In Vitro and Molecular Docking Studies of Antiglycation Potential of Phenolic Compounds in Date Palm (*Phoenix dactylifera* L.) Fruit: Exploring Local Varieties in the Food Industry

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Abstract: The Moroccan date-growing sector is rich in a wide diversity of varieties but faces major challenges, notably the undervaluation of certain varieties intended mainly for animal feed. In this study, our objective was to evaluate the antiglycation activity of four date varieties, including three low-market-value varieties and one high-market-value variety, harvested during two seasons (2021 and 2022). In addition, to improve our knowledge of the antiglycation potential, molecular docking analyses were carried out. The results of the antiglycation activity of the date extracts showed strong activity, particularly for the 'Khalt Khal' variety, which showed a 50% inhibition concentration (IC50) of 1.83 mg/mL and 2 mg/mL in 2021 and 2022, respectively. In addition, the molecular docking analysis also showed the possible link between the bioactive compounds identified and their mechanisms of action. Our findings suggest new evidence for the antiglycation properties of the bioactive compounds present in dates. These results suggest the use of these varieties as a source of bioactive molecules or as a food additive. This could make it possible to create medicines or food products with a high commercial value using dates, which could help to treat the complications associated with glycation.

Keywords: date palm fruit; harvesting season; bioactive compounds; antiglycation activity; docking studies; food

1. Introduction

Dates are produced mainly in the hot desert regions of southwest Asia and North Africa and are marketed around the world as a high-value fruit. With the current uncertainty of the world food supply and the expected increase in demand, date palms should continue to provide a good source of low-cost food [1]. World date production rose from around 6.44 million tons in 2000 to 9.65 million tons in 2021. Egypt, Saudi Arabia, and Iran are the main producing countries, while Morocco ranks 13th, with production of around 150,301 tons in 2021 [2]. The socio-economic value of dates is particularly recognized in oases, where date palms have historically served as a means of exchange between local...
populations. Despite several reports on the chemical composition and nutritional value of dates, there are still many other potential benefits to be explored.

Given the significant global demand for dates and the burgeoning health crisis posed by diseases like diabetes, it is imperative to explore the untapped potential of date varieties, particularly those currently undervalued in the market, through rigorous scientific inquiry.

Dates are rich in phenolic compounds, offering both quality and quantity, thus offering a wide range of potential new uses [3–6].

These compounds, including phenolic acids, flavonoids, anthocyanins, procyanidins, carotenoids, and sterols, provide numerous health benefits [7,8]. Traditional medicine has long utilized various parts of the date fruit, such as its pulp and pit, to treat hypertension and diabetes [9]. Additionally, dates are valued in popular medicine for their laxative effects and therapeutic use in conditions like asthma, fever, fatigue, tuberculosis, loss of consciousness, respiratory diseases, and thoracic pain [10–12]. Extensive preclinical research on date fruit extract has highlighted its wide-ranging health benefits, including antioxidative, antimitogenic, antimicrobial, anticancer, anti-inflammatory, gastroprotective, hepatoprotective, nephroprotective, immunostimulant, anti-diabetic, and antiglycation activities [7,13–15].

Diabetes stands as a significant global health concern, with a staggering impact projected for the coming years. In 2021 alone, an estimated 537 million adults between the ages of 20 and 79 were anticipated to be affected, a number expected to soar to 643 million by 2030 [16,17]. Alarmingly, about 75–80% of people with diabetes die from complications, mostly related to cardiovascular issues [18]. Hyperglycemia is central to the pathogenesis of diabetes, as it subjects plasma proteins such as hemoglobin, serum albumin, and transferrin to prolonged exposure to glucose in patients with inadequate glycemic control. As a result, these proteins undergo glycation, a chemical modification induced by glucose [19,20].

Glycation is a spontaneous, non-enzymatic process that occurs between the amino groups present in the amino acid residues of proteins and reducing sugars. This phenomenon intensifies in the event of hyperglycemia, leading to the formation and accumulation of advanced glycation end products (AGEs) [21,22]. By disrupting proteins and altering their function, AGEs can also interact with the AGE receptor (RAGE), a cell surface receptor [23,24]. This interaction activates various intracellular downstream signaling pathways, resulting in the production of free radicals that contribute to the complications associated with diabetes [25].

In Morocco, date palm cultivation is thriving in the southern Atlas region [26]. Nevertheless, the sector faces a number of challenges, including the low market value of certain varieties intended for animal feed due to their appearance and small shape, which are not much appreciated by consumers [27]. It is therefore essential to exploit them through research into bioactive substances and assessment of the biological activity of these substances to enable these varieties to be used in the pharmaceutical industry as a source of bioactive molecules, to be transformed into a product with high added value, or to be used as a food additive.

