ORIGINAL PAPER

MOLECULAR DOCKING ANALYSIS AND DYNAMICS SIMULATION OF SOME ANTIOXIDANT POLYPHENOLS

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Abstract. *Antioxidant properties of medicinal plants have a very important role in different inflammatory and oxidative stress-related diseases. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) are anti-oxidative enzymes and constitute a very important antioxidant defense against oxidative stress. The purpose of the present study was to elucidate how polyphenolic compounds, specifically chlorogenic acid, rosmarinic acid, and quercetol, present in Acanthus balcanicus, Carduus acanthoides, Tamarix ramosissima, Tragopogon pratensis, and Vaccinium myrtillus, interacts with antioxidant enzymes. This research sought to bridge the gap between theoretical predictions and empirical evidence, providing a comprehensive understanding of the antioxidant capabilities of these polyphenols in the context of diabetes-induced oxidative stress. The interaction between antioxidant enzymes and polyphenols was carried out by using Autodock 4.2 software and SIBIOLEAD software. The results show that all polyphenols owned potent antioxidant capacity and can activate SOD, GPx, and GR, the strongest antioxidant activity being attributed to quercetol, followed by chlorogenic acid and rosmarinic acid. Polyphenols studied can be used as lead compounds in future drug development as antioxidant agents in diabetes.*

Keywords: *antioxidant, molecular docking, superoxide dismutase, glutathione peroxidase, glutathione reductase*

1. INTRODUCTION

Numerous experimental diabetes induction protocols have recently been developed and refined to better understand the pathogenesis of diabetes, and oxidative stress, and to be able to test new therapeutic principles. Discovery of active compounds from natural products have gained enormous importance in the field of drug discovery [1]. Drug discovery from plants involves a multidisciplinary approach combining botanical, ethnobotanical, phytochemical and biological techniques. Drug discovery typically starts with the analysis of target proteins binding sites or identification of structural motifs common to active compounds, *in silico* molecular docking being one of the most powerful techniques to discover novel ligands for receptors [2].

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Superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) are known as antioxidant enzymes. The main function of SOD is to decompose superoxide radicals into molecular oxygen and hydrogen peroxide inside the cells and prevent superoxide toxicity [3]. GPx is an enzyme with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water [4]. GR is an enzyme that catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell [5].

In prior research [6-8], the antioxidant potential of tinctures derived from several plant species, which have been minimally explored in this context, was examined using mice with streptozotocin-induced diabetes. The focus was on evaluating the activities of SOD, GPx, GR in plants such as *Tragopogon pratensis* (goat's beard), *Acanthus balcanicus* (bear's sole or matron), *Tamarix ramosissima* (sea buckthorn), and *Carduus acanthoides* (spinach or red velvet). These species were analyzed in comparison to *Vaccinium myrtillus* (blueberry), a plant widely acknowledged for its antidiabetic and antioxidant properties. Previous HPLC analysis revealed that the hydroalcoholic extract of *Carduus acanthoides* contains chlorogenic acid (85.8 μg/mL), rosmarinic acid (30.4 μg/mL), and quercetol in traces [6]. *Acanthus balcanicus* contains traces of quercetol, and *Vaccinium myrtillus* contains traces of chlorogenic acid [7]. *Tamarix ramosissima* contains quercetol (280.57 μg/mL) and chlorogenic acid in traces. *Tragopogon pratensis* contains chlorogenic acid (270 μg/mL) and rosmarinic acid (258.25 μg/mL) [8]. In the current investigation, the binding energy of the polyphenol-antioxidant enzyme complex was determined utilizing molecular docking and molecular dynamics simulations. This approach aimed to elucidate the antioxidant efficacy of the hydroalcoholic extracts.

2. MATERIALS AND METHODS

2.1 SAMPLE PREPARATION

Vegetable products were harvested from the Botanical Garden of Craiova City (Dolj County, Romania) and prepared as hydroalcoholic extracts manufactured by simple leaching at dry plant material/solvent (70% aqueous ethanol) ratio of 1:5 according to FRX.

