Alpha-glucosidase and α-amylase inhibitory activity of *Pistacia atlantica* Desf. gall extracts and identification of putative bioactives using a combined UPLC fingerprinting and molecular docking approach

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Abstract

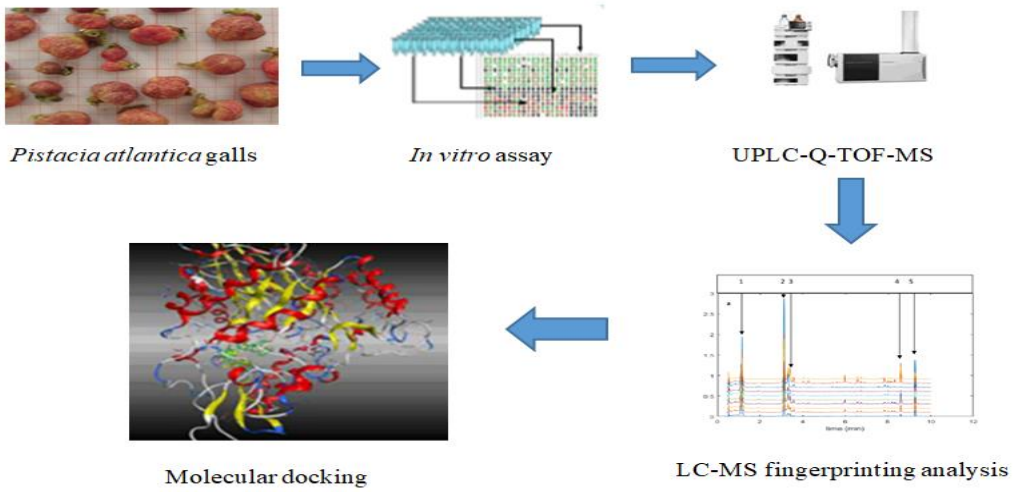
Aims *Pistacia atlantica* Desf. (Anacardiaceae) is a plant widely used in traditional medicine throughout the Mediterranean region. Previous experimental studies have demonstrated the antidiabetic potential of its fruits and leaves. This study was conducted to evaluate the antidiabetic activity of *P. atlantica* galls (PAG) extracts using a combination of in vitro, chemometric, and *in silico* approaches.

Method The antidiabetic activity of the samples were studied by measuring their half-maximal inhibitory concentrations (IC_{50}s) concentrations according to the *in vitro* enzyme inhibition assays and modelled as a function of the LC fingerprints using the partial least squares technique. The peaks potentially responsible for antidiabetic activity of the samples were indicated by studying the regression coefficients of the models**.** Crystal structures of the human pancreatic α-amylase (HPA) and the α-glucosidase homologue isomaltase were obtained from the Protein Data Bank website (http://www.rcsb.org/pdb). Docking simulations and calculations were carried out using AutoDock Vina.

Results PAG extracts inhibited HPA (IC_{50}s ranging from 1.85 to 2.92 mg/mL) and α-glucosidase (IC_{50}s ranging from 34 to 49 μg/mL) activities, with galls collected from male plants showing higher activity than those from female plants. In addition, all PAG extracts were about 60-fold more efficient at inhibiting α-glucosidase than α-amylase. UPLC fingerprinting, linked to chemometric analysis using a partial least squares regression model, putatively identified five compounds (quinic acid, methyl gallate, digalloyl quinic acid, methyl digallate, and valoneic acid dilactone) responsible for this antidiabetic effect. Molecular docking using AutoDock Vina revealed that the identified compounds interacted with key amino acid residues of HPA and α-glucosidase.

Conclusions By employing UPLC fingerprinting combined with chemometric analysis and molecular docking simulations, quinic acid and digalloyl quinic acid were identified from *P. atlantica* gall extract as the most promising ligands for further investigation into their antidiabetic potential.

Keywords: *Pistacia atlantica* galls, α-amylase, α-glucosidase, chromatographic fingerprinting, molecular docking.



Introduction

Diabetes mellitus (DM) is a chronic metabolic disease, characterized by increased blood glucose levels, either caused by a deficiency of insulin production as a result of autoimmune-mediated selective destruction of the insulin-secreting β-cells of the pancreas (Type 1 diabetes mellitus) or by a loss of peripheral tissue response to insulin (Type 2 diabetes). The latter accounts for about 90% of all cases of diabetes and most commonly occurs in middle-aged and older adults, although it is increasingly also affecting children, teenagers and young adults [1-3]. In 2021, it was estimated that more than 500 million people suffered from DM worldwide and this figure is likely to reach 783 million by 2045 [4]. If poorly controlled, the progression of DM can lead to life-threatening vascular complications and damage of vital organs, increasing morbidity and mortality [5-7]. The current management of DM includes the use of insulin and oral hypoglycemic agents intended to boost insulin sensitivity and/or increase its secretion as well as increase glucose excretion and/or its uptake in adipose tissue. Several of these drugs, however, are costly and associated with adverse side effects [8]. In many low and middle-income countries where DM is endemic, plants are widely used as a first choice treatment option for DM as they are easily accessible, believed to be safer than conventional drugs and contain diverse phytochemicals that have already demonstrated antidiabetic activity through a variety of mechanisms [9-13]. Many studies have already identified natural inhibitors of α-amylase and α-glucosidase [14-17]. Such inhibitors have the potential to effectively delay the rise in blood glucose levels in individuals consuming carbohydrate-rich foods, consequently aiding in the management of postprandial hyperglycemia [18]. They have become interesting therapeutic targets in the management of T2DM [19].

Pistacia atlantica Desf. (Anacardiaceae), commonly known as wild pistachio, is an evergreen tree, widespread from the Mediterranean basin to central Asia where it is extensively used in traditional medicine for a wide range of ailments. Previous chemical investigations have reported the presence of volatile compounds, flavonoids, phenolic compounds, fatty acids, tocopherols and phytosterols in this species. Its crude extracts and isolated compounds have demonstrated a wide range of pharmacological properties, including antimicrobial, anti-inflammatory, anticancer, cytotoxic, anticholinesterase, antihypertensive, hepatoprotective, anti-urease, antiparasitic and antidiabetic activities. The majority of these investigations have been conducted on *P. atlantica* leaves and fruits [20]. The purpose of the present work was to screen extracts prepared from *P. atlantica* Desf. galls (PAG) for their α-glucosidase and α-amylase inhibitory activity and investigate the influence of plant gender and seasonality of harvest on such activities. In addition, Ultra-performance liquid chromatography (UPLC) fingerprinting followed by partial least squares (PLS) analysis was performed to characterize the peaks in the chromatograms linked to potential bioactive phytochemicals within the extracts. A molecular docking approach was further employed to predict the binding affinity of these phytochemicals towards the target enzymes.