Our aim was to analyze phenolic compounds using HPLC-UV-VIS (high-performance liquid chromatography with ultraviolet–visible detection) and assess the antiglycation properties of four date varieties, including three of low market value (‘Khalt Khal’, ‘Jdar Lahmer’, and ‘Rasse Tmar’) and one of high market value (‘Majhoul’). These varieties were harvested over two consecutive seasons, in 2021 and 2022. Ultimately, an in silico analysis was conducted to enhance our understanding of how the bioactive compounds identified in dates bind to BSA and RAGE as target proteins. This investigation will facilitate our exploration of the potential to utilize inexpensive dates as a source of molecules applicable in the pharmaceutical and food sectors.
2. Materials and Methods

2.1. Plant Material

During two consecutive seasons (23 October 2021 and 23 October 2022), the fruits (*Phoenix dactylifera* L.) were collected at the Tamar stage in two regions, Zagora and Erfoud. In Morocco, these regions have an arid and semi-arid climate, making them ideal climatic conditions for this tree. Four varieties were considered: ‘Khalt Khal’, ‘Jdar Lahmer’, and ‘Majhoul’ from the Zagora region and ‘Rasse Tmar’ from the Erfoud region.

The palms to be sampled for each variety were selected using the diagonal sampling method. On site, with sampling points arranged diagonally, a constant distance of 10 m was maintained across all study sites. Thanks to a consistent selection of trees, the length and spacing of the trees were uniform across the different sites. Dates were harvested from several plants of the same species, from different clusters.

Immediate steps were taken after harvesting to guarantee the quality of the fruit during transport to the laboratory. The integrity of the samples was preserved by carefully storing them in a cool box. When the harvested fruit arrived at the laboratory at the Semlalia Faculty of Sciences in Marrakech, Cadi Ayyad University, it was quickly preserved at a temperature of $-20^\circ$C. The fast preservation process played an essential role in preserving the quality of the fruit harvested. It was essential to implement this rapid preservation method in order to preserve the phenolic compounds and avoid any deterioration [28].

2.2. Sample Preparation

The ethanol extraction of phenolic compounds was carried out according to the method of [13] with slight modifications. The choice of ethanol as extraction solvent was justified by its polar properties and non-toxic nature, and the setting of the ethanol concentration at 60% was based on its efficiency in solubilizing phenolic compounds [29]. In total, 5 g ($\times$3) of date pulp was ground and macerated at room temperature (4 h) with stirring in 50 mL of 60% ethanol. The mixture was centrifuged at 4000 rpm for 20 min. The supernatant was collected, and the same procedure was repeated three times on the pellet. The supernatant was concentrated under reduced pressure, with the extract recovered and stored at $-20^\circ$C.

2.3. Phenolic Compound Analysis by HPLC-UV-VIS

For the HPLC-UV-VIS analysis, a SHIMADZU high-performance liquid chromatograph was used, according to the method described by Bonifácio-Lopes et al. [30] and Vit et al. [31]. The phenolic compounds were assessed by high-performance liquid chromatography (SHIMADZU, Kyoto, Japan), using a C18 column (250 mm $\times$ 4.6 mm) at 30 $^\circ$C and a UV/VIS PDA, SPD, M20A detector (SHIMADZU, Kyoto, Japan). The mobile phase was 5% formic acid (solvent A) and 5% methanol (solvent B). The mobile phase gradient used was as follows: 0 min, 5% B; 55 min, 100% B; and 55–60 min, 100% B. The flow rate was 1 mL/min, the injection volume was 20 $\mu$L for each extract ($\times$3 replicates), the detection length was 280 nm, and the analysis time was 60 min. Each peak was identified using the retention time of the standards used. The results of this section have already been published [32], and the data from this study were used to establish links between the antiglycation activity and the phenolic compounds present in dates. In addition, these identified phenolic compounds were used in the molecular anchoring study.

2.4. In Vitro Glycation Inhibition Test

For the determination of the glycation inhibitory activity of fructose-induced proteins (AGEs), the method published by Fernando et al. [33] was used with minor modifications. In total, 1 mL of fructose was incubated (1000 mM in 200 mM phosphate buffer pH 7.4) with 1.25 mL BSA (20 mg/mL in 200 mM phosphate buffer pH 7.4), 0.25 mL extract (at different concentrations), and 2.5 mL phosphate buffer (200 mM, pH 7.4) at 37 $^\circ$C for 7 days. A control was prepared using only BSA and fructose (the reagents used in this experiment were purchased from Sigma-Aldrich Products: ‘Sigma-Aldrich, St. Louis, MO, USA’). The fluorescence emission of each mixture was measured with an excitation and emission
wavelength of 370 nm and 445 nm, respectively, using a fluorescence spectrometer (the experiment was conducted in three replicates for each extract and for each concentration). Antiglycation activity was measured using the following equation:

\[
\% I = \left(\frac{\text{Control intensity} - \text{Sample intensity}}{\text{Control intensity}}\right) \times 100
\]

