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Fruit Bioactive Compounds as P-Glycoprotein Inhibitors - An In-Silico Study

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Abstract

Introduction: *P-glycoprotein is a crucial efflux transporter whose overexpression contributes to drug resistance, hindering effective treatment of various diseases.*

Objective: *This study aims to identify the inhibitory bioactive fruit components on p-glycoprotein, which could potentially overcome drug resistance.*

Methodology: *150 bioactive components were identified from 40 regional fruits from India. The druggability and pharmacokinetic profiles of the components were examined, revealing 8 potential inhibitors. Molecular docking was performed to identify the most suitable inhibitor, which was then subjected to simulation to validate its interaction with p-glycoprotein.*

Result: *According to our research, Δ5-Avenasterol (−79.5 kcal/mol) and Phyllanthin (−78.8 kcal/mol) have a comparable binding affinity to the control inhibitor rifampin (−73.6 kcal/mol), making them appropriate inhibitors for p-glycoprotein.*

Conclusion: *The conformational stability of the most effective inhibitory drugs inside the p-glycoprotein structure was evaluated using molecular dynamics simulations. This research may lead to the development of novel natural p-glycoprotein inhibitors, providing a promising strategy for combating multidrug resistance.*

Key words: *P-glycoprotein, inhibitors, fruit components, drug efflux, docking, simulation.*

INTRODUCTION

P-glycoprotein is a transport protein of the ABC-transporter superfamily; whose overexpression results in multidrug resistance (MDR) in infectious, cancerous and other chronic illnesses by actively effluxing therapeutic molecules from cells, thereby reducing drug efficacy (Juvale et al., 2022). Targeting p-glycoprotein to help its efflux activity is a different strategy for overcoming MDR (Pokharel et al., 2017). In healthy tissue, p-gp regulates cellular uptake, distribution, and elimination of foreign substances, affecting drug absorption, metabolism, excretion, and toxicity, reducing effectiveness and bioavailability (Finch and Pillans, 2014). During drug development, ADMET properties helps identify issues early and design molecules with improved therapeutic potential (Guan et al., 2018; Prachayasittikul and Prachayasittikul, 2016). In order to develop novel approaches to restore medication sensitivity in MDR diseases, it is crucial to suppress p-glycoprotein activity. This can be accomplished in one of three ways: by reducing or inhibiting the levels of p-glycoprotein expression; by preventing p-glycoprotein from interacting with the drug of interest; or by preventing the ATPase enzyme, which normally supplies p-glycoprotein with the energy it needs to efflux. (Xue et al., 2017). Certain drugs and other bioactive substances with practical scaffolds can reverse MDR brought on by p-glycoprotein. The combination of fruit ingredients may have a major influence on the absorption and efficacy of medications (Ganesan et al., 2021).

Fruit compounds can interact with the drug transporter p-glycoprotein to modify its efflux activity. Certain drug transporters have been reported to be modulated by citrus fruit juices, especially those from orange, grapefruit and pummelo (Petric et al., 2021). Fruit extracts from mints, apricots, oranges, and strawberries reduce the activity of efflux carriers associated with p-glycoprotein (Deferme et al., 2002). A number of dietary phytochemicals have been assessed for their possible p-glycoprotein inhibitory effects, including the bioflavonoid quercetin, morin and -Epigallocatechin gallate (EGCG) (Kitagawa et al., 2004).

Flavonoids and fruit extracts, especially quercetin, naringenin, and tangeretin, decrease p-glycoprotein activity, enhancing intestinal absorption and bioavailability of drugs that use p-glycoprotein substrates and boosting their potential for therapeutic use (Ghafourian et al., 2020; Varma et al., 2021). The efficacy and safety profiles of these drugs require further research using modern techniques viz., molecular docking, structure-activity relationship analysis and clinical trials (Shukla et al., 2022).