Materials and methods

Chemicals

Methanol, HCl (37%), Na_2SO_4, NaOH pellets, potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$), Na_2HPO_4, NaH_2PO_4, Na_2CO_3, starch, 3.5-dinitrosalicylic acid (DNS), human pancreatic α-amylase (EC 3.2.1.1), *Saccharomyces cerevisiae* α-glucosidase (EC 3.2.1.20) and *p*-nitrophenyl-α-D-glucopyranoside, were purchased from Sigma-Aldrich. UPLC-grade methanol was obtained from Fisher Scientific, Leicestershire, UK. Acetic acid

was obtained from Sigma-Aldrich and ultra-pure water was made in house by a Sartorius Arium pro UV system (Sartorius Stedim Biotec, Goettingen, Germany).

Plant material

The galls of *P. atlantica* Desf trees were harvested every month from July to November 2010. Both female and male trees were sampled from the Laghouat region, south of Algiers, Algeria. Five samples were collected for each gender per region (Fig.S1). The identity of the gall samples was confirmed by Prof S. Belhadj (Department of Agropastoralism, Faculty of Science, Achour Zian University, Djelfa, Algeria), and a voucher specimen (GL032010ULDB) was deposited in the herbarium of the Department of Biology, Faculty of Science, University of Laghouat (Algeria) [21].

Sample preparation

The extraction was performed according to a method described previously [22]. The powdered *P. atlantica* galls (PAG) (2 g) were macerated in 100% methanol (40 mL) for 48 h at room temperature and in the dark. The organic extract was filtered and the residue re-extracted with 30 mL of the same solvent for 24 h, then filtered. The filtrates were combined and the methanol was removed using a rotary evaporator at 45 °C. The final residue was dried, redissolved in 10 mL of pure methanol and kept at 6 °C until analysis.

Human pancreatic α-amylase (HPA) and α-glucosidase inhibition assays

The assays were performed as described previously [23]. The HPA and α-glucosidase inhibitory activity of the extracts were calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_{cont} - (A_{sample} - A_{blank})}{A_{cont}} \times 100 \quad \textbf{(1)}$$

where A_{sample} represents the absorbance of the sample, A_{blank} that of the blank (i.e. buffer instead of enzyme solution) and A_{cont} that of the control (i.e. buffer instead of sample extract). The dose-response curve was obtained by plotting the percentage inhibition versus the concentration. The IC_{50}s, i.e. the sample concentration required to achieve half-maximal inhibition of the enzyme, was determined for each sample using OriginPro v8.6 (OriginLab, Northampton, MA, USA). All PAG extracts were compared on the basis of their IC_{50}s values, estimated from the dose response curves.

UPLC fingerprinting

UPLC fingerprinting was performed following a previously described methodology [24]. The mobile phase consisted of formic acid 0.1% (A) and methanol (B). Column temperature was 25°C, flow rate 0.3 mL/min, injection volume 2 µL, and detection wavelength 254 nm. Partial Least Squares (PLS) regression analysis was performed with data pre-processing, modelling, and validation as described previously [21]. The data matrix X consisted of the samples (rows; $\boldsymbol{n}$=10) and the time points (columns; $\boldsymbol{p}$ = 11501). Negative regression coefficients gave information about the putative bioactive compounds within the extracts responsible for HPA or α-glucosidase inhibitory activity. The optimal model complexity for each inhibitory activity model was selected by determining the leave-one-out cross-validation with combination of the low root mean square error of cross-validation (RMSECV) value (Table S1).

Statistical analyses

All experiments were carried out in triplicate and data were expressed as means ± SD. One-way analysis of variance (ANOVA) followed by Duncan *post-hoc* tests were used to determine the significance (at $p \leq 0.05$) of

the harvest month and plant gender on the HPA and α-glucosidase activities. Paired *t*-tests were used to compare the differences in the averages of HPA and α-glucosidase activities between harvest months and plant genders [21]. All calculations were performed using SPSS version 16 (SPSS, Prentice Hall, Chicago IL, USA, 2007). Data from column centering, normalizing, SNV, and PLS) were analysed using m-files, written in Matlab v7.1 (The MathWorks, Natick, MA, USA).

Docking studies

Target proteins preparation

The crystal structures of HPA (PDB ID: 4GQR) was downloaded from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb). As the crystal structure of α-glucosidase from *Saccharomyces cerevisiae* was unavailable, the crystal structure of the α-glucosidase homologue isomaltase (PDB ID: 3A4A) - whose sequence shows 84% similarity with that of *Saccharomyces cerevisiae* α-glucosidase [25] was retrieved instead from the Protein Data Bank. The server Computed Atlas of Surface Topography of proteins (CASTp) was used to identify the active site of both enzymes (http://sts.bioe.uic.edu/castp/) [26]. In preparation for the docking, both proteins were processed, using the Discovery Studio software v20.1, by removing unnecessary water molecules, heteroatoms and ligands. Polar hydrogens and Gasteiger charges were assigned with AutoDockTools (ADT) v1.5.6.

Preparation of ligands

The secondary metabolites detected following UPLC-MS/MS analysis were used as the ligands for the docking study. The three-dimensional structure of all ligands were retrieved from the PubChem database (http://pubchem.ncbi.nlm.nih.gov) and visualized using UCSF Chimera v1.15 [27].

Molecular docking

The docking simulation and calculations were performed using AutoDock Vina [28]. The grid box for each enzyme was centered on their respective binding site and their size was adjusted to fit the size of ligands. For α-amylase, the grid box values were set to x = 10.37, y = 17.39 and z =41.14 (1 Å grid spacing) with the size adjusted to 26 × 25 × 29. For α-glucosidase, the grid values were x = 18.0, y = -11.0 and z =16.69 (1 Å grid spacing) with the size adjusted to 34 × 42 × 32. All ligands were considered as freely-rotating while the proteins were considered as rigid structures. The ligands showing the lowest binding energy values (highest docking scores) were considered as the most favourable. PyMOL™ v1.7.4.5 (Schrödinger, LLC, New York, NY, USA) and Ligplot^{+} v1.4.5 were used to visualize the interactions between the ligands and the target proteins.

Results and discussion

Influence of harvesting time and plant gender on the α-amylase and α-glucosidase activity of PAG extracts

PAG extracts from male and female plants showed dose-dependent inhibitory activity on HPA at concentrations ranging from 0.5-3.0 mg/mL. The calculated IC_{50}s values of PAG extracts collected from July to October ranged from 1.85 to 2.66 mg/mL and from 2.21 to 2.92 mg/mL for male and female plants, respectively. Extracts prepared from male plants collected in July (LMG07) demonstrated the highest HPA inhibitory activity with the lowest IC_{50}s (1.85± 0.21) of all samples.

Given that the chemical composition of a plant species directly affects its biological activity, samples from the same species gathered at various times of the year may exhibit notable discrepancies in their chemical constituents and, thus, varying pharmacological properties [23, 29]. In this context, the obtained results were

statistically analyzed to check for significant differences and to evaluate the influence of seasonality on the HPA and α-glucosidase activities of *P. atlantica* Desf. galls harvested at different harvests and examine the effect of gender differences on those activities.

One-way ANOVA showed a significant difference in the HPA inhibitory activity between different harvest months within each gender ($p < 0.001$). This was confirmed by Duncan *post-hoc* tests, with male samples collected in July and October showing significant differences in activity from those collected in November while female samples collected in July-August showing significant differences in activity from those collected in September and November.

