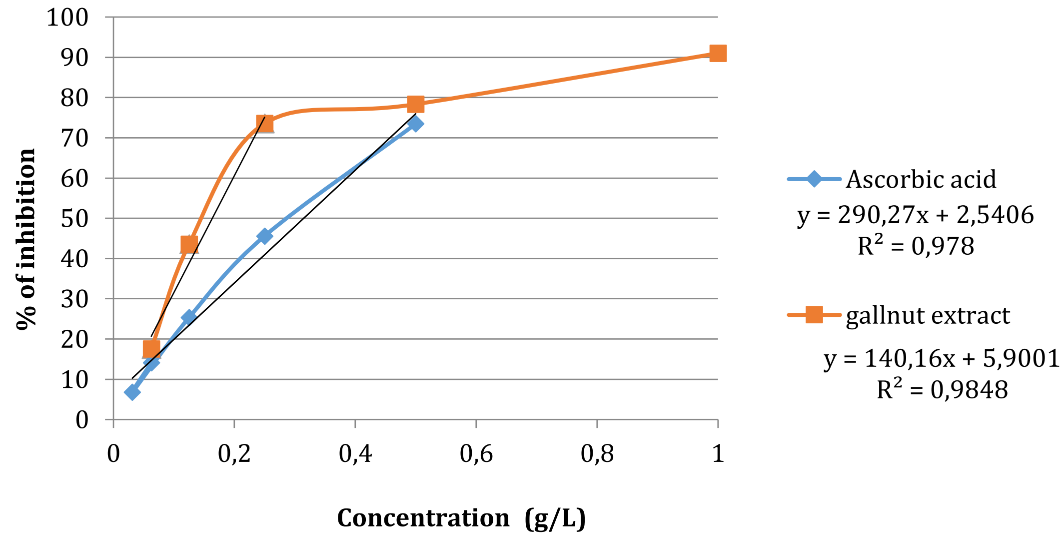
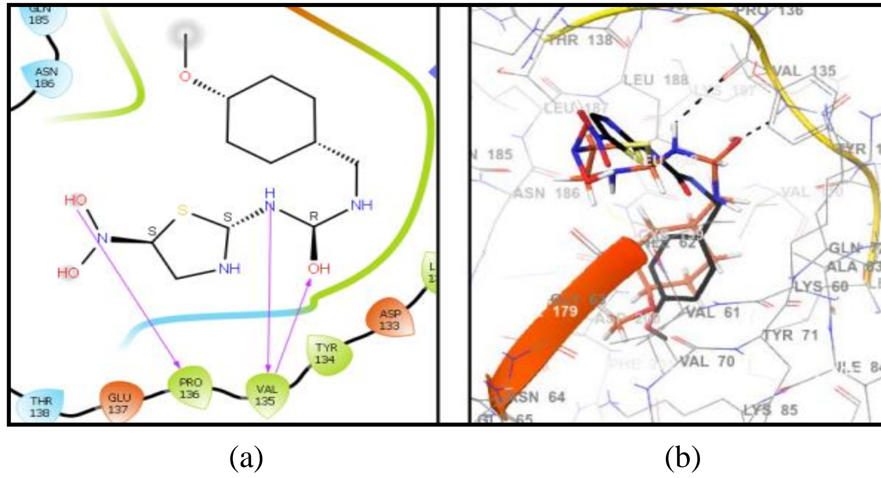


Figure 1. Percentage of DPPH inhibition versus concentration.

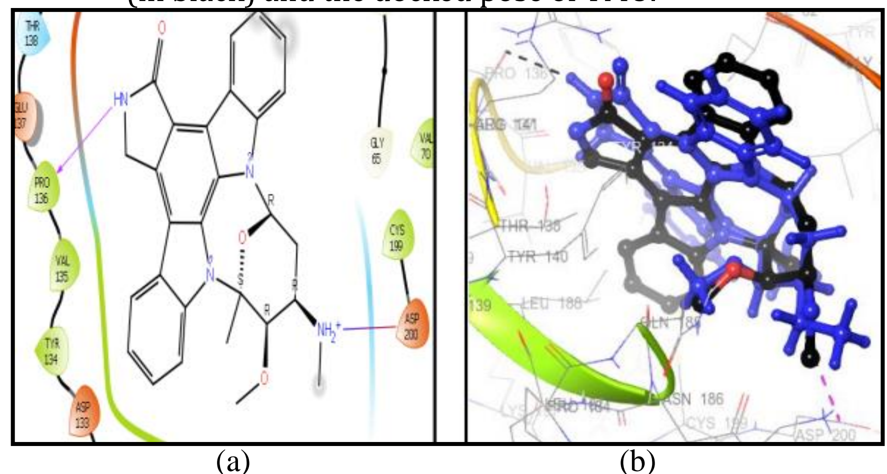
3.4. Molecular docking

The reference ligand (TMU) shares with the active site of the enzyme two "donor-acceptor" bonds with the amino acid Val 135 and a third "donor" bond with the amino acid Pro 136. The interaction energy is estimated at -7.55 Kcal/mol. It was observed that the root-mean-square deviation (RMSD) for the redocking protocol was 1.7164 Å. Similarly, for the cross-docking, the RMSD of staurosporine (STU) was found to be 0.5594 Å. It has an electrostatic bond with Asp 200 and a donor bond with Pro 136. The interaction energy is estimated at -5.25 Kcal/mol. We note that the two RMSDs of the poses are lower than 2 Å, which validates the approach of the docking protocol.