The overexpression of p-glycoprotein leads to reduced intracellular accumulation of various anticancer medicines, thereby diminishing their potency and facilitating MDR (Dewanjee et al., 2017). Studies on the therapeutic potential of natural products in preventing or curing illnesses by modifying p-glycoprotein activity, are underway to develop targeted inhibitors of the p-glycoprotein transporter in order to combat MDR. Fruit phytochemicals not only improve health but also revolutionize drug delivery and discovery by offering innovative and sustainable solutions to a range of pharmacological problems (Heneman and Zidenberg, 2008).

Thus, the present study investigates the potential of fruit-derived bioactive components as p-glycoprotein inhibitors, aiming to identify the most effective candidates for inhibition of p-glycoprotein.

METHODOLOGY

40 Indian fruits were selected, from which 150 bioactive components were chosen to identify potential p-glycoprotein inhibitors.

1. Preparation of protein molecule

The crystal structure of human p-glycoprotein (PDB ID: 6C0V) (https://www.rcsb.org/structure/6C0V) was obtained using RCSB PDB (https://www.rcsb.org/) for molecular docking. Energy minimization and structural optimization were carried out using “protein preparation” protocol of Biovia Discovery Studio (DS) version 4.5 (2021). Biovia DS assisted in side chain optimization of the protein structure prior to docking.

2. Preparation of Ligand molecule

The Simplified Molecular Input Line Entry System (SMILES) is a species-specific line notation system used to describe the structure of chemical compounds. The canonical SMILES of these bioactive components were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and verified using ChemSpider-(https://pubchem.ncbi.nlm.nih.gov/source/ChemSpider).

3. Calculation of molecular properties

The Molinspiration Cheminformatics software (https://molinspiration.com/) was used to analyze the molecular characteristics of bioactive components.

4. ADMET properties analysis

To evaluate the pharmacokinetic properties of each compound that meets Lipinski’s Rule of Five, the ADMET characteristics were analyzed using the pkCSM platform. For each active component of fruits, several properties were evaluated, including lipophilicity (Log P), molecular weight, and the number of hydrogen bond donors and acceptors. When a substance contains more than five hydrogen bond donors, more than ten hydrogen bond acceptors, a molecular weight larger than 500 Daltons, or a Log P value greater than five, Lipinski's Rule of Five predicts poor absorption or permeability. The analysis allows for at most one violation of these criteria (Lipinski et al., 1996).

It provides insights into a potential of compound behaviour in a biological system, including its absorption in the gastrointestinal tract, distribution across various tissues, metabolic stability, elimination routes, and possible toxic effects. By integrating these predictions, pkCSM aids in assessing the drug-likeness and therapeutic potential of bioactive molecules.

5. Molecular Docking

Protein-ligand flexible docking was carried out with the CDOCKER tool of Biovia software. The lowest energy conformation for every ligand was chosen and examined using the 'Analyzed Ligand Program'.

Molecular Dynamic Simulation

The two best receptor-ligand complexes, identified through molecular docking based on their lowest binding energy, were subjected to simulation using BIOVIA DS software and prepared with a CHARMM-based force field (Roy and Ghosh, 2023).

RESULT

Fruit components have distinct pharmacokinetic and structural characteristics that make them useful in a range of therapeutic settings. Research suggests that bioactive fruit compounds can inhibit p-glycoprotein, potentially enhance drug efficacy and overcome drug resistance. The bioactive compounds of fruits and their uses are presented in Table 1.

Table 1: Bioactive components of fruits and their uses.