A paired-*t*-test, used to evaluate the statistical significance of gender differences on the HPA inhibitory activity of the samples, revealed that the HPA inhibitory activity of galls differed significantly (p=0.018) depending on the plant gender (Fig.1a, Fig.S2, Table 1). Our findings show that the HPA inhibitory activity profiles of the Laghouat region appear to differ between male and female plant. The reason for this difference can be explained by the fact that *P. atlantica* synthesizes and decomposes various compounds to cope with environmental factors, including biotic (pathogens and herbivory) and abiotic factors (light, temperature, nutrients, and water availability), as well as geographical conditions [29]. According to this hypothesis, prolonged exposure of *P. atlantica* to different environmental factors could lead to genetic modifications between male and female galls. This could result in different biosynthetic pathways and variations in the qualitative and quantitative accumulation of secondary metabolites, which may directly impact the inhibitory activities of *P. atlantica* galls against HPA. Furthermore, galls form as a response of plant tissues to infection by gall-forming insects. To maximize the content of bioactive compounds associated with α-amylase and α-glucosidase inhibition, it is crucial to understand the effects of *P. atlantica*-pathogen and *P. atlantica*-insect interactions, as well as various environmental stresses.

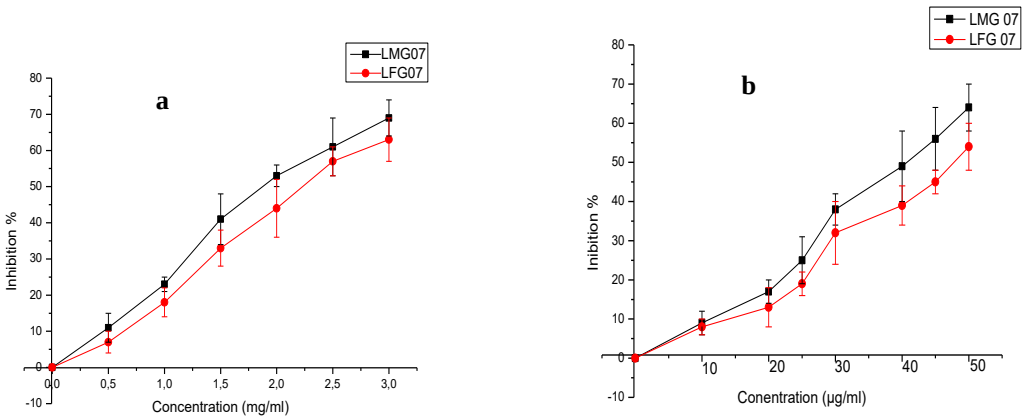


Fig.1 HPA (**a**) and α-glucosidase (**b**) inhibitory activity (expressed as % inhibition) of various concentrations of gall extracts of *P. atlantica* male and female plants collected during the month of July. LMG: Male galls from Laghouat - LFG: Female galls from Laghouat.

Table 1 *In vitro* inhibitory activity of PAG extracts from male and female plants against HPA and α-glucosidase collected in various months, expressed as IC_{50}s values in mg/mL and µg/mL, respectively.

Samples	Months	HPA inhibition IC_{50}s (mg/mL)	α-glucosidase inhibition IC_{50}s (µg/mL)
LMG07	July	1.85^{a}± 0.21	40±8
LMG08	August	2.37^{ab}± 0.32	37±6
LMG09	September	2.17^{ab}± 0.16	34±7
LMG10	October	2.06^{a}± 0.17	42±5
LMG11	November	2.66^{b}± 0.26	39±6
LFG07	July	2.21^{a}± 0.17	49±6
LFG08	August	2.55^{b}± 0.13	42±3
LFG09	September	2.86^{c}± 0.10	44±11
LFG10	October	2.31^{ab}± 0.15	47±7
LFG11	November	2.92^{c}± 0.17	45±4

Values represent mean ± standard deviation of triplicate analyses. Values in one column with a different superscript differ significantly ($p < 0.05$) according to the Duncan post-hoc test. Superscripts a→ b→ c → d: indicate decreasing activities (increasing IC_{50}s). LMG: Laghouat male gall, LFG: Laghouat female gall

PAG extracts from both male and female *P. atlantica* plants also dose-dependently inhibited α-glucosidase at concentrations ranging from 10-50 µg/mL. The IC_{50}s values of PAG extracts collected from July to October ranged from 34 to 42 µg/mL and from 42 to 49 µg/mL for male and female plants, respectively. Extracts prepared from male plants collected in September (LMG09) demonstrated the highest α-glucosidase inhibitory activity with the lowest IC_{50}s (34± 7) of all samples.

One-way ANOVA and Duncan *post-hoc* tests showed no significant difference in the α-glucosidase inhibitory activity between different harvest months within each gender. Likewise, the paired-*t*-test showed no statistical significance (p =0.071) of gender differences on the α-glucosidase inhibitory activity of the samples (Fig. 1b, Fig. S2, Table 1). Overall, galls collected from male plants showed a higher potential to inhibit HPA and α-glucosidase than those from female plants. This suggests that the antidiabetic properties of both genders of *P. atlantica* galls are linked to their antioxidant activity. Previous studies have reported that male galls exhibit higher antioxidant activity than female galls [21]. These antioxidants in *P. atlantica* galls are mainly phenolic compounds. Taken together, these results indicate that the inhibitory activities against these enzymes observed in our experiments could be attributed to the presence of phenolic compounds in the gall extracts. The health-promoting mechanisms of these compounds seem to be related to their antioxidant capacity and their ability to inhibit certain enzymes associated with chronic diseases. Our findings showed that the influence of gender on the HPA and α-glucosidase inhibitory activities of *P. atlantica* galls was more significant than the harvest time. In addition, both male and female PAG extracts were about 60-fold more efficient at inhibiting α-glucosidase than HPA, in agreement with previous studies [23]. We have previously reported the inhibitory activity of PAG against HPA [22]. This difference in inhibition is beneficial because, as reported by Kim et al [30], high HPA inhibition is undesirable as it could lead to intestinal disorders. However, the high α-glucosidase and mild HPA

inhibitory properties of *Pistacia atlantica* galls may provide the foundation for a particularly effective therapy for postprandial hyperglycemia with minimal side effects such as flatulence, abdominal distention, meteorism, and diarrhea. Our results are consistent with claims that plant-derived phytochemicals exhibit lower HPA inhibitory activity and stronger inhibition potential against α-glucosidase [10, 23, 30].

To the best of our knowledge, the present study is the first to report on the inhibitory activity of PAG against an α-glucosidase homologue, including information on the best harvesting period to maximize bioactivity.

UPLC fingerprinting of PAG extracts and identification of potential active metabolites

UPLC chromatograms from 10 samples were optimized and developed using the procedure outlined in the method section. Correlation optimized warping (COW) was employed to align these chromatograms (Fig. 2). Column centering (CC), normalization, and standard normal variate (SNV) were then applied [23]. Following the pre-treatment of the chromatograms, PLS calibration was used to model the HPA /α-glucosidase inhibitory activities of the studied extracts and identify the peaks in the LC-chromatograms corresponding to potential active metabolites (Figs. 3 and 4). Negative regression plots provide insights into the bioactive compounds responsible for α-amylase and α-glucosidase activities in *P. atlantica* gall extracts. The optimal model complexity for each antidiabetic model was determined by selecting the leave-one-out cross-validation with a low RMSECV value (Tables S1).