2.2 TESTING IN VIVO ANTIOXIDANT ACTIVITIES

Tests for the antioxidant activity were performed using streptozotocin-diabetic adult Swiss albino mice distributed in seven groups.
Group I: mice with normal pancreatic function (intactcontrol, C);
Group II: diabetic mice (D);
Group III: diabetic mice treated with *Tragoponis pratensis folium* 20% at 150 mg/kg b.w. (5.25 – 6.75 mg extract dissolved in 0.3 mL distilled water);
Group IV: diabetic mice treated with *Tamaricis ramosissimae folium et flos* 20% at 150 mg/kg b.w. (5.25 – 6.75 mg extract dissolved in 0.3 mL distilled water);
Group V: diabetic mice treated with *Cardui acanthoiditis folium* 20% at 150 mg/kg b.w. (5.25 – 6.75 mg extract dissolved in 0.3 mL distilled water);
Group VI: diabetic mice treated with *Acanthi balcanici herba* 20% at 150 mg/kg b.w. (5.25

– 6.75 mg extract dissolved in 0.3 mL distilled water);
Group VII: diabetic mice treated with *Myrtilli fructus* 20%, 150 mg/kg b.w (5.25 – 6.75 mg extract dissolved in 0.3 mL distilled water), a plant product recognized for its antioxidant effect.

At the end of experiments, the animals were sacrificed and the activities of SOD, GPx, and GR were tested, using commercially available kits from Randox using streptozotocin diabetic adult Swiss albino mice as was previously reported [9]. Kits produced by Randox Laboratories were used for the quantitative *in vitro* determination of the activity of antioxidant enzymes. The measurement method was colorimetric, performed using a Beckman UV-VIS spectrophotometer, model DU-65 (Beckman, Potsdam – Germany).

2.3 DOCKING PROTOCOL AND MOLECULAR DYNAMICS SIMULATION

The CAS no and Canonical SMILES of polyphenol compounds were retrieved from the PubChem database (www.ncbi.nlm.nih.gov/pubchem). The three-dimensional (3-D) structure and mol2 file of each polyphenol was generated using the Gaussian program suite at DFT/B3LYP/6-311G level of theory. Three-dimensional structures of the target proteins have been retrieved from protein data bank (PDB) having PDB IDs: 1CB4 for SOD, 2P31 for GPx, and 1XAN for GR (Fig. 1).

The blind molecular docking analysis was performed using the Autodock 4.2.6 software together with the AutoDockTools. The docking between polyphenols and targets involves adding all the polar hydrogens, and computing the Gasteiger charge; a grid box was created using Autogrid 4 with 120×120×120 Å in x, y, and z directions with 0.375 Å spacing from the target center. For the docking process, the Lamarckian genetic algorithm with a population size of 150 and several 30 runs was chosen. All other parameters were used with the default values.

The calculations were realized in duplicate and the results were expressed as averages. For the final visualization stage of the formed inclusion complexes, the PyMol (Schrodinger) software [10] and Discovery Studio Visualization (Biovia) software [11] were selected.

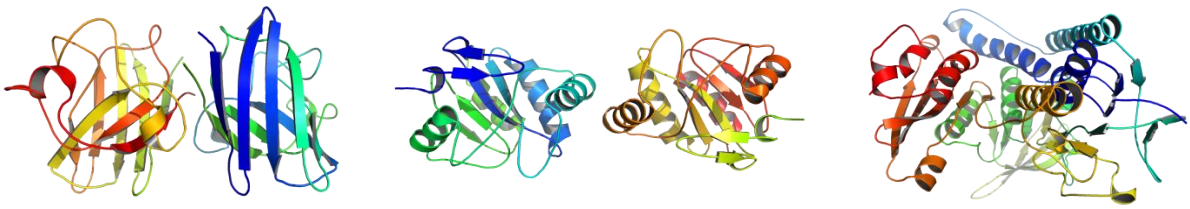


Figure 1. X-ray molecular structure of selected targets (SOD-left, GPx-center, GR –right)

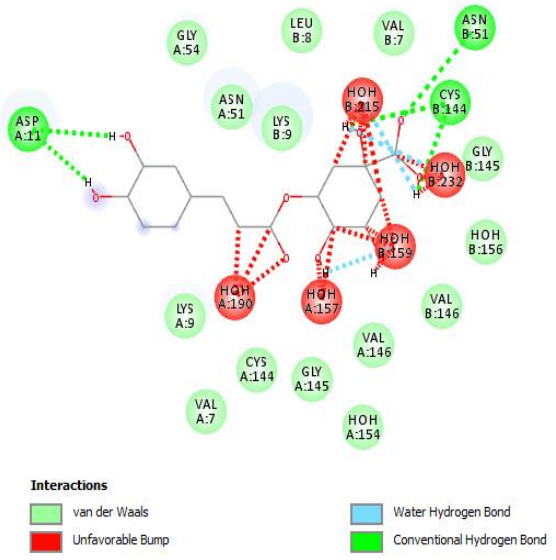
Molecular dynamics simulations were executed utilizing the MD Simulation tool integrated within SiBIOLEAD, which employs the GROMACS simulation software [11]. This web-based platform operationalizes a quintet of procedural steps comprising preprocessing, energy minimization, equilibration, production dynamics, and trajectory analysis. The simulation workflow was initiated using top-ranking ligand-receptor complexes, specifically those involving enzyme-ligand interactions. These complexes were strategically positioned within a cubic simulation box, which was then populated with an SPC water model to mimic aqueous conditions. To achieve charge neutrality, the protein complex environment was supplemented with NaCl ions at a concentration of 0.15 M. Energy minimization was conducted using the steepest descent method, encompassing a total of 5000 steps to refine the system's potential energy landscape. After energy minimization, the system underwent