2.5. Molecular Docking Studies

2.5.1. Ligand Preparation

The chemical structures of 10 ligands detected in date fruits by HPLC-UV-VIS analysis (gallic acid, trans-ferulic acid, 4-hydroxyphenylacetic acid, caffeic acid, vanillic acid, ellagic acid, epicatechin, catechin, vanillin, and kaempferol) were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/ (accessed on 3 April 2024)) in SDF format, then converted into a 3D pdb file using the Open Babel graphical interface (version 2.3.1).

2.5.2. Preparation of the Target Protein

The three-dimensional (3D) structures of the target proteins, namely the crystalline structure of bovine serum albumin (BSA) (PDB ID: 4OR0) and the receptor for advanced glycation end products (PDB ID: 2MOV), were obtained from the Protein Data Bank of the Research Collaboratory for Structural Bioinformatics (RCSB) (https://www.rcsb.org/ (accessed on 3 April 2024)). The PyRx software (Version: PyRx. 0.8) was utilized to prepare the aforementioned proteins and perform molecular docking. The proteins were prepared for docking by removing all heteroatoms and water molecules, adding polar hydrogen atoms, and subsequently assigning Kollman charges.

2.5.3. Molecular Docking between Ligand and Protein

The graphical interface of the PyRx software was used to execute the molecular docking study. The first step was to import and prepare the protein and ligand in the interface and then perform the molecular docking using a grid box dimension of \(x = 144.94, y = 64.26,\) and \(z = 88.72\) and \(x = 28.98, y = 51.61,\) and \(z = 31.43\) with a grid center of \(x = 35.16, y = 23.98,\) and \(z = 98.04\) and \(x = 2.30, y = -6.14,\) and \(z = -12.94\) for BSA and RAGE, respectively. The ligand with the lowest binding energy score was selected for further study. Discovery Studio software version 2021 was used to visualize the docking results.

2.6. Statistical Analysis

Statistical analyses were performed using R software, version (R i386 4.0.5) (R is a programming language and free software for data processing and statistical analysis). The results are presented as the mean of three replicates ± standard deviation.

3. Results

3.1. Antiglycation Activity

The interaction between the aldehyde function of reducing sugars and the free \(\text{NH}_2\) function of an amino acid within a protein or other molecule, in a non-enzymatic way, induces the formation of advanced glycation end products. The accumulation of AGEs in the human body has been correlated with the development of various conditions, such as insulin insensitivity and other cardiovascular complications [34–37]. This interaction also activates several downstream intracellular signaling pathways, initiating the production of free radicals that contribute to the complications associated with diabetes [25].

Our research evaluated the antiglycation efficacy of date palm fruit extracts, including three low-market-value varieties and one high-market-value variety harvested in two seasons, in inhibiting the formation of advanced glycation end products. Based on the intensity of the fluorescence emitted by the samples at 345 nm, indicating the formation of the BSA/fructose complex (Figure 1), our results indicated that the four varieties studied exhibited concentration-dependent antiglycation activity. Higher extract concentrations resulted in greater inhibition of glycation product formation.
Our research evaluated the antiglycation efficacy of date palm fruit extracts, including tannins, phenolic acids, and flavonoids, which have both antioxidant and antiglycation potential, are more effective in the treatment of diabetes mellitus [38]. Consequently, there is great interest in identifying new sources of phytochemicals capable of effectively scavenging free radicals and reducing non-enzymatic glycation.

The analysis of the phenolic compounds presents in the four date varieties harvested in two successive seasons (2021 and 2022) using high-performance liquid chromatography is presented in Table 1 [32]. The standards used in this analysis include gallic acid,
tyrosol, trans-ferulic acid, 4-hydroxyphenylacetic acid, caffeic acid, vanillic acid, ellagic acid, epicatechin, catechin, quercetin, vanillin, and kaempferol.

Table 1. Content of individual phenolic compounds determined by HPLC-UV-VIS, for Moroccan varieties harvested in two successive seasons (mg/100 g DW).