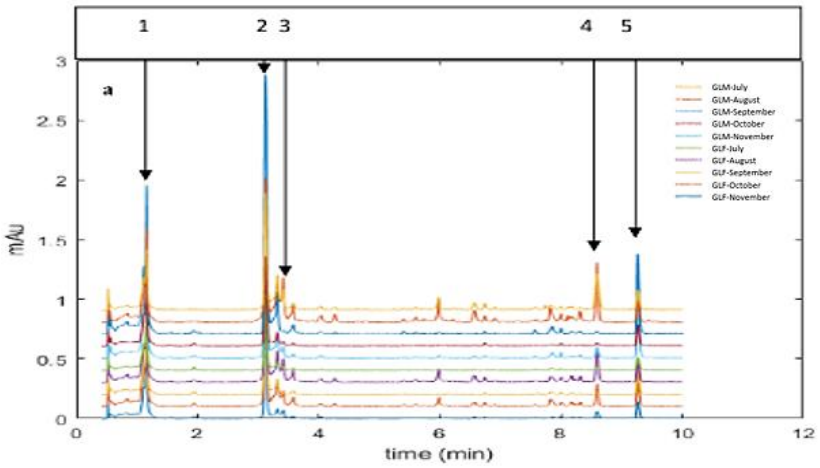


Fig.2 Pre-processed chromatograms of 10 PAG extracts, acquired on an Acquity BEH C18 UPLC column. See the Materials and methods section for the experimental conditions.

Peaks labelled as **2**, **3**, **4,** and **5** (eluting at 3.12, 3.39, 8.61 and 9.2 min, respectively) displayed negative regression coefficients in the HPA and the α-glucosidase inhibition models, suggesting their putative inhibitory activity on both enzymes. On the other hand, the peak labelled as **1** (eluting at 1.16 min) had a negative regression coefficient in the α-glucosidase model but a positive one in the HPA model, suggesting selective inhibitory activity on α-glucosidase only (Figs. 3 and 4).

The information obtained from Figs. 3-4 also reveals that the number of compounds inhibiting α-amylase (5 compounds) was higher than those inhibiting α-glucosidase (4 compounds). These results suggest that the relationship between anti-amylase or anti-glucosidase compounds and antidiabetic activity is complex. The presence of numerous antidiabetic compounds does not necessarily correlate with a high measured antidiabetic activity. The structure of the anti-amylase and anti-glucosidase compounds plays a crucial role in their enzyme

inhibitory effects. Hence, the *P. atlantica* gall extract containing a high number of anti-amylase compounds did not necessarily yield the lowest IC_{50}s value (Table 1).

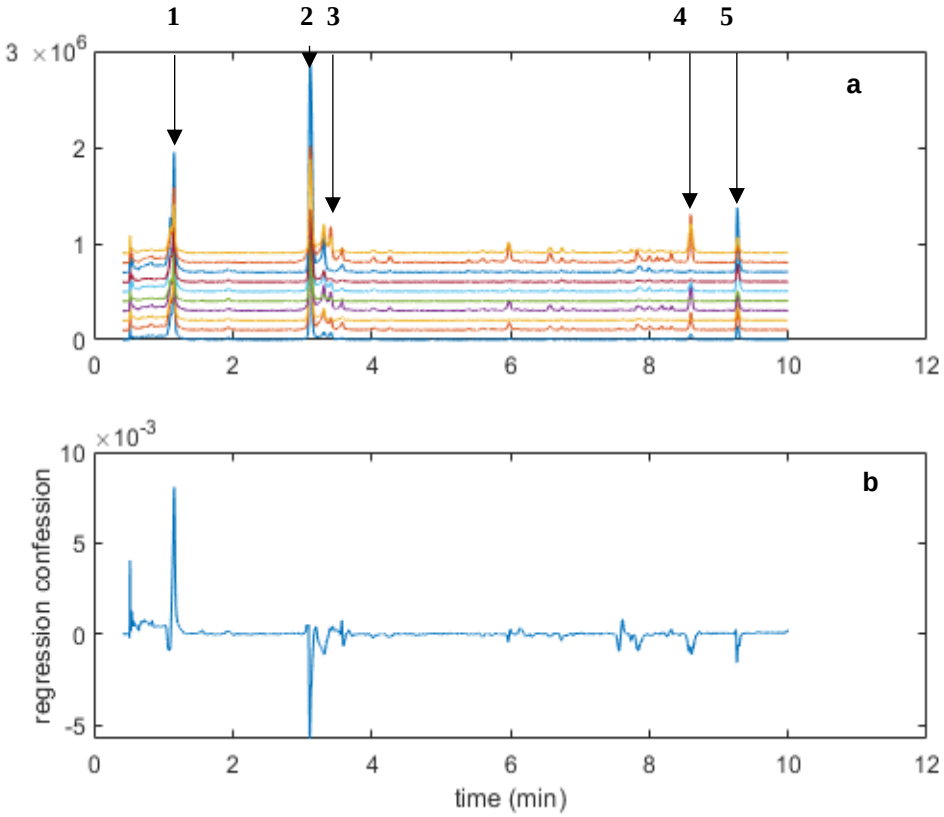


Fig. 3 (a) UPLC chromatogram and (b) PLS regression coefficients after SNV and column centering of the 10 PAG extracts evaluated in the HPA inhibitory activity model.

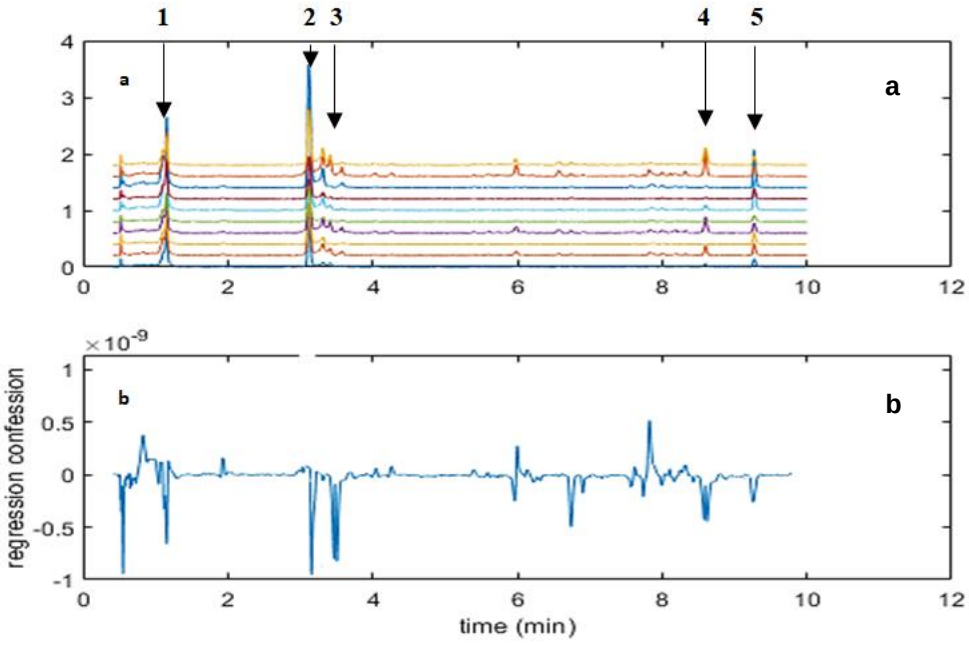
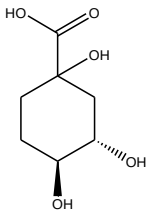


Fig. 4 (a) UPLC chromatogram and (b) PLS regression coefficients after SNV and column centering of the 10 PAG extracts evaluated in the α-glucosidase inhibitory activity model.