(a) (b)

Figure 2. a) 2D visualization of the co crystallized and docked poses of TMU
b) 3D visualization of the superposition of the co crystallized pose (in black) and the docked pose of TMU.



(a) (b)

Figure 3. a) 2D visualization of the docked poses of STU
b) 3D visualization of the superposition of the co crystallized (black) and docked poses of STU

Table 3. Scoring results

Ligand	Glid score (Kcal/mol)	State penalty
Ellagic acid	-10.13	0.07
Isocryptomerin	-8.89	0.04
Propylgallat	-8.43	0.05
Ethylgallat	-8.31	0.05
Méthylgallat	-7.8	0.05
Syringic acid	-6.64	0
Amentoflavone	-6.46	0.12
Methylbutulate	-3.64	0
Nycthantic acide	-2.96	0
Roburic acid	-2.75	0
β-sitosterol	-2.61	0
Gallic acid	-1.56	0.01
Methyleolealonate	-1.31	0

Table 4. Detected interactions of the top five scoring ligands

Ligand	Detected interactions from 2D diagram
Ellagic acid	O Val135 NH (1HD) NH Val135 O (1HA) NH Lys85 O (1HA)
Isocryptomerin	O Val135 NH (1HD) NH Val135 O (1HA) NH Lys60 O (HA) Phenyl Arg141 (HI)
Propylgallat	O Val135 NH (1HD) NH Val135 O (1HA)
Ethylgallat	O Val135 NH (1HD)
Methylgallat	O Val135 NH (1HD) NH Val135 O (1HA)

The scoring results obtained and detected interactions of complexes exhibiting significantly lower energy than those obtained by the reference ligand are shown in Tables 3 and 4.

According to these results, the complexes formed with ellagic acid, isocryptomerin, propylgallate, ethylgallate, and methylgallate show energies significantly lower than those obtained by the reference ligand (-7.55 Kcal/mol). Ellagic acid gave the best score (-10.13 Kcal/mol), suggesting that the polyphenol family has strong inhibitory power. Propylgallate, ethylgallate, and methylgallate show scores of -8.43, -8.31, -7.80 Kcal/mol, respectively, these increasing values depend on the length of the esterified alkyl chain. In fact, a three-carbon chain has a higher score than a one-carbon chain.

All ligands with a higher score than the reference share hydrogen bonds with the Val 135 residue. According to El Kerdawy et al. (2019), this binding must be retained to achieve effective inhibition of GSK-3β kinase. Interactions with other residues at the binding site will only increase the strength of the binding interaction (El Kerdawy et al., 2019).

In addition to the two bonds with Val 135, ellagic acid has a hydrogen bond with Lys 85. As for isocryptomerin, it presents, in addition to the two bonds with Val 135, a hydrogen bond with Lys 60 and a hydrophobic interaction between the phenyl group and Arg 141. The latter two interactions further strengthen the binding of the ligand to the active site of the enzyme. According to M. Arfeen et al., the study of the most active and selective compound showed that selectivity is determined by residues Lys 85, Arg 141, Thr 138, and Cys 199 thus allowing the design of selective GSK-3β inhibitors (Arfeen et al., 2016).

Ellagic acid and isocryptomerin share hydrogen bonds with residues Lys 85 and Arg 141 respectively, which allows them to be classified as selective inhibitors.

4. Conclusion

In vitro antioxidant activity and in-silico wound healing activity of *Quercus infectoria* dry extract were determined and presented in this paper. The extract obtained shows a high tannin content, representing 62.63% of total polyphenols. This content explains most of the antioxidant activity of our extract. The final step in our work concerns the study of gallnut healing activity *in silico* via inhibition of GSK-3β by molecular docking. Score energy and molecular interactions are the criteria for inhibitor selection. This study revealed that ellagic acid, isocryptomerin, propyl gallate, ethyl gallate, and methyl

gallate were likely inhibitors, justified by the low score energy these ligands possess towards the reference inhibitor.

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Author Contribution

All authors declare equal contribution to the design and experimental work, interpretation of the results and editing the manuscript.

Conflicts of Interest

The authors declared no conflict of interest.

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