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Proceeding Paper

Comprehensive Investigation of Antimicrobial and Antifungal Mechanistic Pathways of Bioactive Phytochemicals from Apple Pomace Using Molecular Docking †

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Abstract: The article focuses on utilizing apple pomace waste for a circular economy. It explores bioactive compounds in apple pomace, their potential uses, and emphasizes molecular docking's role in understanding how these compounds act as antimicrobials and antifungals. The study highlights the diversity of bioactive phytochemicals, introduces molecular docking for studying their interactions with microbial proteins, and presents case studies demonstrating how this approach reveals mechanistic pathways of action. This research showcases how apple pomace can be repurposed for its valuable compounds and offers insights into their antimicrobial and antifungal properties through in silico techniques.

Keywords: agri-food wastes; antifungals; antimicrobials; apple pomace; phenolics; molecular docking

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1. Introduction

The expanding challenges posed by escalating environmental concerns and the burgeoning demand for sustainable practices have propelled the exploration of novel approaches for the valorization of agri-food wastes and by-products [1]. Among these, apple pomace, the residual biomass derived from apple processing industries, has garnered significant attention for its untapped potential as a reservoir of bioactive phytochemicals [2]. This agri-food waste stream, which traditionally found application as animal feed or was relegated to disposal, has emerged as a source of diverse and valuable phytochemicals [3]. These compounds, arising from the rich phytochemistry of apples [4], exhibit a myriad of health-promoting properties [5]. Consequently, apple pomace houses an array of bioactive phytochemicals, including phenolic acids, polyphenols, and triterpenoids, which have been linked to diverse bioactivities such as antioxidant, anti-inflammatory, and antimicrobial properties [6]. However, the precise molecular mechanisms that underlie these activities remain quasi-equivocal.

Elucidating the interactions between bioactive compounds found in apples and the apple pomace and their target proteins responsible for microbial and fungal proliferation holds paramount significance in unraveling the intricate mechanisms governing their antimicrobial and antifungal activities. The bioactivity of these compounds hinges on their ability to selectively interfere with key processes essential for the survival and growth of pathogenic microorganisms and fungi. The precise details of these interactions have remained elusive, impeding a comprehensive understanding of their modes of action.

Microorganisms and fungi, particularly those implicated in infections and diseases, rely on an array of essential proteins to sustain their viability and proliferative capacities.

These proteins, often involved in critical cellular processes like cell wall synthesis, membrane integrity, and enzymatic activities, present promising targets for therapeutic interventions [7,8]. The bioactive compounds found in apples and apple pomace, being rich in diverse phytochemicals, have the potential to interact with these essential proteins, disrupting their functions and thereby impeding the survival of pathogens.

Molecular docking, as a computational technique, offers a unique vantage point to explore these interactions at a molecular level. By virtually simulating the binding of bioactive compounds to target proteins, molecular docking provides insights into the intricate molecular contacts, binding orientations, and binding affinities governing their interactions [9]. This enables researchers to identify the specific amino acid residues and chemical moieties that play pivotal roles in the formation of stable complexes. Such information is invaluable not only for understanding the binding mechanisms but also for facilitating the rational design of new and improved antimicrobial and antifungal agents based on the structure of natural bioactive compounds [10]. This article is poised to shed light on this intricate molecular tango through the application of molecular docking, an advanced computational tool that provides insights into the binding affinities, interaction modes, and key molecular contacts within complex biological systems.

2. Materials and Methods

The molecular docking technique was used to predict the position and orientation of ligands (the bioactive compounds found in apples and the apple pomace) bound to a selected list of protein receptors (targets).

Three classes of ligands were selected by cross-referencing the literature data [11–17] with PubChem (https://pubchem.ncbi.nlm.nih.gov) [18]: phenolic acids (caffeic acid, gallic acid, ferulic acid, p-coumaric acid, chlorogenic acid, syringic acid, ellagic acid, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid and cinnamic acid), polyphenols ((+)-catechin, (-)-epi-catechin, quercetin, kaempferol, rutin, myricetin, phloretin, procyanidin, and phlorizin), and triterpenoids (ursolic and oleanolic acids). The structure-data files (SDF file format) for selected ligands were obtained from PubChem [18], then tridimensional (3D) optimized and converted in Tripos MOL2 file format (MOL2), as required for molecular docking. MarvinSketch was used for 3D optimization and generation of all ligand files, MarvinSketch version 23.12.0, 2023, ChemAxon (https://chemaxon.com/).

Target selection was made by cross-referencing the literature data with *The Research Collaboratory for Structural Bioinformatics Protein Data Bank* (RCSB PDB) [19–22]. Three structural files (RCSB PDB IDs) solved by X-ray crystallography were selected to perform three different docking runs: (1) 5TZ1 (Lanosterol 14-alpha demethylase from *Candida albicans*—fungus) [23]; (2) 2W9H (Dihydrofolate reductase from *Staphylococcus aureus*—gram-positive bacterium) [24]; and (3) 3MZF (D-alanyl-D-alanine carboxypeptidase DacA from *Escherichia coli*—gram-negative bacterium) [25].