<table>
<thead>
<tr>
<th>Phenolic Compound</th>
<th>Khalt Khal 2021</th>
<th>Khalt Khal 2022</th>
<th>Jdar Lahmer 2021</th>
<th>Jdar Lahmer 2022</th>
<th>Rasse Tmar 2021</th>
<th>Rasse Tmar 2022</th>
<th>Majhoul 2021</th>
<th>Majhoul 2022</th>
</tr>
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<tbody>
<tr>
<td>Gallic acid</td>
<td>9.26 ± 1.55</td>
<td>23.63 ± 0.05</td>
<td>15.55 ± 0.16</td>
<td>8.29 ± 0.02</td>
<td>15.86 ± 0.11</td>
<td>18.46 ± 0.52</td>
<td>10.83 ± 0.59</td>
<td>6.14 ± 0.08</td>
</tr>
<tr>
<td>Trans-ferulic acid</td>
<td>2.67 ± 0.10</td>
<td>nd</td>
<td>1.42 ± 0.02</td>
<td>nd</td>
<td>0.40 ± 0.04</td>
<td>0.77 ± 0.02</td>
<td>0.64 ± 0.04</td>
<td>0.34 ± 0.17</td>
</tr>
<tr>
<td>4-Hydroxyphenylacetic acid</td>
<td>6.09 ± 1.74</td>
<td>9.42 ± 0.60</td>
<td>0.83 ± 0.05</td>
<td>1.13 ± 0.75</td>
<td>4.59 ± 0.01</td>
<td>7.48 ± 0.09</td>
<td>6.89 ± 2.40</td>
<td>1.62 ± 0.36</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>2.26 ± 1.23</td>
<td>1.28 ± 0.23</td>
<td>0.81 ± 0.08</td>
<td>0.89 ± 0.01</td>
<td>1.71 ± 0.14</td>
<td>0.38 ± 0.10</td>
<td></td>
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</tr>
<tr>
<td>Vanillic acid</td>
<td>16.12 ± 3.39</td>
<td>24.36 ± 0.42</td>
<td>9.56 ± 0.71</td>
<td>7.08 ± 2.02</td>
<td>4.36 ± 0.22</td>
<td>3.38 ± 0.20</td>
<td>6.43 ± 1.10</td>
<td>2.78 ± 0.60</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>14.63 ± 5.40</td>
<td>27.33 ± 2.86</td>
<td>5.35 ± 1.76</td>
<td>6.54 ± 0.32</td>
<td>12.23 ± 0.23</td>
<td>9.75 ± 0.23</td>
<td>0.44 ± 0.12</td>
<td>2.13 ± 0.02</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>5.58 ± 1.34</td>
<td>5.30 ± 0.70</td>
<td>1.16 ± 0.10</td>
<td>2.06 ± 0.04</td>
<td>2.81 ± 0.33</td>
<td>1.60 ± 0.02</td>
<td>1.56 ± 0.40</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Catechin</td>
<td>5.37 ± 1.56</td>
<td>9.28 ± 0.53</td>
<td>2.11 ± 0.36</td>
<td>1.28 ± 0.62</td>
<td>2.31 ± 0.71</td>
<td>0.60 ± 0.03</td>
<td>2.59 ± 1.13</td>
<td>0.44 ± 0.20</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1.32 ± 0.20</td>
<td>2.98 ± 0.67</td>
<td>0.26 ± 0.04</td>
<td>0.20 ± 0.03</td>
<td>1.04 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
<td>0.96 ± 0.10</td>
<td>0.51 ± 0.21</td>
<td>0.99 ± 0.43</td>
<td>0.16 ± 0.05</td>
<td>3.53 ± 0.73</td>
<td>2.72 ± 0.15</td>
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<td></td>
</tr>
</tbody>
</table>

Each value in the table is the mean ± standard deviation (n = 3). Letters (a–f) indicate significant differences at p < 0.001 (same letters indicate non-significant differences, and different letters indicate significant differences); 2021 and 2022: harvest date; nd: not detected.

The analysis revealed that the varieties studied had a very significant variation in terms of the phenolic compounds detected by HPLC-UV-VIS (p < 0.001). This variation is probably attributable to genotype. At the same time, the difference observed between harvesting seasons for the varieties was not very significant (p < 0.05), suggesting that the effect of season on date phenolics is limited. At the same time, the analysis showed that tyrosol and quercetin were not detected in any of the varieties in either the 2021 or 2022 samples [32]. In addition, it can also be noted that the three varieties with low market value have high concentrations of phenolic compounds, comparable to those of the ‘Majhoul’ variety, which is considered a variety with high market value. This confirms the underestimated opportunities in these low-market-value varieties, in particular the ‘Khalt Khal’ variety, widely used in livestock feed in Moroccan oases. This variety has higher concentrations of phenolic compounds than any of the other varieties studied. With regard to the main phenolic compounds, HPLC-UV-VIS analysis revealed that gallic acid, vanillic acid, ellagic acid, and 4-hydroxyphenylacetic acid are among the predominant compounds in the date varieties studied. For samples harvested in 2021, these major compounds vary, respectively, from 9.266 to 15.865 mg/100 g dry weight (DW), from 4.357 to 16.116 mg/100 g DW, from 0.441 to 14.628 mg/100 g DW, and from 0.832 to 6.890 mg/100 g DW. In contrast, for samples harvested in 2022, the variations in these major compounds are 6.140 to 23.629 mg/100 g DW, 2.784 to 24.360 mg/100 g DW, 2.134 to 27.325 mg/100 g DW, and 1.129 to 9.419 mg/100 g DW, respectively [32].

