ICSI Analytics, National Institute for Research and Development for Cryogenic and Isotopic Technologies – ICSI Rm. Vâlcea

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

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Introduction (1/3)

- 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.
- Apple pomace has garnered significant attention for its untapped potential as a reservoir of bioactive phyto-chemicals.
- 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.

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Introduction (2/3)

- 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.

Introduction (3/3)

- 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.
- 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.

Materials and Methods (1/3)

- 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 se-lected list of protein receptors (targets).
- Three classes of ligands were selected by cross-referencing the literature data with **PubChem**:
  - **Phenolic acids:** caffeic acid, gallic acid, ferulic acid, p-coumaric acid, chlorogenic acid, syringic acid, ellagic acid, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, cinnamic acid;
  - **Polyphenols:** (+)-catechin, (-)-epi-catechin, quercetin, kaempferol, rutin, myricetin, phloretin, procyanidin, phlorizin;
  - **Triterpenoids:** ursolic and oleanolic acids.

Materials and Methods (2/3)

- Target selection was made by cross-referencing the literature data with ***The Research Collaboratory for Structural Bioinformatics Protein Data Bank*** (RCSB PDB)
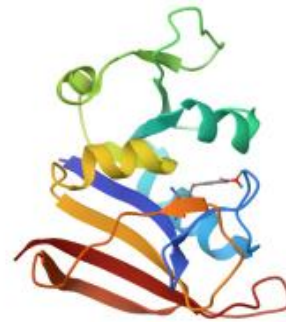

5TZ1
Lanosterol 14-alpha demethylase
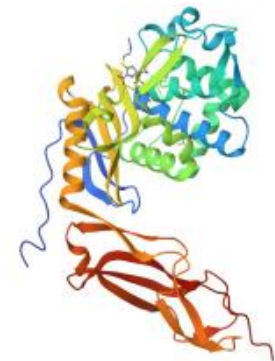*Candida albicans*
(fungus)

2W9H
Dihydrofolate reductase
Staphylococcus aureus
(gram-positive bacterium)

3MZF
D-alanyl-D-alanine carboxypeptidase DacA
Escherichia coli
(gram-negative bacterium)

Materials and Methods (3/3)

- Three individual molecular docking runs were executed for each identified molecular target utilizing AutoDock Vina v.1.2.0.
- PyRx—Python Prescription v.0.9.2 was employed as the control interface.
- All molecular docking runs were conducted within a search space encompassing less than 27,000 Å³ (a region centered around the binding site of the co-crystallized ligands derived from the X-ray structures of the respective molecular targets).
- 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.

Results (1/4)

| 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

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Results (2/4)

The best binders in each ligand category against 5TZ1

Ligand	BA (kcal/mol)	Color
Phenolic acids		
Chlorogenic acid	-8.4	🟢
Polyphenols		
Procyanidin	-9.9	🟦
Triterpenoids		
Oleanolic acid	-9.9	🟧
Reference compounds		
Oteseconazole (co-crystallized)	N/A	⬛
Oteseconazole (re-docking)	-10.3	🟪
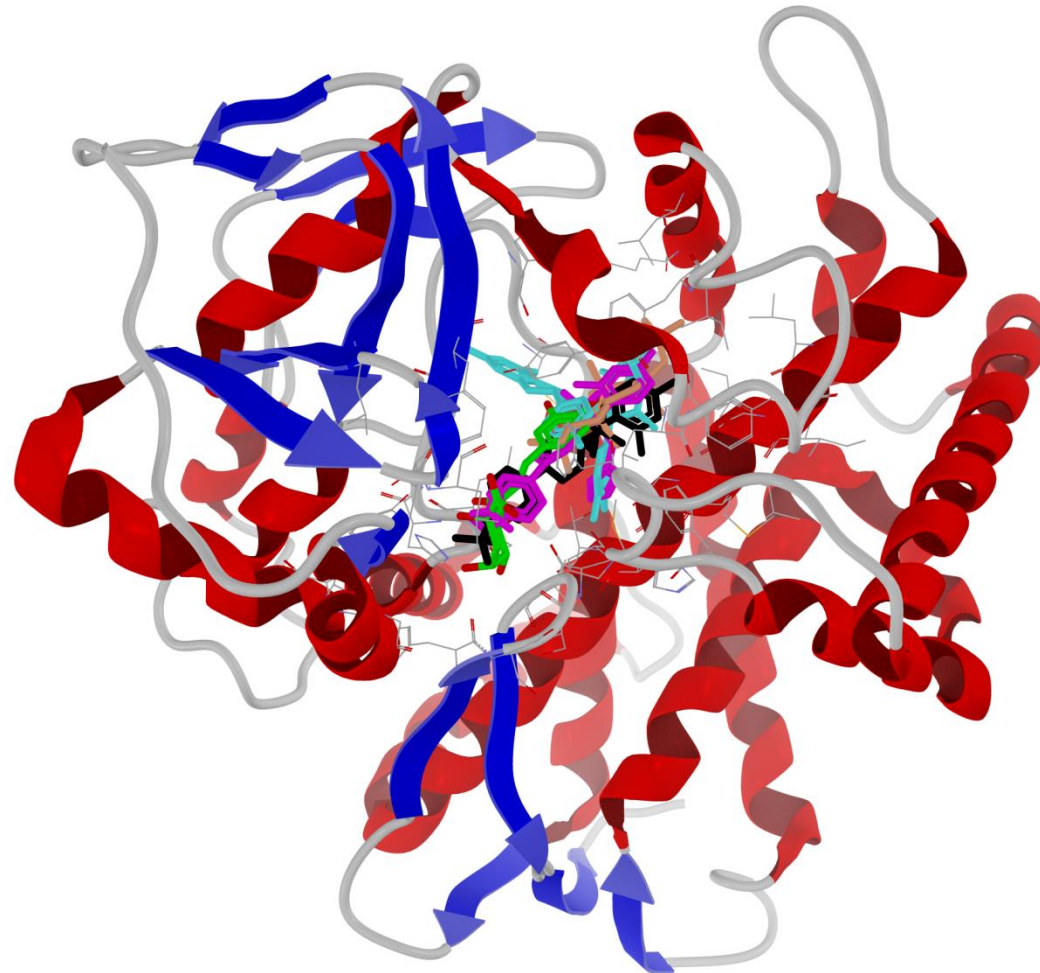
Results (3/4)