The galls on *P. atlantica* leaves occur as a result from attacks from insects such as chalcid wasps and aphids [21]. As plants co-evolved to interact with such insect pests, it is reasonable to assume that the activity observed for the PAG extracts in the present study is linked to the presence of defensive metabolites providing protection to *P. atlantica* against HPA and α-glucosidase in insects [31, 32].

LC is a useful method to determine the chemical composition (fingerprinting) and to assess the quality of herbal extracts [31]. UPLC quadrupole-time-of-flight (QToF) mass spectrometry combined with multivariate calibration techniques such as PLS analysis has previously been employed to rapidly screen for the presence of potential bioactive compounds from complex plant mixtures [23]. In our previous work, we have successfully adopted such methodology to screen *P. atlantica* for putative bioactive compounds [23, 31, 33, 34] and tentatively identified peaks **1**, **2**, **3**, **4**, and **5** as quinic acid, methyl gallate, digalloyl quinic acid, methyl digallate and valoneic acid dilactone, respectively [21] (Fig.5).

Among these phenolic compounds, quinic acid, methyl gallate and digalloylquinic acid were previously detected in *P. atlantica* leaf extracts and predicted to possess inhibitory activity on HPA and/or α-glucosidase [23]. Studies have previously demonstrated that several natural extracts rich in phenolic compounds are able to effectively inhibit HPA and α-glucosidase [35-39] and that many polyphenols can bind to (and inhibit) amylolytic enzymes and/or to starch itself, forming complexes that delay starch digestibility and in turn glucose absorption during food digestion [40].



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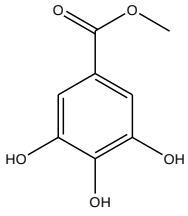

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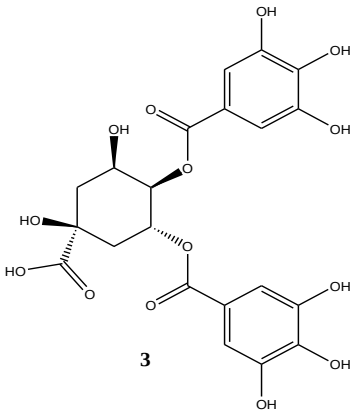

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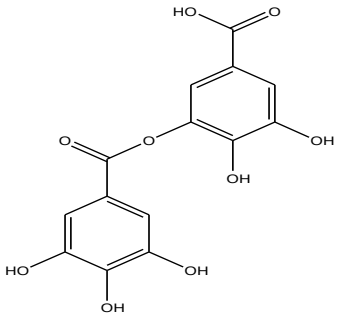

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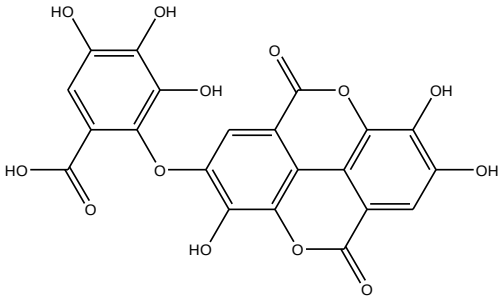

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Fig. 5 Chemical structures of the phenolic compounds, quinic acid **(1)**, methyl gallate **(2)**, digalloylquinic acid **(3)**, methyl digallate **(4)**, and valoneic acid dilactone **(5)**, identified from *P. atlantica* galls.

Molecular docking studies

The binding affinity of compounds (**1-5**) for the α-glucosidase homologue ranged between -6.5 and -10.4 kcal/mol, with valoneic acid dilactone (**5**) showing the best docking score (-10.4 kcal/mol). The specific molecular interactions of the best fit docked poses for (**1-5**) with the α-glucosidase homologue are detailed in Table 2 and Fig. 6.

Table 2 Docking scores (binding energies) and molecular interactions of compounds (**1-5**) with the α-glucosidase homologue.

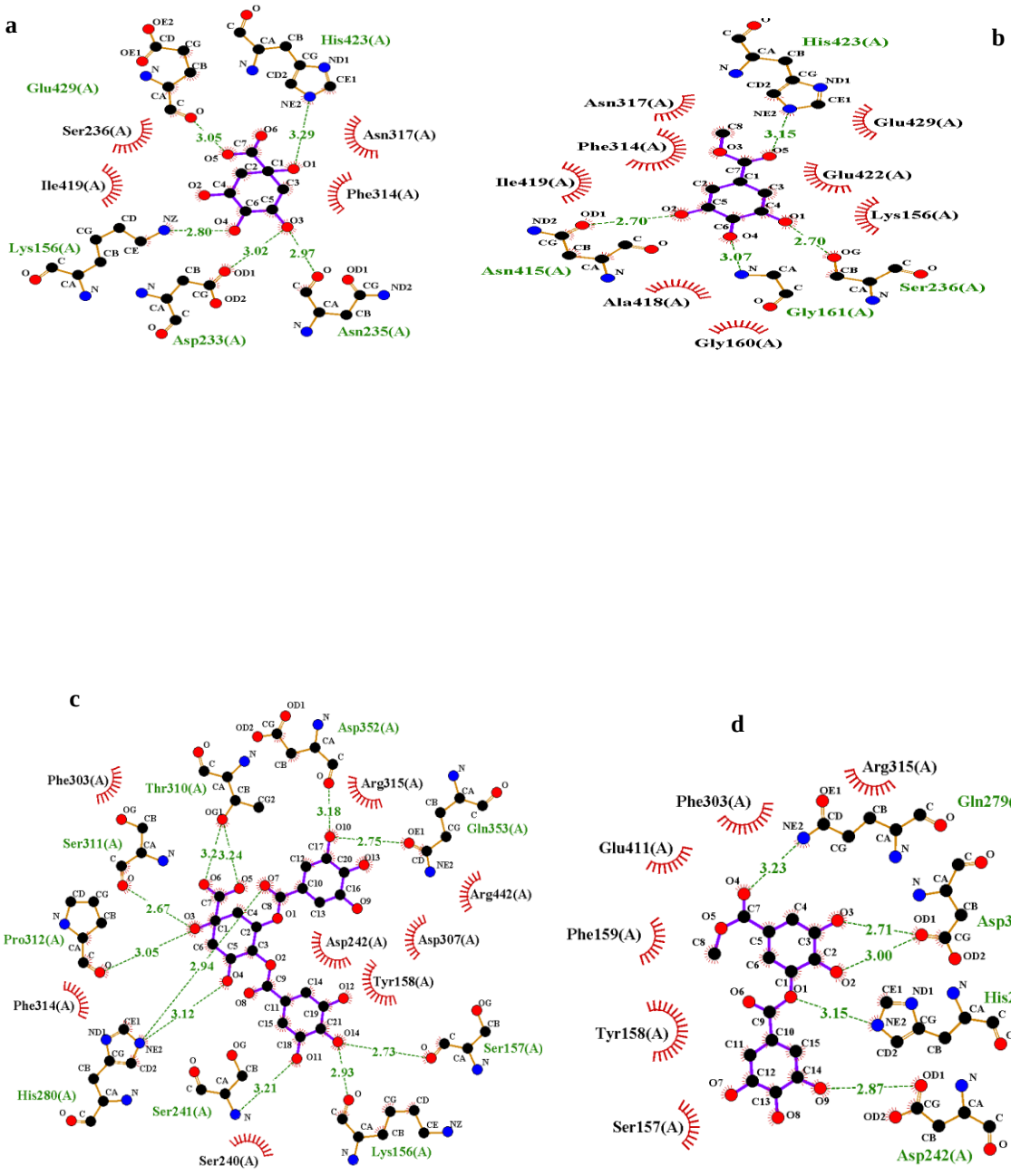
Compound	Docking score (kcal/mol)	Category	Interacting residues	Distance (Å)
Quinic acid (1)	-6.6	Conventional H-Bond	Lys156	2.80
			Asp233	3.02
			Asn235	2.97
			His423	3.29
			Glu429	3.05
		Hydrophobic	Ser236	
			Phe314	
			Asn317	
			Ile419	
Methyl gallate (2)	-6.5	Conventional H-Bond	Gly161	3.07
			Ser236	2.70
			Asn415	2.70
			His423	3.15