Fruit	Bioactive components	Uses
Amla (Phyllanthus emblica)	Phyllanthin	Phyllanthin is known for its role in modulating p-glycoprotein activity (Mirunalini and Krishnaveni, 2010; Marques et al., 2021).
Coconut (Cocos nucifera)	Δ5-Avenasterol	Three coconut-derived sterols, similar to cholesterol, have been studied for their antioxidant and anti-inflammatory properties, indicating their potential pharmaceutical relevance (Evtyugin et al., 2023).
Orange (Citrus sinensis)	Tangeretin, Sinensetin, and Nobiletin	Polymethoxyflavones are known for their potent anti-cancer and anti-inflammatory properties, and their ability to inhibit p-glycoprotein enhances their therapeutic potential in overcoming drug resistance in cancer therapy (Arafa et al., 2021).
Jackfruit (Artocarpus heterophyllus)	Cycloartenol, Cycloartenone, and Cycloartenyl Acetate	Jackfruit triterpenoids exhibit anti-inflammatory and cholesterol-lowering properties (Kaur et al., 2024).

Drug likeness analysis

The bioactive components exhibited acceptable drug-like properties, suggesting their potential for satisfactory oral bioavailability based on Lipinski's Rule of Five, a widely used criterion for evaluating drug-likeness. (Table 2).

Table 2: Drug-Like Characteristics of p- glycoprotein inhibitors

Sl No.	COMPOUNDS	MW	LogP	nHBA	nHBD	n violation
1	Δ5- Avenasterol	412.7	7.9	1	1	1
2	Phyllanthin	418.5	4	6	0	0
3	Tangeretin	372.3	3.5	7	0	0
4	Cycloartenol	426.7	8.1	1	1	1
5	cycloartenone	424.7	8.37	1	0	0
6	Sinensetin	372.3	3.5	7	0	0
7	Cycloartenyl acetate	468.7	8.7	2	0	1
8	Nobiletin	402.3	3.5	8	0	0

Here, molecular weight (MW), lipophilicity (LogP), number of hydrogen bond donors (nHBD), number of hydrogen bond acceptors (nHBA) and number of violations (n violation) are presented as per Lipinski's rule of five.

Most compounds exhibit favourable properties for drug development, with minimal violations, indicating potential bioavailability and drug-like characteristics.

Additionally, by evaluating the absorption, distribution, metabolism, excretion and toxicity profiles of these bioactive inhibitors, the ADMET prediction findings offered additional detailed information about their pharmacokinetic characteristics, which supported their drug-likeness assessment.

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In-silico ADMET prediction analysis

Table: 3 depicts that Tangeretin and Nobiletin have high intestinal absorption but poor BBB and CNS permeability. Cycloartenol and cycloartenone have good BBB permeability. Phyllanthin has poor skin and CNS permeability but high intestinal absorption. Δ5-Avenasterol have similar profiles.

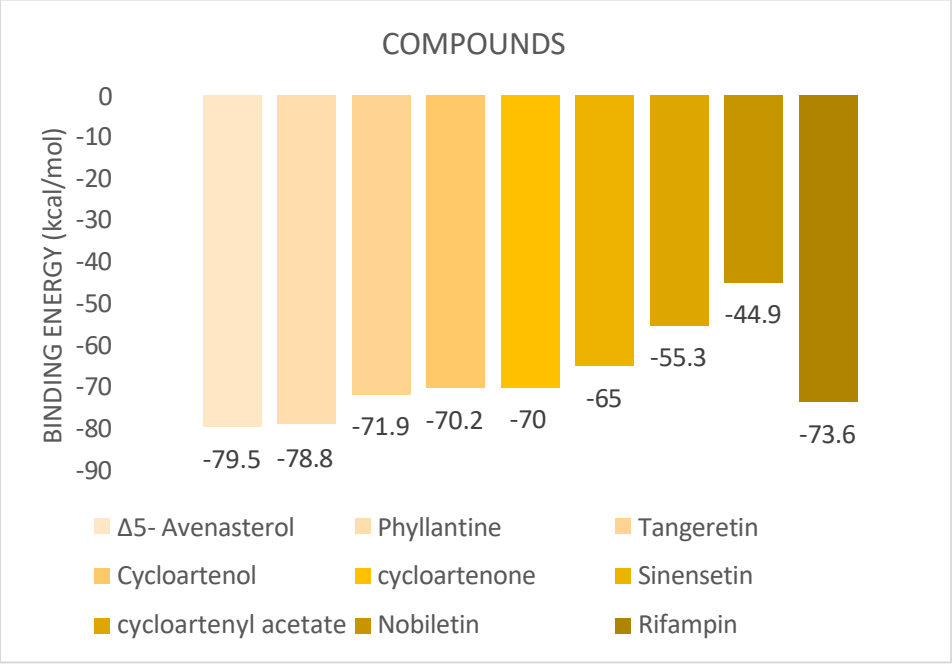
Table 3: ADMET predictions for p-glycoprotein inhibitors