equilibration employing the canonical (NVT) and isothermal-isobaric (NPT) ensembles at a standard temperature of 300 K and pressure of 1 bar throughout 100 ps, preparing the system for the production phase of dynamics. The production dynamics phase was executed over a 100 ns timeframe, leveraging the Leapfrog integrator to facilitate temporal progression, with data sampling resulting in 5000 discrete frames for comprehensive trajectory analysis.

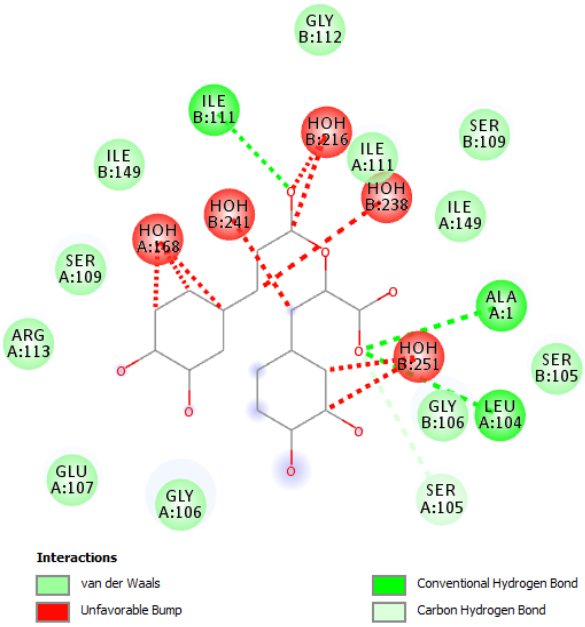
3. RESULTS AND DISCUSSION

For decades, selected plants have been used as herbal medicine for treating different diseases but not for their antioxidant properties [13-15]. In our previous studies, was observed that the hydroalcoholic extracts of selected medicinal plants induced antioxidant activity after administration [6,7]. As was reported, diabetic mice had the lowest mean value of GR (46.3 ± 2.12 U/mL), GPx (3415,5 ± 98.29 U/mL) and SOD (191.7 ± 10.75 U/mL) activities. The highest GR activity was obtained for diabetic mice treated with *Tamaricis ramosissimae folium et flos* 20% extract (89.6 ± 0 U/mL). The highest GPx activity was observed also in this group (7124 ± 246.07 U/mL). The therapeutic efficacy of the antioxidant potential of the tinctures resulted from the average value of the plasma level of SOD activity was in ascending order of the following potency: *Myrtilli fructus* plant extract (194.15 ± 3.18 U/mL), *Acanthi balcanici herba* extract (195.1 ± 2.27 U/mL), *Cardui acanthoiditis folium* extract (212.45 ± 5.30 U/mL), *Tamaricis ramosissimae folium et flos* extract (227.65 ± 10.39 U/mL). The antioxidant enzymes exhibited higher activities in mice treated with *Tamaricis ramosissimae folium et flos* extract, this strong antioxidant activity could be related to the high quercetol content of the plant extract.

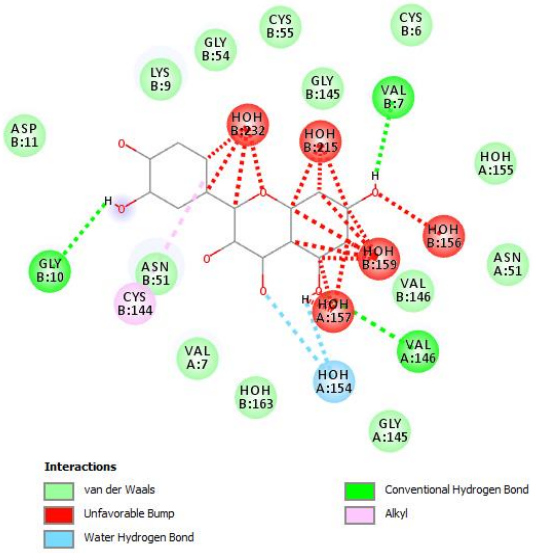
The results of molecular docking simulation indicate that the interaction of the established polyphenols (i.e., ligands) with the targets was energetically favorable. Figs. 2-4 presents the values obtained for the binding energy of each tested compound and the bidimensional map of the ligand (polyphenol) interaction with each target (antioxidant enzyme).