The best binders in each ligand category against 2W9H

Ligand	BA (kcal/mol)	Color
Phenolic acids		
Chlorogenic acid	-8.7	■
Ellagic acid	-8.7	■
Polyphenols		
Quercetin	-8.8	■
Phlorizin	-8.8	■
Triterpenoids		
Ursolic acid	-6.1	■
Reference compounds		
Trimethoprim (co-crystallized)	N/A	■
Trimethoprim (re-docking)	-7.4	■
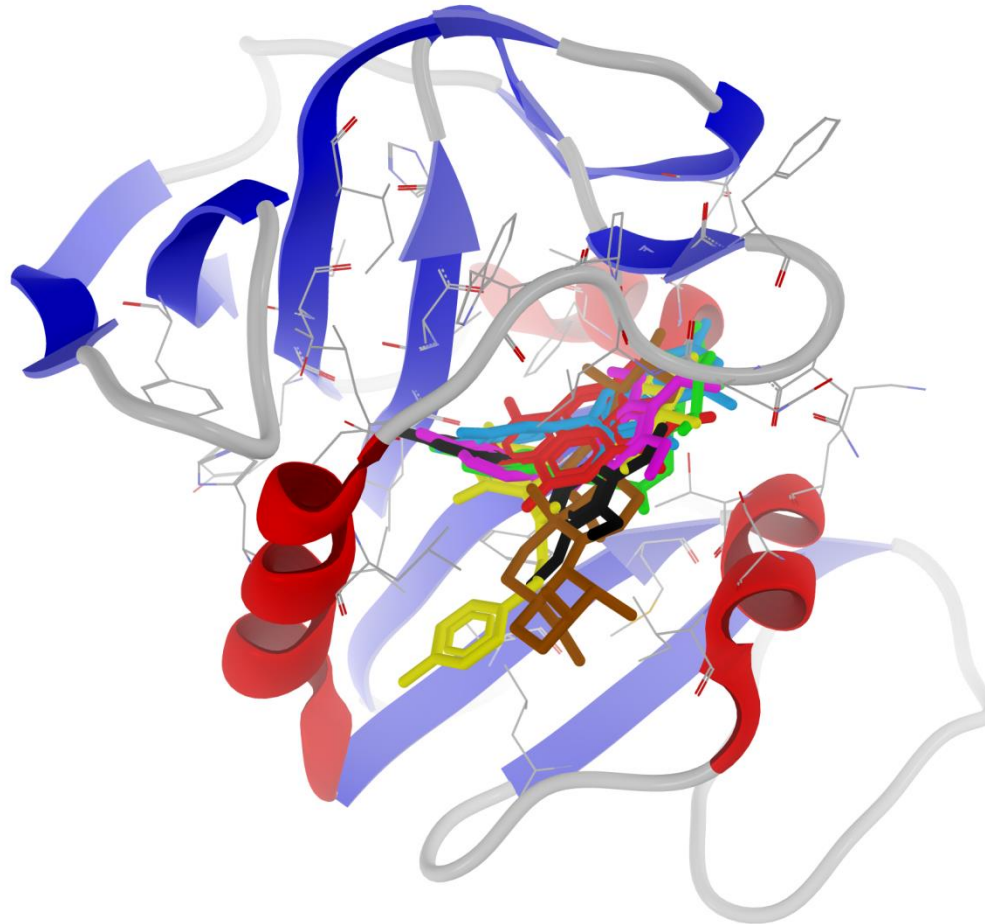
Results (4/4)