		Hydrophobic	Lys156	
			Gly160	
			Phe314	
			Asn317	
			Ala418	
			Ile419	
			Glu422	
			Glu429	
Digalloylquinic acid (3)	-9.4	Conventional H-Bond	Lys156	2.93
			Ser157	2.73
			Ser241	3.21
			His280 (2)	2.94/3.12
			Thr310 (2)	3.23/3.24
			Ser311	2.67
			Pro312	3.05
			Asp352	3.18
			Gln353	2.75
		Hydrophobic	Tyr158	
			Ser240	
			Asp242	
			Phe303	
			Asp307	
			Phe314	
			Arg315	
			Arg442	
Methyl digallate (4)	-8.0	Conventional H-Bond	Asp242	2.87
			Gln279	3.23
			Asp307 (2)	2.71/3.00
			His280	3.15
		Hydrophobic	Ser157	
			Tyr158	
			Phe159	
			Phe303	
			Arg315	

			Glu411	
Valoneic acid dilactone (5)	-10.4	Conventional H-Bond	Tyr158	2.89
			Ser240	3.07
			Glu277	3.04
			His280	2.87
			Asp352	2.70
			Glu411(2)	2.89/3.13
		Hydrophobic	Lys156	
			Phe159	
			Phe178	
			Asp242	
			Phe303	
			Arg315	
			Gln353	
			Arg442	

Quinic acid (**1**) showed five H-bond interactions with five amino acids residues of the α-glucosidase homologue (Lys156, Asp233, Asn235, His423, Glu429) at distances ranging from 2.80 to 3.29 Å. It also showed hydrophobic interactions with Ser236, Phe314, Asn317 and Ile419 (Fig. 6a). Methyl gallate (**2**) formed four H-bonds with four amino acids (Gly161, Ser236, Asn415, His423) at distances ranging from 2.70 to 3.15 Å. It also interacted with the α-glucosidase homologue via hydrophobic interactions with Lys156, Gly160, Phe314, Asn317, Ala418, Ile419, Glu422 and Glu429 (Fig. 6b). Digalloyl quinic acid (**3**) formed eleven H-bonds with nine amino acids (Lys156, Ser157, Ser241, His280, Thr310, Ser311, Pro312, Asp352, Gln353) at distances ranging from 2.67 to 3.24 Å. It also interacted with Tyr158, Ser240, Asp242, Phe303, Asp307, Phe314, Arg315 and Arg442 through hydrophobic interactions (Fig. 6c). Methyl digallate (**4**) showed five H-bond interactions with four amino acids (Asp242, Gln279, His280 and Asp307) at distances ranging from 2.71 to 3.23 Å. It also showed hydrophobic interactions with Ser157, Tyr158, Phe159, Phe303, Arg315 and Glu411 (Fig. 6d). Valoneic acid dilactone (**5**) formed seven H-bond interactions with six amino acids (Tyr158, Ser240, Glu277, His280, Asp352, Glu411) at distances ranging from 2.70 to 3.13 Å. It also showed hydrophobic interactions with Lys156, Phe159, Phe178, Asp242, Phe303, Arg315, Gln353 and Arg442 (Fig. 6e).

Previous reports have revealed that Asp69, His112, Arg213, Asp215, Glu277, His351, Asp352, Arg442, and Glu411 were the key amino acid residues involved in the catalytic activity of the α-glucosidase homologue [41-43]. Here, we observed that valoneic acid dilactone (**5**) formed a high number of H-bond interactions (7 in total) with the target protein and also had the highest number of H-bond and hydrophobic interactions with key active site residues (Glu277, Asp352, Glu411 and Arg442). This may explain its high docking score (predicted biding affinity) for the α-glucosidase homologue.


a
Glu429(A)
His423(A)
Ser236(A)
Asn317(A)
Ile419(A)
Phe314(A)
Lys156(A)
Asp233(A)
Asn235(A)
3.05
3.29
2.80
3.02
2.97
b
His423(A)
Asn317(A)
Glu429(A)
Phe314(A)
Glu422(A)
Ile419(A)
Lys156(A)
Asn415(A)
Ala418(A)
Gly161(A)
Ser236(A)
Gly160(A)
3.15
2.70
2.79
3.07
c
Asp352(A)
Phe303(A)
Thr310(A)
Arg315(A)
Gln353(A)
Ser311(A)
Arg442(A)
Pro312(A)
Asp242(A)
Asp307(A)
Tyr158(A)
Phe314(A)
Ser157(A)
His280(A)
Ser241(A)
Ser240(A)
Lys156(A)
3.18
3.23
3.24
2.75
2.67
3.05
2.94
3.12
2.73
2.93
3.21
d
Arg315(A)
Phe303(A)
Gln279(A)
Glu411(A)
Phe159(A)
Asp307(A)
Tyr158(A)
His280(A)
Ser157(A)
Asp242(A)
3.23
2.71
3.00
3.15
2.87