3.3. Correlation and Principal Component Analysis (PCA)

A Pearson correlation study was conducted to explore the links between antiglycation activity and the levels of various phenolic compounds measured by HPLC-UV-VIS (Figure 3). The main objective was to highlight the compounds responsible for antiglycation activity and to visualize the opportunities for adding value to the date fruits studied. The results of the analysis reveal a negative correlation between all the individual phenolic compounds, with the exception of kaempferol, and antiglycation activity expressed as IC50 mg/mL. However, a robust and statistically significant correlation was evident between this activity and catechin (r = −0.58), vanillic acid (r = −0.65), epicatechin (r = −0.40),
vanillin ($r = -0.30$), and trans-ferulic acid ($r = -0.46$). These results confirm that these phenolic compounds are among those responsible for inhibiting protein glycation.

**Figure 3.** Antiglycation activity (AG); gallic acid (GA); catechin (CAT); 4-hydroxyphenylacetic acid (4-HPA); caffeic acid (CA); vanillic acid (VA); epicatechin (EPIC); vanillin (Van); trans-ferulic acid (TFA); ellagic acid (EA); kaempferol (Kam).

Principal component analysis (PCA) was used to explore a set of 11 variables associated with four date varieties harvested during two successive seasons, in 2021 and 2022. This analysis allows conclusions to be drawn about the underlying causes of the antiglycation activity of date varieties based on their chemical composition. The graphical results of the PCA performed on the four date varieties indicate that 78.73% of the total variability can be explained by the first two principal components. More precisely, F1 explains 55.80% of the total variance of the data set, while F2 explains the remaining 22.93% (Figure 4a,b).

**Figure 4.** Biplot obtained from PCA of variables and individuals. (a) Application of PCA to generate groupings of varieties harvested in two seasons (2021, 2022). (b) Segregation of date varieties according to their bioactive compounds and antiglycation activity; S1: harvest season 2021; S2: harvest season 2022.

The distribution of date varieties revealed a high degree of variability between the four varieties studied (Figure 4a), as they are distributed in distinct locations, with the exception of points of the same variety harvested in two successive seasons, which are very close to each other. It can therefore be concluded that the variability between these four varieties is mainly attributable to the genotype and that the impact of the seasons on this variability is negligible. The results of this analysis reveal three distinct groups: the first group includes the ‘Rasse Tmar’ variety, the second group includes the ‘Khalt Khal’ variety, and the third group includes the two varieties ‘Jdar Lahmer’ and ‘Majhoul’.
Figure 4b reveals a negative correlation between the three varieties ‘Rasse Tmar’, ‘Jdar Lahmer’, and ‘Majhoul’ and potential compounds responsible for antiglycation activity, such as catechin, vanillic acid, epicatechin, vanillin, and trans-ferulic acid. In other words, the lower concentrations of these compounds in the three mentioned varieties elucidate their reduced antiglycation activity compared to the ‘Khalt Khal’ variety. Conversely, the ‘Khalt Khal’ variety exhibits the highest antiglycation activity, presumably due to its increased content of phenolic compounds, which correlates positively with this particular variety.

3.4. Molecular Docking Study of Identified Compounds

To clearly visualize the detailed mechanism by which the compounds identified in the date extracts bind to BSA and RAGE and to explain the difference observed between the antiglycation activity of the four date varieties studied, a molecular docking study was also conducted. The docking process is based on the prediction of the position, orientation, and binding affinity of the ligand in the active sites of the targets [39]. The docking results are presented in Table 2, while the interactions between the most active compound and the targets are illustrated in Figures 5 and 6.

![Figure 5](image_url) Figure 5. The 2D detailed view shows the interaction between ellagic acid (a), kaempferol (b), epicatechin (c), and catechin (d) with neighboring residues of BSA.