The best binders in each ligand category against 3MZF

Ligand	BA (kcal/mol)	Color
Phenolic acids		
Ellagic acid	-7.1	🟥
Polyphenols		
Rutin	-7.7	🟧
Triterpenoids		
Ursolic acid	-5.9	🟫
Reference compounds		
Imipenem (co-crystallized)	N/A	⬛
Imipenem (re-docking)	-5.9	🟪
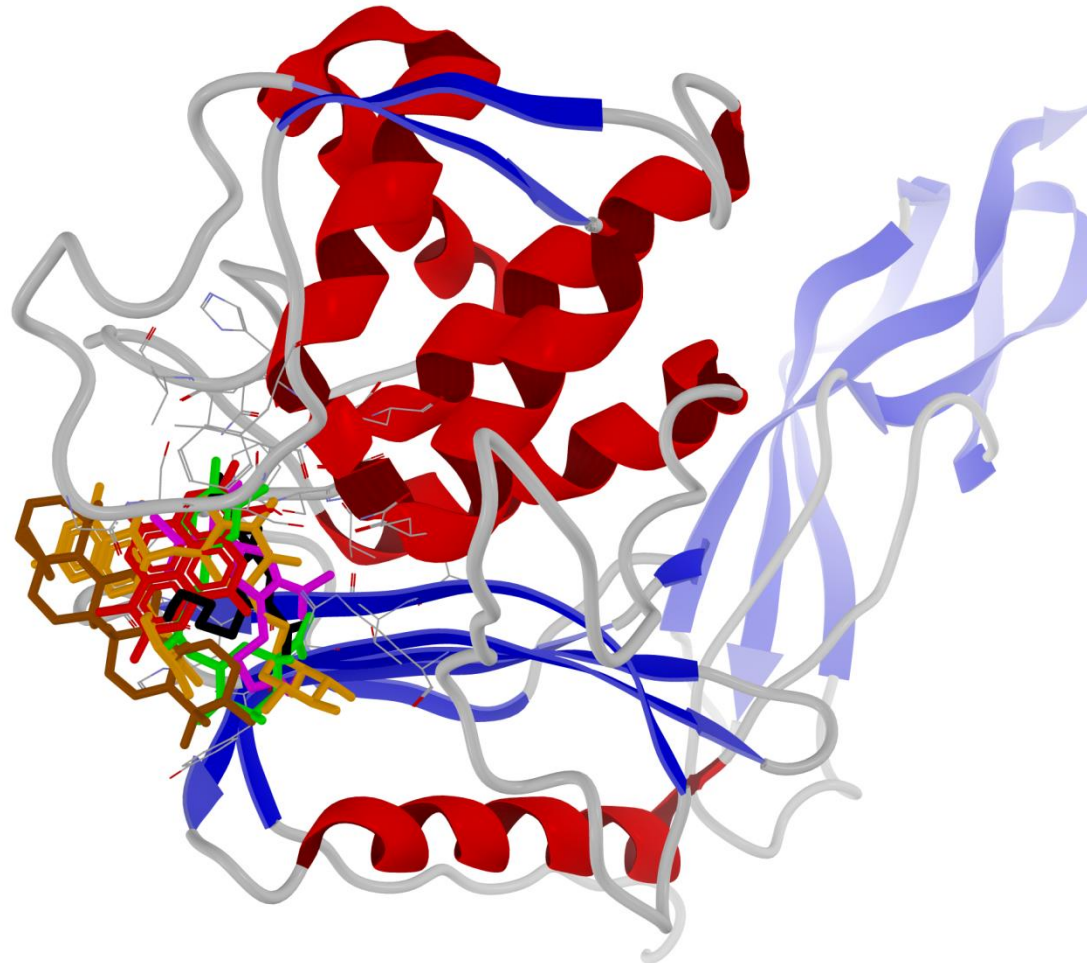
Discussion (1/3)

- The results of the molecular docking simulations reveal promising interactions be-tween 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.

Discussion (2/3)

- ***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.
  - Quercetin, phlorizin and kaempferol showed substantial BAs across the protein targets, underscoring their promising inhibitory properties.

- ***Triterpenoids:***
  - Ursolic acid 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.

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Discussion (3/3)

- ***Reference compounds:***
  - **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.

- 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.

Conclusions

- 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:

The development of effective therapies against microbial infections and fungal diseases.

The development of functional foods that can positively impact public health and well-being.

THANK YOU FOR YOUR ATTENTION!

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