Sl No.	COMPOUNDS	Log S	Intestinal absorption	LogKp cm/s	BBB permeability	CNS permeability	Total Clearance	Max tolerance dose	hepatotoxicity	Skin sensitivity
1	Δ5- Avenasterol	-6.7	94.6	-2.7	0.7	-1.6	0.6	-0.6	NO	NO
2	Phyllanthin	-5.7	98.3	-5.8	-1.3	-3	0.5	0.4	NO	NO
3	Tangeretin	-4.8	98.5	-6.4	-1.0	-3.0	0.7	0.3	NO	NO
4	Cycloartenol	-5.8	95.2	-1.9	0.7	-1.7	0.2	-0.4	NO	NO
5	Cycloartenone	-5.9	97.7	-2.7	0.8	-1.5	0.2	-0.2	NO	NO
6	Sinensetin	-4.7	98.5	-6.0	-1.0	-3.0	0.7	0.2	NO	NO
7	cycloartenyl acetate	-5.9	97.3	-2.7	0.7	-1.7	0.1	-0.3	NO	NO
8	Nobiletin	-4.9	98.9	-6.4	-1.2	-3.1	0.7	0.443	NO	NO

Solubility (Log S), intestinal absorption, skin permeability (Log Kp), permeability of the central nervous system (CNS) and blood-brain barrier (BBB), total clearance, maximum tolerated dose and safety hazards are some of the key characteristics shown in the table.

Most drugs have strong oral bioavailability and high intestinal absorption (>90%). Compounds such as Δ5-Avenasterol shows potential CNS action despite differences in BBB and CNS permeability. The absence of hepatotoxicity or skin sensitization among the compounds indicates a favourable safety profile. Tangeretin and nobiletin are notable for their outstanding absorption abilities.

Figure: 1: Graphical representation of the p-glycoprotein inhibitors according to binding energy



The interactions between the bioactive components and the target protein were examined using molecular docking analysis, which provided information on the underlying mechanisms of action and possible effectiveness of the components. The binding energies (in kcal/mol) of the bioactive compounds are shown in Figure 1. Among the examination of the displayed compounds, at a binding energy of −79.5

kcal/mol, Δ5-avenasterol had the greatest binding affinity, while nobiletin displayed the least binding affinity at −44.9 kcal/mol.

Molecular docking Result

Table 4 gives a summary of the binding energies, hydrogen bonds, hydrophobic interactions and binding sites of the docked bioactive components. Binding energy analysis revealed that Δ5-Avenasterol had the lowest binding energy (-79.5 kcal/mol), succeeded by Phyllanthine (-78.8 kcal/mol). Notably, both components surpassed the binding energy of the control drug rifampin (-73.6 kcal/mol).