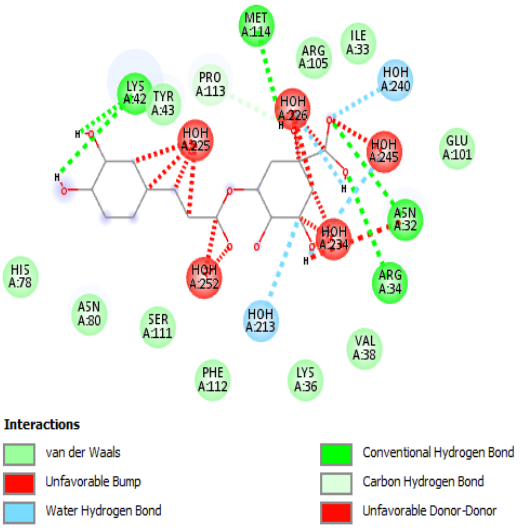
Complex: SOD-chlorogenic acid
Binding energy: -3.39 kcal/mol



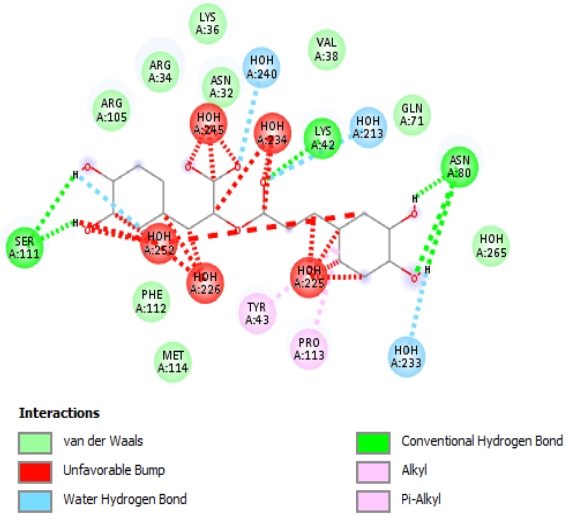
Complex: SOD-rosmarinic acid
Binding energy: -2.96 kcal/mol



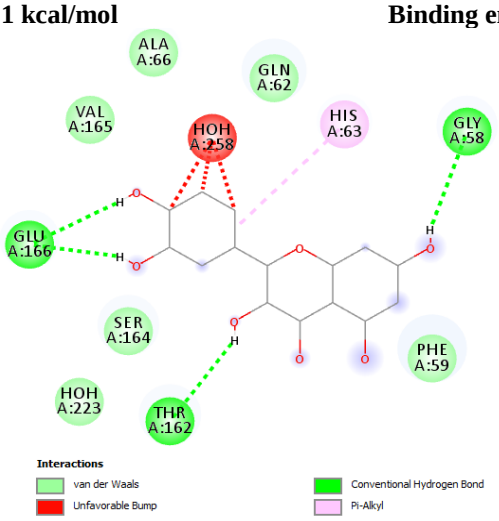
Complex: SOD-quercetol
Binding energy: -4.92 kcal/mol
Figure 2. The binding energy and bidimensional map of polyphenol-SOD complexes



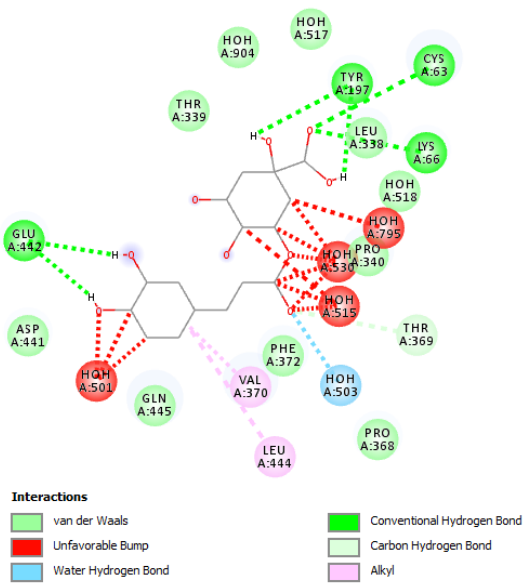
Complex: GPx-chlorogenic acid
Binding energy: -3.51 kcal/mol