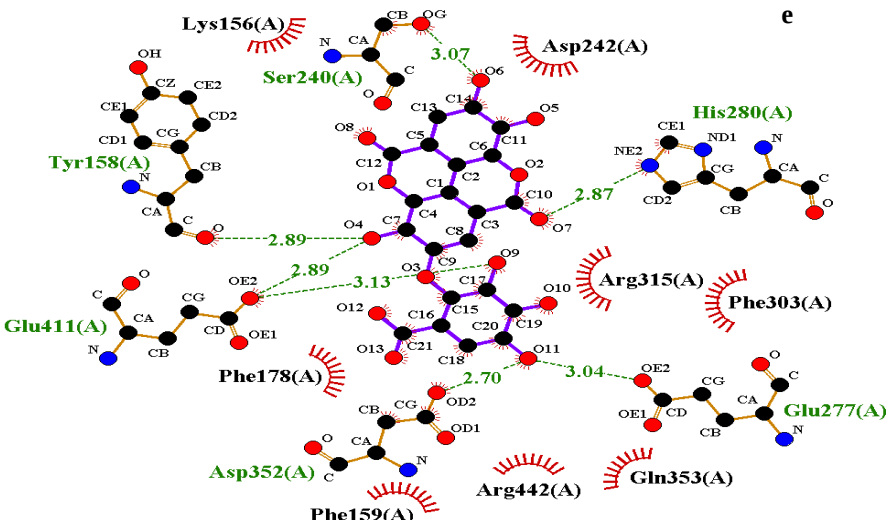


Fig. 6 Molecular docking analysis showing the interactions of quinic acid (**a**), methyl gallate (**b**), digalloyl quinic acid (**c**), methyl digallate (**d**) and valoneic acid dilactone (**e**) with the binding site of the α-glucosidase homologue. Green dotted lines show H-bondings with amino acids in green. Red spikes show hydrophobic interactions with amino acids in black.

The binding affinity of compounds (**1-5**) for HPA ranged between -5.4 and -8.7 kcal/mol, with valoneic acid dilactone (**5**) showing the best docking score (-8.7 kcal/mol). The specific molecular interactions of the best fit docked poses for (**1-5**) with HPA are detailed in Table 3 and Fig. 7.

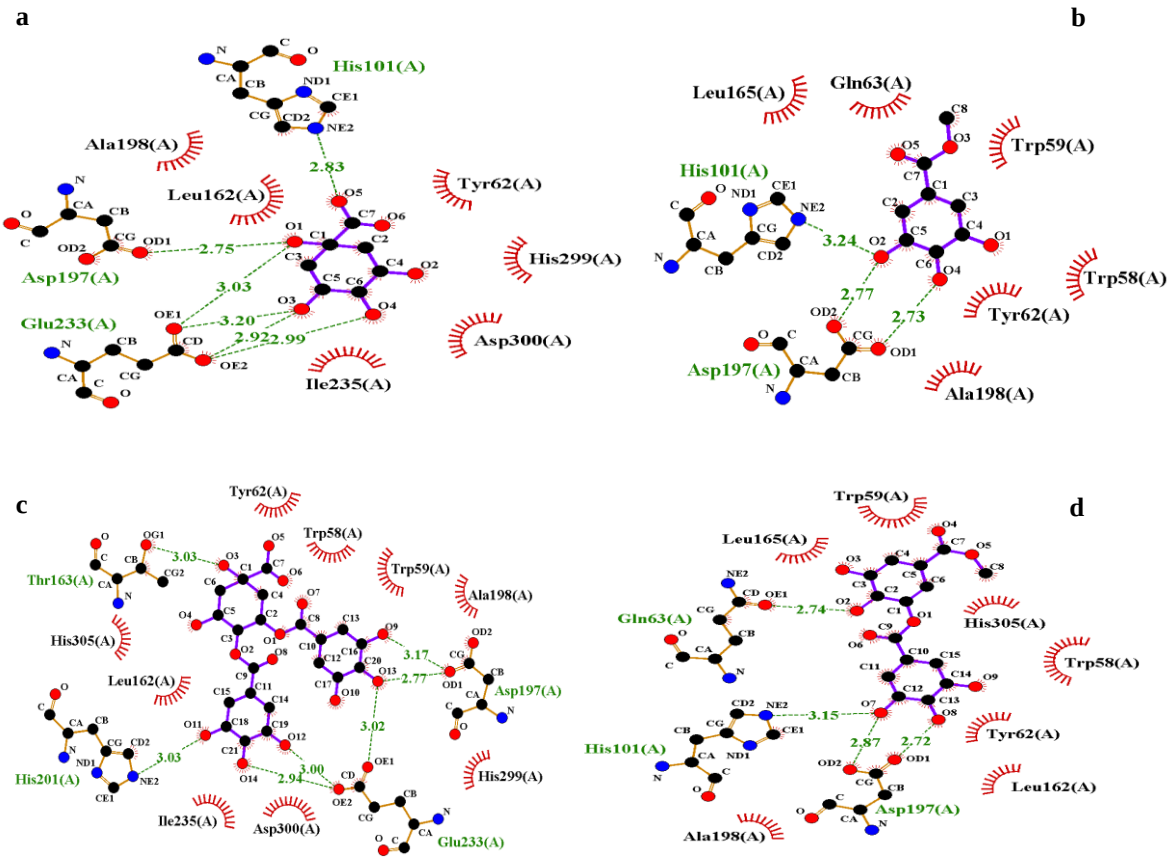
Table 3 Docking scores (binding energies) and molecular interactions of compounds (**1-5**) with HPA.

Compound	Docking score (kcal/mol)	Category	Interacting residues	Distance (Å)
Quinic acid (1)	-5.5	Conventional Hydrogen Bond	His101	2.83
			Asp197	2.75
			Glu233 (4)	2.92/2.99/3.03/3.20
		Hydrophobic	Tyr62	
			Leu162	
			Ala198	
			Ile235	
			His299	
			Asp300	
Methyl gallate (2)	-5.4	Conventional Hydrogen Bond	His101	3.24
			Asp197(2)	2.73/2.77
		Hydrophobic	Trp58	
			Trp59	
			Tyr62	
			Gln63	

			Leu165	
			Ala198	
Digalloylquinic acid (3)	-7.6	Conventional Hydrogen Bond	Thr163	3.03
			Asp197(2)	2.77/3.17
			His201	3.03
			Glu233 (3)	2.94/3.00/3.03
		Hydrophobic	Trp58	
			Trp59	
			Tyr62	
			Leu162	
			Ala198	
			Ile235	
			His299	
			Asp300	
			His305	
Methyl digallate (4)	-7.5	Conventional Hydrogen Bond	Gln63	2.74
			His101	3.15
			Asp197(2)	2.72/2.78
		Hydrophobic	Trp58	
			Trp59	
			Tyr62	
			Leu162	
			Ala198	
			His305	
Valoneic acid dilactone (5)	-8.7	Conventional Hydrogen Bond	Glu233	3.10
		Hydrophobic	Trp58	
			Trp59	
			Thr163	
			Leu165	
			Ile235	
			His299	

	Asp300
	His305
	Asp356

Quinic acid (**1**) showed six H-bond interactions with three amino acids residues of HPA (His101, Asp197 and Glu233) at distances ranging from 2.75 to 3.20 Å. It also showed hydrophobic interactions with Tyr62, Leu162, Ala198, Ile235, His299 and Asp300 (Fig.7a). Methyl gallate (**2**) formed four H-bonds with two amino acids (His101 and Asp197) at distances ranging from 2.73 to 3.24 Å. It also interacted with HPA via hydrophobic interactions with Trp58, Trp59, Tyr62, Leu162, Ala198, Ile235, His305, His299 and Asp300 (Fig.7b).