The results show that among the twelve phenolic compounds determined by HPLC-UV-VIS, four compounds, ellagic acid, kaempferol, epicatechin, and catechin, inserted perfectly into the binding site of BSA, as well as RAGE, with the lowest binding energy of, respectively, −8.9, −8.6, −8.4, and −8.3 kcal/mol for BSA and −7, −6.7, −6.6, and −6.9 kcal/mol for RAGE (Table 2). In general, a high level of binding energy negativity is more effective, indicating that the compound could be used to control glycation processes. This means that the four phenolic compounds ellagic acid, kaempferol, epicatechin, and catechin can inhibit protein glycation with sugars and also inhibit RAGEs, especially as the affinity between the two advanced glycation products and RAGE is low compared to ellagic acid, kaempferol, epicatechin, and catechin (binding energy: pyrraline −5.3 kcal/mol and pentosidine −6.1 kcal/mol). In contrast, a higher binding energy was obtained with 4-hydroxyxynphenylactic acid and vanillic acid towards BSA and RAGE, suggesting that these two compounds may not play as essential a role in antiglycation activity compared to the other identified compounds.
Figure 5. The 2D detailed view shows the interaction between ellagic acid (a), kaempferol (b), epicatechin (c), catechin (d), pyrraline (e), and pentosidine (f) with neighboring residues of BSA.

Figure 6. The 2D detailed view shows the interaction between ellagic acid (a), kaempferol (b), epicatechin (c), catechin (d), pyrraline (e), and pentosidine (f) with neighboring residues of RAGE.

Figure 5a shows the results of molecular docking involving ellagic acid, kaempferol, epicatechin, and catechin as ligands with BSA. Various bonds are formed between these ligands and the residues of the active site, but of particular interest is the interaction between the amino acids of this site, which plays a role in protein glycation, and the ligands. Previous studies have confirmed that the main amino acid residues involved in the glycation process are lysine and arginine [40,41].

Ellagic acid is linked to several arginine residues by conventional hydrogen bonds and Pi–Anion and Pi–Alkyl interactions. Kaempferol, on the other hand, forms six bonds with the amino acids ARG B:256, ARG B:196, ARG B:217, ALA B:290, and SER B:191 of BSA. The bonds established with arginine include conventional hydrogen bonds, Pi–Cation, and Pi–Hydrogen Donor. In contrast, epicatechin forms four bonds with the BSA amino acids ARG B:458, ARG B:196, ASP B:108, HIS B:145, ALA B:193, LEU B:189, and ILE B:455. The types of bonds formed with arginine include Pi–Cation and Pi–Alkyl. Catechin also forms four bonds with the BSA amino acids ARG B:458, ARG B:196, ASP B:108, ALA B:193, LEU B:189, and THR B:190. The types of bonds formed with arginine are Pi–Cation and Pi–Alkyl. The results of our study indicate that the four compounds examined by molecular docking (Figure 5) systematically establish bonds with arginine, an element involved in protein glycation. With regard to the antiglycation activity observed in date extracts and between different varieties, our research also confirms that the presence of abundant phenolic compounds is not sufficient to generate this activity. It is also necessary to consider the specific nature of the compounds capable of inhibiting protein glycation, even when their concentration is low.
**Table 2.** The structural details and data for compounds identified by HPLC-UV-VIS in date fruit extracts and two molecules considered as advanced glycation products (pyrraline and pentosidine), as well as docking score values for individual protein ligands with bovine serum albumin (4OR0) and advanced glycated end-product receptors (2MOV).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Information</th>
<th>Chemical Structure</th>
<th>Docking Score (kcal/mol)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>BSA</td>
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<tr>
<td>Gallic acid</td>
<td>MW: 170.12 g/mol</td>
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<td></td>
<td>MF: C7H5O5</td>
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<td>PubChem CID: 370</td>
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<td><em>Trans</em>-ferulic acid</td>
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The formulae and information concerning the molecules are taken from the National Library of Medicine; MF, molecular formula; MW, molecular weight.
With regard to the interactions of the four phenolic compounds and the two AGE phenolic compounds on the RAGE active site, several bonds were observed. Ellagic acid showed hydrogen bonds with ASN A:34, GLU A:105, and GLY A:75 of RAGE, as well as a Pi–Anion bond with GLU A:74, and GLU A:105 and a Pi–Alkyl bond with PRO A:101. However, some interactions were unfavorable (Figure 6a). Kaempferol formed a hydrogen bond with VAL A:95, GLY A:75, and LYS A:103, as well as hydrophobic interactions with ILE A:76, ARG A:94, and PRO A:101 (Figure 6b). For epicatechin, hydrogen bonds were observed with GLY A:36 and ILE A:6, as well as a van der Waals bond with GLY A:75 and hydrophobic interactions with ARG A:94, ARG A:96, and GLU A:74 (Figure 6c). For catechin, hydrogen bonds were established with ARG A:37, GLU A:105, GLY A:75, and VAL A:95, as well as hydrophobic interactions (Pi–Sigma and Pi–Alkyl) with ILE A:76, ARG A:94, and PRO A:101 (Figure 6d). While the two AGES bind to RAGE and induce the activation of several reactions linked to inflammation and oxidative stress, hydrogen bonds were observed with ARG A:37, GLY A:75, VAL A:95, GLU A:105, ASN A:34, and pyrraline. Similar bonds were observed between GLY A:36 and ILE A:6 and pentosidine. The two compounds also formed hydrophobic bonds with PRO A:101 and pyrraline and between ARG A:96 and pentosidine (Figure 6e,f).