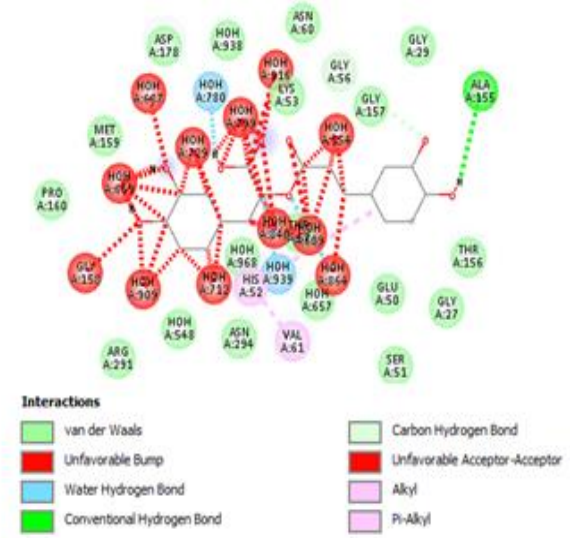
Complex: GPx–rosmarinic acid
Binding energy: -3.27 kcal/mol



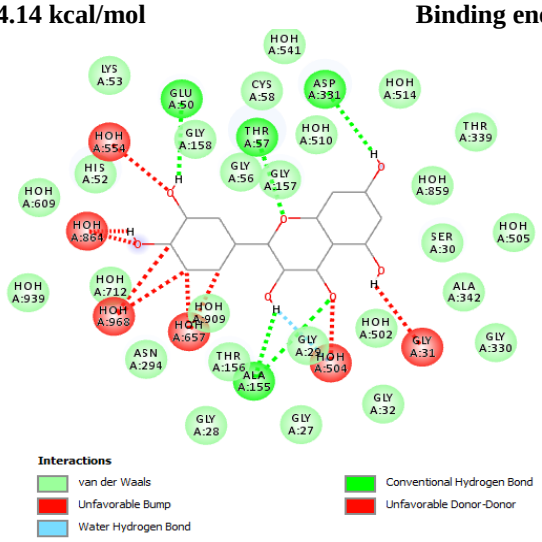
Complex: GPx –quercetol
Binding energy: -5.89 kcal/mol
Figure 3. The binding energy and bidimensional map of polyphenol-GPx complexes



Complex: GR-chlorogenic acid
Binding energy: -4.14 kcal/mol



Complex: GR-acid rosmarinic
Binding energy: -3.11 kcal/mol



Complex: GR -quercetol
Binding energy: -6.15 kcal/mol

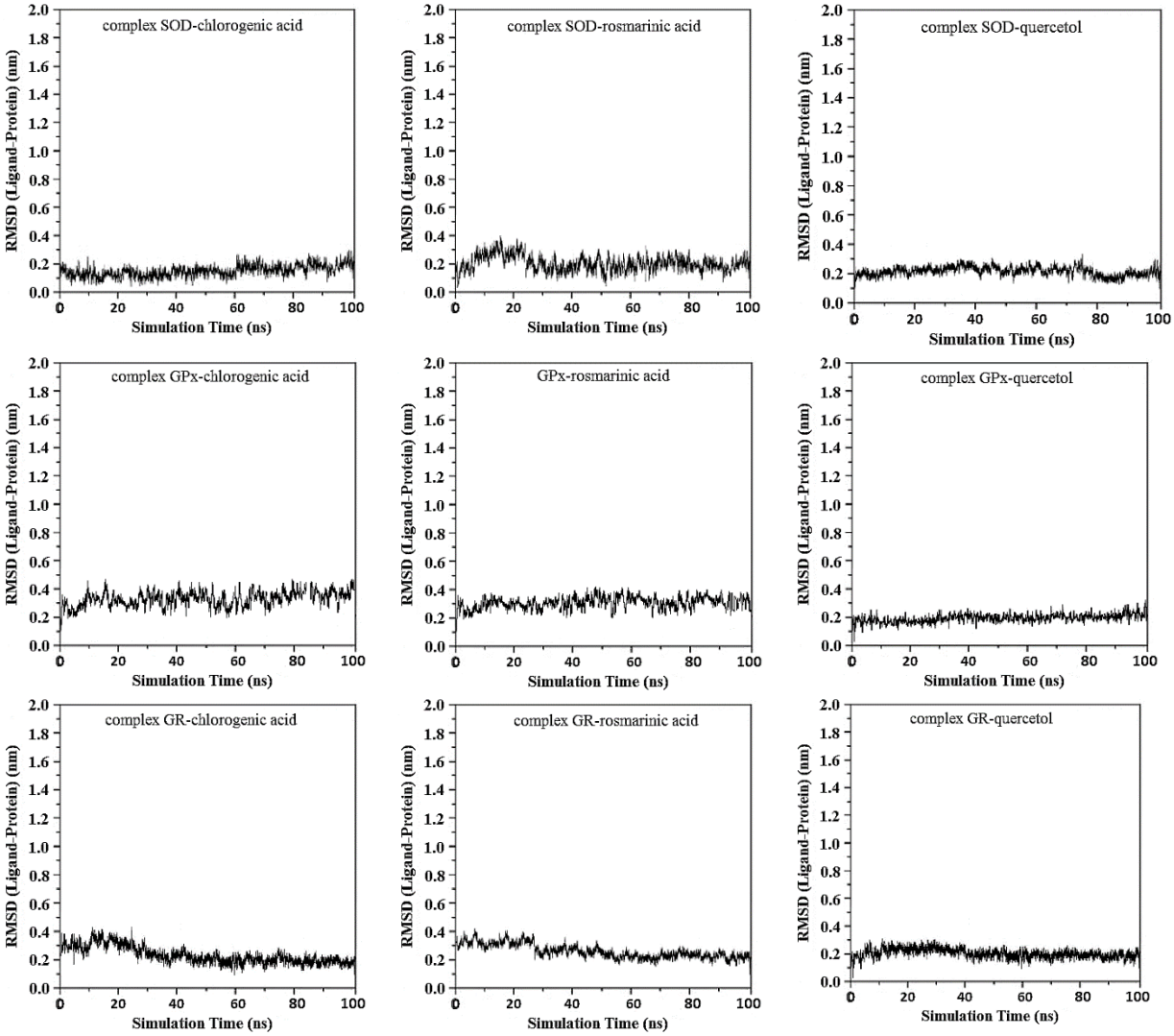
Figure 4. The binding energy and bidimensional map of polyphenol-GR complexes