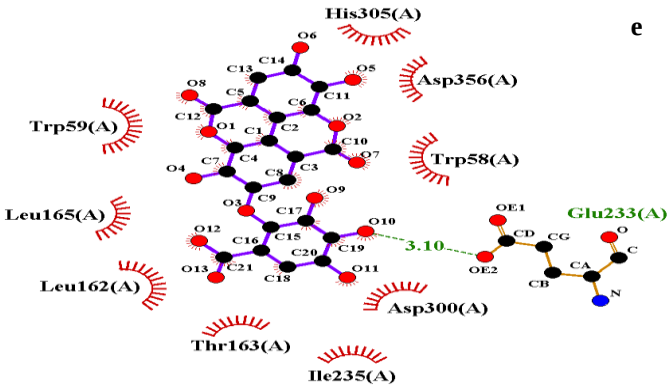


Fig.7 Molecular docking analysis showing the interactiona of quinic acid (**a**), methyl gallate (**b**), digalloyl quinic acid (**c**), methyl digallate (**d**) and valoneic acid dilactone (**e**) with the binding site of HPA. Green dotted lines show H-bondings with amino acids in green. Red spikes show hydrophobic interactions with amino acids in black.

Digalloyl quinic acid (**3**) formed seven H-bonds with four amino acids (Thr163, Asp197, His201, Glu233) at distances ranging from 2.77 to 3.17 Å. It also interacted with the target enzyme through hydrophobic interactions with Trp58, Trp59, Tyr62, Leu162, Ala198, Ile235, His299, Asp300 and His305 (Fig.7c). Methyl digallate (**4**) showed four H-bond interactions with three amino acids (Gln63, His101, Asp197) at distances ranging from 2.72 to 3.15 Å. It also showed hydrophobic interactions with Trp58, Trp59, Tyr62, Leu162, Leu165, Ala198 and His305 (Fig.7d). Valoneic acid dilactone (**5**) formed one H-bond interaction with Glu233 with a bond length of 3.10 Å. It also formed several hydrophobic interactions with Trp58, Trp59, Leu162, Thr163, Leu165, Ile235, Asp300, His305 and Asp356 (Fig.7e).

Previous reports have indicated that Asp197, Glu233 and Asp300 are three essential residues of the catalytic site of HPA that are involved in the hydrolysis of carbohydrates [44-46]. Other important residues that have showed interactions to HPA in previous docking studies involving natural products include Trp58, Trp59, Tyr62, Gln63, His101, Ala106, Tyr151, Thr163, Gly164, Leu165, Arg195, His201, Ile235 and His299 [47, 48]. Our results revealed that although all compounds showed interactions with at least one of the residues of the catalytic triad (Asp197, Glu233, and Asp300), only quinic acid (**1**) and digalloyl quinic acid (**3**) interacted with each of these residues. Digalloyl quinic acid (**3**) formed the highest number of interactions (two H-bonding and five hydrophobic interactions) with other important residues of this enzyme.

Inhibitors of HPA and α-glucosidase exert their effect by binding either to amino acids within the active site of these enzymes (i.e. competitive inhibition) or to residues nearby, effectively blocking entrance to the active site (i.e non-competitive inhibition). This binding is influenced by the number and type of molecular (hydrogen and hydrophobic) interactions between ligands and specific amino acids [49].

Structural activity relationships (SARs)

The structure-inhibitory potency relationships of the identified compounds suggests that the hydroxyl group of aryl ring and galloyl groups may play a major role in α-glucosidase and α-amylase inhibition. For instance, when comparing the binding results of quinic acid and galloyl derivatives of quinic acid with α-glucosidase and α-amylase, we found that the binding affinity raised as the number of galloyl groups on the quinic increased, such as in the digalloyl quinic acid. This increase may be due to the galloyl group possesses three hydroxyl groups, which are potentially important for hydrogen bonding, and an aromatic moiety, which is important for hydrophobic interactions. Methyl digallate with five hydroxyl group attached to an aromatic rings and two

carboxylic acids esterified with gallic acid and methyl alkyl was observed to be more efficient than methyl gallate (Fig.5), proving an advantageous influence of galloyl group on the scavenging α-glucosidase and α-amylase activities of gallic acid derivatives. It is noted worthy that quinic acid had activity comparable to that of the methyl gallate, despite the absence of galloyl groups. This result established that the carboxylic acid esterified and free carboxylic group did not influence the inhibitory effect on the enzyme. This is probably because the activity is related to the hydroxyl groups present in both compounds. As shown in Fig. 5, the number of hydroxyl groups in the aryl ring of the identified compounds of *P. atlantica* gall extract are ranked in the following order: valoneic acid dilactone = digalloyl quinic acid > Methyl digallate > Methy gallate, which was consistent with the results of the binding affinities. Our results confirmed that the five identified compounds of *P. atlantica* gall extract had the ability to enter into the active site of the α-glucosidase and HPA and further inhibited the catalytic action of the enzyme through hydrogen bonding and hydrophobic interactions, which was consistent with a previous study reporting that the α-glucosidase and HPA inhibitors possess multiple hydroxyl group attached to an aromatic rings that become the reason for their high activity [50-52].

Conclusion

This study demonstrated the HPA and α-glucosidase inhibitory potential of *Pistacia atlantica* gall extracts. It was observed that inhibition against HPA and α-glucosidase was more influenced by plant gender than by harvest time, with galls collected from male plants showing a higher inhibitory potential. The screened extracts were found to be approximatively 60-fold more efficient at inhibiting α-glucosidase than HPA. UPLC-QTOF-MS fingerprinting of the studied extracts, linked to PLS regression analysis, enable the putative identification of five compounds, namely quinic acid (**1**), methyl gallate (**2**), digalloyl quinic acid (**3**), methyl digallate (**4**) and valoneic acid dilactone (**5**), with potential inhibitory activity on HPA and/or α-glucosidase. Compound **5** showed the best predicted biding affinity for the α-glucosidase homologue, interacting with key residues of the active site of this enzyme. It also showed the best docking score against HPA. Quinic acid (**1**) and digalloyl quinic acid (**3**) showed interactions with each of the residues of the catalytic triad of this enzyme. To the best of our knowledge, this is the first report on the prediction of the binding affinity of PAG phytochemicals towards HPA and α-glucosidase. Further in-depth investigations including *in vitro* and *in vivo* work on the active compounds identified in this study are warranted to confirm the potential of *P. atlantica* galls in the management of diabetes.

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Contributors' statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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