This molecular docking investigation between RAGE and compounds recognized as ligands indicates that the ligand’s affinity relies on the type and quantity of bonds established between the receptor’s active site and the ligand. Specifically, ellagic acid demonstrates a pronounced affinity with RAGE, indicating its potential to impede the binding of AGES to RAGE.

4. Discussion

The antiglycation activity of the four date fruit varieties examined compares favorably with that of other fruits such as peach, kiwi, persimmon, and grapefruit, which show activities of 1.327 mg/mL, 1.450 mg/mL, 1.031 mg/mL, and 3.451 mg/mL, respectively [42]. In addition, the antiglycation activity of six fresh fruits, including papaya, bael, guava, mango, pomegranate, and amla, is significantly lower compared to that in our study. Their percentage inhibition of protein glycation for an extract concentration of 40 mg/mL is about 33.36%, 55.73%, 46.63%, 11.42%, 50.17%, and 71.29%, respectively [43].

The formation of α-dicarbonyl compounds during the Maillard reaction is a crucial step in the generation of AGES (advanced glycation end products), among which methylglyoxal (MG) emerges as one of the most reactive carbonyl species (RCS) in the human body. The accumulation of MG in the body can lead to the development of various complications associated with diabetes in individuals [44].

In this context, it is possible that the antiglycation activity observed in the date fruits studied is attributable to the presence of phenolic compounds. Research has highlighted that polyphenols present in these fruits could inhibit AGE biosynthesis due to their antioxidant properties, ability to chelate metals, interaction with proteins, scavenging of methylglyoxals (MGs), and/or blocking of the receptor for advanced glycation end products [43,45]. Our study proved that gallic acid, vanillic acid, and ellagic acid are among the major compounds in dates. These three compounds have a high antiglycation activity according to several studies.

Recently, the antiglycation function of gallic acid has also been confirmed in both in vivo and in vitro experiments; in addition to inhibiting AGE-induced inflammatory reactions [46], it could also attenuate cardiac complications caused by AGES [47]. Another study examined AGE formation by incubating superoxide dismutase with glucose, methylglyoxal (MG), or a combination of glucose and MG, which presented specific AGES. Meanwhile, incubating superoxide dismutase for 10 days at 37 °C with glucose, MG, or a combination of the two, plus an increasing concentration of ellagic acid, resulted in a progressive decrease in AGES in a concentration-dependent manner [48]. Vanillic acid has a hydroxyl group that can provide a nucleophilic center that attacks electrophilic reactive carbonyl species to form a hemiacetal adduct instead of glycated products [49], thus signifi-
cantly reducing MG-induced cytotoxicity [50]. In addition to phenolic compounds, another mechanism of diabetic retinopathy involves the increased production of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) by the interaction of AGE with receptors for advanced glycation end products in the lens epithelium. This inhibition of RAGE may reduce the subsequent development of oxidative stress and inflammation [45,51,52].

Our results confirm the feasibility of incorporating dates with a low market value into the design of nutritional foods with a high market value. They are also proving to be suitable thickening or gelling agents for processed products, such as confectionery, jams, table jellies, soft cheeses, and yoghurts [53,54]. At the same time, several studies have corroborated the possibility of extracting date syrup rich in bioactive compounds, used as a sugar substitute in concentrated drinks, chocolates, ice creams, sweets, snacks, bakery products, and health foods [55,56].

According to [5,57,58], gallic acid is among the major compounds in dates harvested in Oman, Saudi Arabia, and Morocco, with contents ranging from 7.0 to 19.14 mg/100 g dry weight (DW), from 1.61 to 11.23 mg/100 g DW, and from 4.379 to 31.411 mg/100 g DW, respectively. However, El Sohaimy et al. [13] observed an extremely low gallic acid concentration (0.52 mg/100 g DW) in the ethanolic extract of an Egyptian variety. From our results and those found by other researchers, it appears that phenolic compounds do not depend solely on genotype but are attributed to several factors, including environmental conditions, variety, ripening stage, harvest season, geographical origin, fertilizer application, soil type, extraction medium, and extraction conditions [22,59–65].