Table 4: Docking Analysis of the p-glycoprotein inhibitors

SL NO.	COMPOUNDS	Binding energy (kcal/mol)	No of H bonds	Binding Site	Number of hydrophobic interactions	Binding Site
1	Δ5-Avenasterol	-79.5	0	-	11	PHE983, LEU976, LEU975, ILE736, PHE732, ALA729, PHE336, LEU332, ILE328, PHE79, PHE72
2	Phyllanthin	-78.8	2	GLU972, LEU332	3	ILE736, PHE732, ALA729
3	Tangeretin	-71.9	2	SER979, LEU976	3	PHE732, LEU 332, PHE72
4	Cycloartenol	-70.2	1	SER979	8	LEU976, LEU975, ILE736, ALA729, LEU332, ILE328, PHE79, PHE72
5	Cycloartenone	-70	0	-	8	LEU976, LEU975, ILE736, PHE336, LEU332, PHE72, PHE73, PHE79
6	Sinensetin	-65	1	GLU972	5	LEU976, ILE736, PHE732, LEU332, PHE72
7	Cycloartenyl acetate	-55.3	0	-	6	LEU976, LEU975, ILE736, LEU332, PHE79, PHE72
8	Nobiletin	-44.9	5	SER979, LEU976, GLU972, ALA729, LEU332	5	LEU976, ILE736, LEU332, PHE79, PHE72

Table 4 represents the results of docking experiments, which were used to evaluate hydrophobic interactions, hydrogen bonding and binding energy (kcal/mol) of bioactive compounds at the protein binding site. The compounds are arranged according to their binding energy: Δ5-Avenasterol (-79.5 kcal/mol) demonstrated the strongest binding, forming eleven hydrophobic interactions, while nobiletin (-44.9 kcal/mol) exhibited the weakest binding, forming only five hydrogen bonds. The Ligand-protein binding stability is maintained by the participation of important residues in hydrogen bonding and hydrophobic interactions, including LEU332, PHE72, PHE732 and LEU976.

Figure 2: Two-dimensional and Three-dimensional molecular interactions between p-glycoprotein and bioactive components

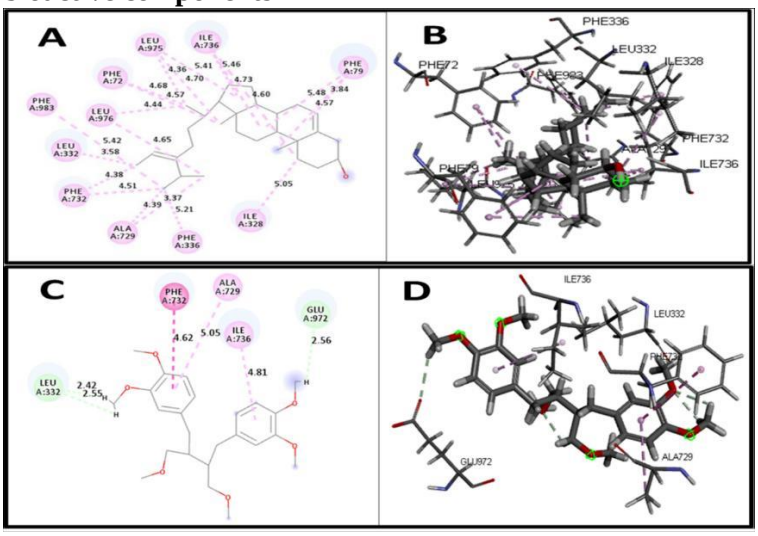


Figure 2 illustrates both two-dimensional and three-dimensional molecular interactions between the bioactive compounds and the protein binding site. Panels A (2D structure of Δ5-Avenasterol) and B (3D structure of Δ5-Avenasterol) highlight key hydrophobic interactions with residues viz., PHE72, PHE336, LEU332, and ILE736. Panels C (2D structure of Phyllanthin) and D (3D structure of Phyllanthin) show that Phyllanthin forms hydrogen bonds with GLU972, LEU332 along with hydrophobic interactions with PHE732, ALA729 and ILE736. The 2D diagrams (A and C) display hydrogen bonds and hydrophobic contacts with corresponding bond lengths, while the 3D diagrams (B and D) provide spatial orientation within the binding pocket, emphasizing the role of specific residues in ligand stability. To further validate the binding interactions and stability of the ligand-protein complexes observed in the molecular docking analysis, molecular dynamics (MD) simulations were performed to assess the conformational behaviour and dynamic properties of the system over time.