Figs. 2-4 elucidate that GR forms the most stable complex with quercetol, exhibiting a binding energy of -6.15 kcal/mol. This stability is attributed to the formation of five hydrogen bonds with the amino acids GLU_{A50}, THR_{A57}, ALA_{A155}, and ASP_{A331}, showcasing the specificity of interaction sites within the enzyme structure for quercetol. The investigation into GR's interaction with various polyphenols reveals the formation of complexes of differing stabilities, highlighting the nuanced interaction landscape of GR with polyphenolic compounds. Furthermore, the interaction of quercetol with GPx is noted as the most stable, with a binding energy of -5.89 kcal/mol, surpassing those formed with rosmarinic acid (-3.27 kcal/mol) and chlorogenic acid (-3.01 kcal/mol). The stabilization of the GPx-quercetol complex is facilitated by four hydrogen bonds involving the amino acids GLY_{A58}, THR_{A162}, and GLU_{A166}. Interestingly, chlorogenic acid and rosmarinic acid share a common binding site on GPx, involving a distinct set of amino acids, which underscores the competitive and complex nature of enzyme-ligand interactions among different polyphenols.

The molecular docking analyses further reveal quercetol as exhibiting the highest stability in its interaction with SOD, marked by a binding energy of -4.92 kcal/mol. This

finding is significant in understanding the pronounced in vivo antioxidant activity observed with the *Tamarix ramosissima* extract, potentially attributed to the high quercetol content. It is noted that chlorogenic acid exhibits a stronger binding affinity compared to rosmarinic acid, facilitated by specific interactions involving five hydrogen atoms. Notably, chlorogenic acid and quercetol are observed to interact at the same active site on SOD, while rosmarinic acid binds at an allosteric site, suggesting a potential mechanism for allosteric activation of SOD by rosmarinic acid. This implies a regulatory role for rosmarinic acid in enhancing SOD activity, which could contribute to disease prevention through the modulation of the antioxidant enzymatic system. The study also highlights the occurrence of unfavorable interactions, such as steric clashes, which may compromise the stability of protein-ligand complexes. These observations underscore the complexity of molecular docking simulations and the need for careful interpretation of interaction dynamics.

Table 4. RMSD enzymes-polyphenols



The RMSD of the GPx-chlorogenic acid complex appears to stabilize after an initial rise, maintaining an average fluctuation below 0.4 nm throughout the majority of the simulation. This indicates that after the complex reaches equilibrium, the overall conformation of the complex experiences minor deviations, suggesting a stable interaction between GPx and chlorogenic acid under the simulated conditions. Such a pattern of RMSD behavior typically suggests that the ligand-protein complex achieves structural stability relatively early in the

simulation process, and the maintained level of deviation implies a consistent conformational state.