Three individual molecular docking runs were executed for each identified molecular target utilizing AutoDock Vina v.1.2.0 [26]. PyRx—Python Prescription v.0.9.2 [27] was used as the control interface. AutoDock Vina, as part of its functionality, automatically computed grid maps and subsequently organized the docking outcomes in a transparent manner, enhancing the user experience. All molecular docking runs were conducted within a search space encompassing less than 27,000 Å^3, a region centered around the binding site of the co-crystallized ligands derived from the X-ray structures of the respective molecular targets, as advocated by the developers of the software [26]. To enhance the precision of the docking process, the exhaustiveness parameter was configured to 100, a significant increase from the default exhaustiveness value of 8 in PyRx v.0.9.2. As a reference and control measure, the co-crystallized ligands associated with each identified molecular target were subjected to re-docking in their respective docking runs.

3. Results

The summary of the three docking runs is provided concisely in Table 1, showcasing the binding affinities of the best docking poses from each run. A detailed graphical depiction of binding patterns of the best binders in each ligand category against their molecular targets is provided in the Supplementary Materials (Figure S1).

Table 1. Docking results: binding affinities of the best docking poses against selected targets.

Ligands		Targets (BA)		
Name	**IDs**	**5TZ1**	**2W9H**	**3MZF**
Phenolic acids				
Caffeic acid	689043	−6.6	−6.3	−5.4
Gallic acid	370	−5.7	−5.6	−5.2
Ferulic acid	445858	−6.2	−6.4	−5.4
p-Coumaric acid	637542	−6.5	−6.2	−5.1
Chlorogenic acid	1794427	−8.4	−8.7	−7.0
Syringic acid	10742	−5.5	−5.7	−5.4
Ellagic acid	5281855	−7.5	−8.7	−7.1
4-Hydroxybenzoic acid	135	−5.5	−5.4	−4.7
3,4-Dihydroxybenzoic acid	72	−5.8	−5.4	−5.2
Cinnamic acid	444539	−6.6	−5.9	−5.0
Polyphenols				
(+)-Catechin	9064	−8.3	−8.6	−6.6
(-)-Epicatechin	72276	−8.2	−8.3	−6.6
Quercetin	5280343	−8.5	−8.8	−6.6
Kaempferol	5280863	−8.3	−8.7	−6.5
Rutin	5280805	−9.5	−8.5	−7.7
Myricetin	5281672	−7.6	−8.7	−6.6
Phloretin	4788	−7.7	−8.0	−6.3
Procyanidin	107876	−9.9	−8.2	−7.2
Phlorizin	6072	−8.4	−8.8	−7.0
Triterpenoids				
Ursolic acid	64945	−9.5	−6.1	−5.9
Oleanolic acid	10494	−9.9	−5.2	−5.4
Reference compounds				
Oteseconazole	77050711/VT1	−10.3	N/A	N/A
Trimethoprim	5578/TOP	N/A	−7.4	N/A
Imipenem	5288621/IM2	N/A	N/A	−5.9

BA: binding affinity expressed in kcal/mol; IDs: for natural bioactive compounds were used the PubChem Compound Identification (CID) records, while for the reference compounds were used both CIDs and RCSB PDB identifiers (PDB IDs); N/A: not applicable.

4. Discussion

The results of the molecular docking simulations reveal promising interactions between bioactive compounds from apple pomace and selected protein targets. Notably, several phenolic acids, polyphenols, and triterpenoids displayed strong BAs with the selected protein receptors.

Phenolic acids: Among the phenolic acids, chlorogenic acid demonstrated the highest binding affinity against the fungal target, while ellagic acid was the best binder against the gram-positive bacterial target. Both compounds exhibited identical binding affinity for the gram-negative bacterial target. Their strong binding affinities suggest their potential as broad-spectrum antimicrobial and antifungal agents. Caffeic acid, ferulic acid, and

p-coumaric acid also displayed notable BAs, indicating their potential therapeutic relevance.

Polyphenols: Procyanidin and rutin exhibited good binding affinities against the selected protein receptors. Those compounds strong interactions with selected targets suggest their potential as potent antimicrobial and antifungal agents. Additionally, quercetin, phlorizin and kaempferol showed substantial BAs across the protein targets, underscoring their promising inhibitory properties.

Triterpenoids: Ursolic acid, one of the triterpenoids investigated in this study, displayed impressive binding affinities, particularly against the fungal target (5TZ1). This finding suggests that ursolic acid may be a valuable candidate for antifungal drug development. Oleanolic acid exhibited even better BS against the fungal target but showed varying interactions with the two bacterial targets.

Oteseconazole, a known antifungal drug, demonstrated the highest BA against its target (5TZ1), validating the reliability of our molecular docking approach. Trimethoprim and imipenem, both known antibacterial drugs, demonstrated substantial binding affinities against their respective targets (2W9H and 3MZF), confirming the docking runs' accuracy.

5. Conclusions

Molecular docking provides valuable mechanistic insights into the interactions between these compounds and microbial proteins, aiding in the rational design of novel therapeutic agents and/or the formulation of functional foods for the benefit of human health. However, future research directions could include experimental validation of these in silico findings through in vitro and in vivo studies. Additionally, the identification of (other) specific pathways and molecular mechanisms through which these bioactive compounds exert their antimicrobial and antifungal effects should be pursued, enabling a deeper understanding of their mode of action.

In conclusion, this study highlights the potential of repurposing apple pomace for its bioactive compounds and emphasizes the utility of molecular docking as a powerful tool for investigating the antimicrobial and antifungal mechanisms of natural products. This research contributes to the broader goal of sustainable resource utilization and: (1) the development of effective therapies against microbial infections and fungal diseases; and (2) the development of functional foods that can positively impact public health and well-being.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Binding patterns of the best binders in each ligand category against their molecular targets.

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