Molecular Dynamic Simulation

MD simulations are essential for concluding in silico research. The evaluations included the radius of gyration (Rg) and root means square fluctuations (RMSF). MD simulations of the docked complexes were performed in order to have a better understanding of the dynamic behaviour of the lead compounds inside the active areas of the protein structure. Phyllanthin shows a slightly stronger binding affinity compared to Δ5-Avenasterol, indicated by the more negative binding energy. Both compounds have similar electrostatic and van der Waals energy values but phyllanthin has a slightly higher van der Waals energy, which may contribute to its stronger binding affinity. The potential energy values are very close, indicating similar stability and both compounds have identical radius of gyration (47.9), indicating they have similar molecular compactness.

Table 4: Molecular dynamics simulation analysis of Δ5-Avenasterol and Phyllanthine

Sl No.	Compounds	Binding energy (Kcal/mol)	Electrostatic energy (Kcal/mol)	Van der Waals energy (Kcal/mol)	Potential energy (Kcal/mol)	Kinetic energy (Kcal/mol)
1	Δ5- Avenasterol	-36.6	-209106	7681.4	-201452	58298.8
2	Phyllanthin	-40.5	-209751	8459.4	-201894	58385.4

Table 4 shows that Phyllanthine, with a lower binding energy (-40.5 kcal/mol) than Δ5-Avenasterol (-36.6 kcal/mol), shows higher binding stability. Van der Waals interactions (7681.4–8459.4 kcal/mol) and electrostatic energy (Δ5-Avenasterol: -209,106 kcal/mol; Phyllanthine: -209,751 kcal/mol) also contribute considerably to the total potential energy for both compounds. The kinetic energy (~58,000 kcal/mol) and potential energy profiles (-201,452 to -201,894 kcal/mol) demonstrate the stable molecular connections of the system. The ligand-protein complexes appear to be similarly compact and structurally stable throughout the simulation, as shown by the constant radius of gyration (47.9 Å).

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Figure: 3 The graphical presentation of root mean square fluctuations (RMSF) of Δ5-Avenasterol (left) and Phyllanthin (right) with respect to p-glycoprotein