The low and stable RMSD trajectory suggests that the GPx-quercetol complex maintains a consistent conformational state throughout the simulation. In the line graph, the RMSD values exhibit minor variations throughout the simulation, maintaining a remarkably low level, predominantly under 0.3 nm. This indicates a stable binding and suggests that the chosen simulation parameters are capable of preserving the structural integrity of the complex over the simulated timeframe. The consistent RMSD trajectory observed for the GPx-quercetol complex can be interpreted as evidence of the complex's conformational stability during the simulation period. This stability is critical when evaluating the interaction dynamics and the potential for quercetol to act as a stable ligand for the GPx enzyme. The results depicted in the graph could support the hypothesis that quercetol forms a stable complex with GPx, which may be relevant for further research into the therapeutic or biological significance of this interaction.

This conveys that the GPx-rosmarinic acid complex remains relatively stable, with little deviation in its structural conformation throughout the simulation. The line graph illustrates the RMSD values across the simulation time, showing a pattern of minor fluctuation with RMSD values generally below 0.4 nm. The stability indicated by the low RMSD values is significant for computational studies in drug design and protein-ligand interactions, as it suggests that rosmarinic acid consistently maintains a stable complex with the GPx enzyme. Such information is vital for understanding the dynamic stability and potential biological activity of the complex, providing insights that could be essential for downstream applications such as inhibitor design or understanding the mechanistic aspects of enzyme function.

The low RMSD values suggest that the complex achieves structural stability quickly and maintains this stable conformation throughout the simulation period. The line graph reveals that the RMSD of the GR-chlorogenic acid complex exhibits a stable profile with most of the values hovering around or below 0.3 nm. Such a stable RMSD pattern implies that the binding between GR and chlorogenic acid is consistently maintained, indicating that the molecular interaction is strong and persistent under the conditions simulated. This type of data is critical for biochemical studies focusing on enzyme activity regulation, drug design, or the structural biology of ligand-receptor interactions, as it provides evidence for the conformational integrity and potential functional efficacy of the complex.

The graph depicts a relatively stable RMSD for the GR-quercetol complex, with the majority of the values tightly clustered around 0.2 nm. This narrow range of fluctuation indicates that the complex maintains a consistent structure throughout the simulation period, without significant deviations in the ligand or protein conformations. The stability reflected in the RMSD trend is indicative of a strong and stable interaction between GR and quercetol within the simulated environment. The maintenance of a low RMSD value throughout the simulation suggests that the binding between the protein and ligand is robust, which is valuable information for fields such as pharmacology and enzymology, where the understanding of ligand-binding dynamics is essential.

For GR-rosmarinic acid, the RMSD plot indicates minimal fluctuation, with values largely consolidating around the 0.2 nm mark, showcasing a stable ligand-protein interaction throughout the simulation. Such a profile suggests that the GR-rosmarinic acid complex achieves and sustains conformational stability relatively quickly, maintaining this stable state across the entire simulation timeframe. The graph demonstrates that the molecular interaction between GR and rosmarinic acid is characterized by a low degree of structural deviation, implying a potential for strong binding affinity and functional significance in biological contexts. This data is particularly pertinent for computational studies in drug discovery and

enzyme mechanism elucidation, as it provides a quantitative measure of the dynamic stability and structural fidelity of the ligand-protein complex under study.

The graph illustrates that the RMSD for the SOD-chlorogenic acid complex remains consistently low, predominantly oscillating slightly above 0.2 nm, which signifies a stable interaction between the enzyme and the ligand during the simulation. This consistent low level of RMSD suggests that the complex retains its structural integrity over time without significant conformational changes. Such stability, as shown by the narrow fluctuation in RMSD, is critical in the context of pharmacological and enzymatic studies, as it indicates that the ligand may effectively maintain interaction with the enzyme under simulated physiological conditions. This information can be foundational for further investigation into the therapeutic potentials of chlorogenic acid as a modulator of SOD activity.

The RMSD trajectory presented in the graph is characterized by low fluctuation, with most values maintaining close to the 0.2 nm mark. This pattern indicates a stable conformation for the SOD-quercetol complex across the duration of the simulation, suggesting that the complex retains its structural integrity and does not experience significant conformational changes. The depicted stability of the RMSD values over time implies a potentially strong and stable binding interaction between SOD and quercetol. Data of this nature is crucial for understanding the molecular dynamics of ligand binding and can provide valuable insights for studies related to antioxidative therapies and the pharmacological applications of quercetol.