The phenolic compounds present in dates have various effects, including antitumor, antioxidant, and anti-inflammatory properties, as well as properties in the prevention of cardiovascular disease [66,67]. Studies on antiglycation activity have also shown that gallic acid, catechin, and quercetin can inhibit the formation of Amadori products [19]. In addition, these phenolic compounds can inhibit RAGEs, which are linked to pathologies such as Alzheimer’s disease and endothelial cell dysfunction [22,60]. The presence of these bioactive compounds in dates opens up promising opportunities for the development of pharmaceutical products aimed at inhibiting the advanced glycation of proteins from dates, particularly those of low market value, or for using them as nutritional food ingredients to limit the formation of AGEs in the food industry.

Our results are in line with those of several studies; research on Lamiaceae species showed that phenolic compounds are responsible for antiglycation activity [68]. Similar conclusions were reported in a study conducted on Chinese olives, analyzed for their antiglycation properties, where the results revealed the involvement of phenolic compounds in this activity [69]. A number of mechanisms contribute to the inhibition of the formation of advanced glycation products (AGEs). These include the inhibition of glycation intermediates by phenolic compounds such as methylglyoxal and the blocking of AGE receptors (RAGEs) present on B lymphocytes and certain neurons [22,59,60]. In addition, phenolic compounds can also inhibit free radicals, which oxidize Schiff bases into reactive carbonyl and dicarbonyl groups during the early stages of the Maillard reaction [20,45]. In addition, syringic acid has been identified as a potent inhibitor of AGEs, as it forms bonds with the amino group of lysine, preventing their reaction with sugars [70]. Some compounds, such as epigallocatechin-3-gallate and theaflavin-3, 3′-digallate, capture methylglyoxal, which is responsible for neuronal cell apoptosis [60].

The reduction in the formation of glycation, thanks to protein binding by certain phenolic compounds, as well as binding with RAGE, has been demonstrated in our study, as well as in other research carried out in [40,71,72]. The binding of phenolic acids to BSA via the hydrogen bonds of amino acids, particularly arginine, is consistent with the observations of Yildirim-Elikoglu and Erdem [73] as well as Brudzynski and Maldonado-Alvarez [74]. These studies demonstrated the formation of hydrogen bonds between the electronegative nitrogen or oxygen atoms of the amino (NH\(_2\)) and hydroxyl (OH) groups of proteins and the positively charged hydrogen atoms of the hydroxyl groups of polyphenols. However, the structure of phenolic acids influences their affinity for BSA. On
the other hand, the presence of an aromatic ring on a drug (ligand) is often decisive in its interaction with the target protein [75,76].

Several studies have shown that gallic acid, catechin, epigallocatechin, and caffeic acid, present mainly in certain fruits, attenuate the expression of RAGEs [47,77]. Molecular docking studies have also revealed that certain polyphenols, such as curcumin, can bind strongly to RAGEs, blocking their interaction with ligands [51]. In addition, polyphenols have effects on the sirtuin family protein, which can inhibit the NF-κB signaling pathway responsible for RAGE gene transcription [78].

5. Conclusions

A comparative study of four date varieties, three with low market value and one with high market value, harvested in two consecutive seasons, was conducted to assess their antiglycation activities, followed by a molecular anchoring study. Among all the varieties studied, the ‘Khalt Khal’ variety showed the highest content of phenolic compounds as well as the highest antiglycation activity with an IC50 of 1.83 mg/mL in 2021 and 2 mg/mL in 2022. In addition, the molecular anchoring study revealed that ellagic acid, kaempferol, epicatechin, and catechin were the main polyphenols contributing to the antiglycation activity of the date varieties studied. In the light of these results, it is advisable to use date varieties, particularly the three low-value varieties, to improve or develop food products (extracts from these fruits can be used as food additives to provide specific technological functions such as texturizing agents, preservatives, colorings, nutritional additives, and flavorings agents), and the bioactive compounds present in dates also offer a promising opportunity for the development of phytopharmaceutical products targeting diseases associated with advanced protein glycation. In addition, these compounds could be used as nutritional food ingredients to limit the formation of AGEs during industrial food processing.

Author Contributions: Conceptualization, M.A., A.A. and I.E.; methodology, A.O. and M.O.; software, A.O. and M.O.; validation, M.A., A.A. and I.E.; formal analysis, A.O.; resources, M.A. and I.E.; writing—original draft preparation, A.O.; visualization, M.A., A.A. and I.E.; supervision, M.A., A.A., A.O. and I.E.; revised and finalized this manuscript, M.A., A.A., A.O. and I.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within this article.

Acknowledgments: The authors would like to thank the Laboratory of Agro-Food, Biotechnologies and Valorization of Plant Bioresources, Agrobiotechnology and Bioengineering Center, CNRST Labeled Research Unit (AgroBi-otech-URL-CNRST-05 Center), 40000 Marrakech, Morocco, for the provision of infrastructure and research facilities.

Conflicts of Interest: The authors declare no conflicts of interest.

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