The graph depicts the RMSD values for the SOD-rosmarinic acid complex, which display a stable trend, with most of the RMSD data points fluctuating around a narrow range close to 0.2 nm. This low and steady level of RMSD indicates a stable ligand-protein interaction with minimal structural changes occurring throughout the duration of the simulation. This stability in the RMSD is indicative of a robust and consistent conformation for the SOD-rosmarinic acid complex, suggesting that the complex maintains its structural integrity and that rosmarinic acid remains well bound to SOD over the simulated time frame. This information is particularly valuable for further biochemical and pharmacological research on the therapeutic uses of rosmarinic acid, especially in the context of diseases where oxidative damage is a concern and SOD is a target of interest (Table 4).

The paper contributes to the field of drug discovery by highlighting the potential of natural polyphenols as antioxidant agents. By combining molecular docking and dynamics simulation, it provides their mechanisms of action at the molecular level. Future research could include exploring their effects in other models of disease characterized by oxidative damage and expanding the range of polyphenols and enzymes studied.

4. CONCLUSIONS

An approach to virtual screening under computational biology along with receptor ligand binding affinity can be an easy screening method prior to identify the efficacy of lead compounds that have potent therapeutic efficacies. Polyphenols (chlorogenic acid, rosmarinic acid, and quercetol) have shown promising binding affinities towards enzyme targets, quercetol having the highest affinity for the antioxidant enzymes. From this docking studies can be concluded that the binding of polyphenols to the antioxidant enzymes may lead to increase in their activities and reduce the oxidative stress in induced diabetes mellitus. With the help of molecular docking, this study can correlate the antioxidant effectiveness of the hydroalcoholic extracts with the content in polyphenols. *Tamarix ramosissima* showed a strong antioxidant effect. In addition, the diabetic mice treated with its hydroalcoholic extract

showed the highest values of SOD, GPx, and GR enzymatic activity due to the high quercetol content of this plant extract. The molecular dynamics simulations of GPx, GR, and SOD complexes with chlorogenic acid, quercetol, and rosmarinic acid have consistently indicated a high degree of conformational stability. This stability is evidenced by low RMSD values observed across all ligand-protein interactions studied, signifying minimal structural deviations and suggesting robust binding affinities.

REFERENCES

[1] Li, W., Yuan, G., Pan, Y., Wang, C., *Frontiers in Pharmacology*, **8**, 74, 2017.
[2] Pinzi, L, Rastelli, G., *International Journal of Molecular Sciences*, **20**, 4331, 2019.
[3] Kurutas, E.B., *Nutrition Journal*, **15**, 71, 2016.
[4] Muthukumar, K., Rajakumar, S., Sarkar, M.N., Nachiappan, V., *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **99**, 761, 2011.
[5] Deponte, M., *Biochimica et Biophysica Acta*, **5**, 3217, 2013.
[6] Varut, R.M., Gîrd, C.E., Rotaru, L.T., Varut, M.C., Pisoschi, C.G., *Pharmaceutical Chemistry Journal*, **51**, 1088, 2018.
[7] Popescu, R.M., Mogoşanu, G.D., Berbecaru-Iovan, A., Neamțu, J., Popescu, F., Pisoschi, C., *Romanian Journal of Biochemistry*, **51**, 37, 2014.
[8] Rotaru, L.T., Văruţ, R.M., Amzoiu, E., Mormoe, M., Nicolaescu, O., Amzoiu, M.O., Udrescu, L., *Pharmaceutical Chemistry Journal*, **53**, 964, 2020.
[9] Bandegi, A.R., Rashidy-Pour, A., Vafaei, A.A., Ghadrdoost B., *Advanced Pharmaceutical Bulletin*, **4**, 493, 2014.
[10] *Protein Preparation Wizard*; Epik, Schrödinger, LLC, New York, NY, 2021; *Impact*, Schrödinger, LLC, New York, NY; *Prime*, Schrödinger, LLC, New York, NY, 2021.
[11] BIOVIA, Dassault Systèmes, *BIOVIA Workbook*, Release 2020; BIOVIA Pipeline Pilot, Release 2020, San Diego: Dassault Systèmes, 2022.
[12] Ragunathan, V., Tiwari, P., *Journal of Biomolecular Structure and Dynamics*, **40**, 585, 2022.
[13] Radulescu, C., Olteanu, R.L., Nicolescu, C.M., Bumbac, M., Buruleanu, L.C., Holban, G.C., *Foods*, **10**(8), 1856, 2021.
[14] Radulescu, C., Buruleanu, L.C., Nicolescu, C.M., Olteanu, R.L., Bumbac, M., Holban, G.C., Simal-Gandara, J., *Plants-Basel*, **9**(11), 1470, 2020.
[15] Radulescu, C., Olteanu, R.L., Stihi, C., Florescu, M., Stirbescu, R.M., Stanescu, S.G., Nicolescu, C.M., Bumbac, M., *Journal of Chemometrics*, **34**(6), e3234, 2020.