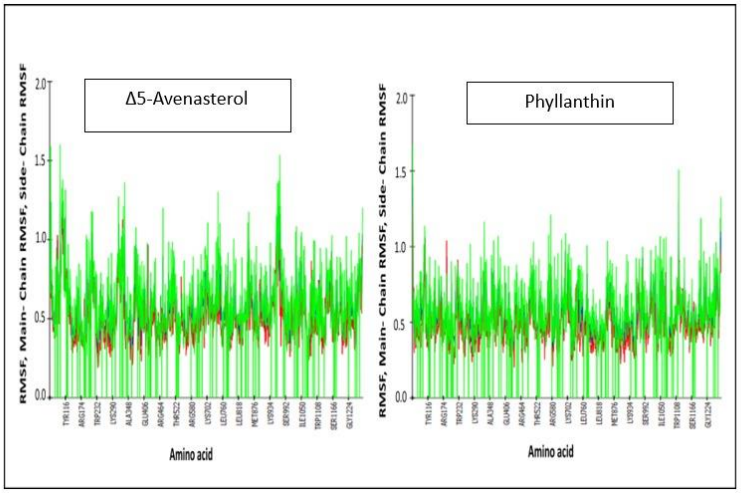
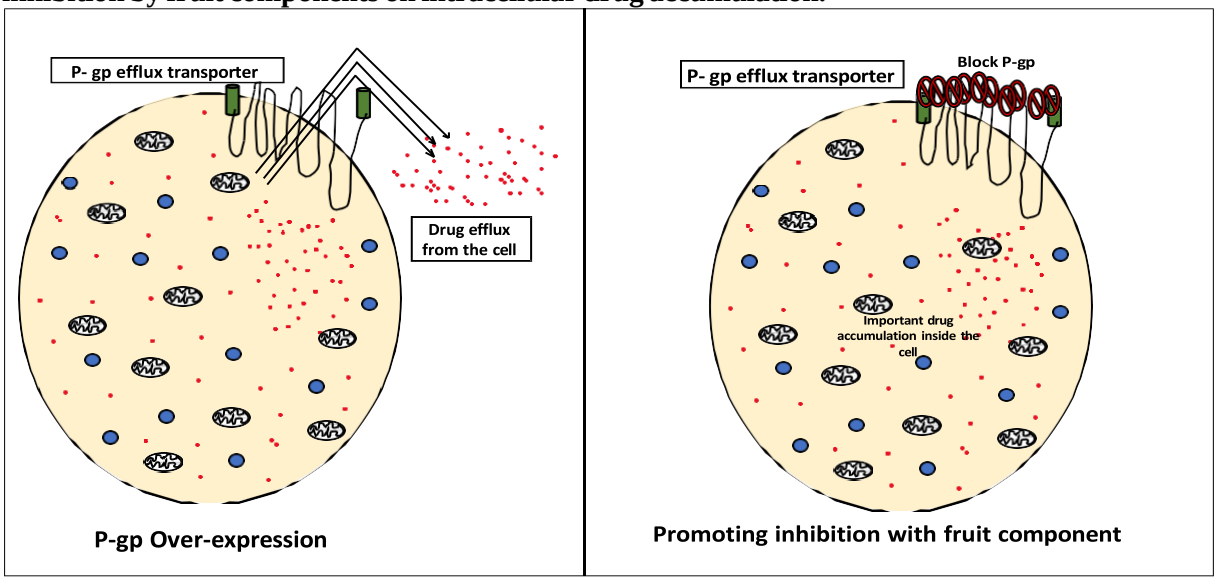


Figure 3 illustrates the use of RMSF to calculate the average displacement of an atom from its time-averaged position, providing insights into the dynamics and flexibility of protein residues. The RMSF plot shows the relative flexibility of protein residues, with higher values indicating greater flexibility and lower values indicating stability. The main-chain RMSF values are consistently lower than the side-chain values, indicating that the backbone of the protein is more stable.

DISCUSSION

Multidrug resistance (MDR) primarily arises from the overexpression of p-glycoprotein, an efflux transporter, which reduces the accumulation of drugs within cells. Inhibiting p-glycoprotein activity can modify the pharmacokinetics of drug substrates, potentially improving their bioavailability and ability to penetrate tissues.

Figure: 4 Schematic representations showing the effect of p-glycoprotein overexpression and its inhibition by fruit components on intracellular drug accumulation.



In case of P-gp over expression in figure: 4, the efflux transporters may actively pump therapeutic drugs out of the cell (left panel). This reduces intracellular drug accumulation and may result in drug resistance. Fruit-derived bioactive components may inhibit P-gp by blocking its efflux activity. (right panel). This leads to improved intracellular drug retention and perhaps increased therapeutic efficacy.

Since natural compounds are a safe substitute for manufactured drugs, which are frequently linked to harmful side effects and toxicity, they are attracting attention as possible p-glycoprotein inhibitors

(Abdallah et al., 2016). Fruit ingredients have the ability to directly change the activity of p-glycoprotein by interacting with either the substrate-binding site or the ATP binding site.

Numerous studies have shown that components derived from fruit can modify p-glycoprotein-driven efflux. For example, because of its interaction with p-glycoprotein, orange juice decreases digoxin transport by 60–70% at a 50% concentration (Lim et al., 2008). Additionally, it has been discovered that orange juice contains a number of active compounds that function as p-glycoprotein inhibitors, such as 3,3′,4′,5,6,7,8-heptamethoxyflavone, tangeretin, and nobiletin (Takanaga et al., 2000). Citrus fruits are also a great source of bioactives that have long been utilized to treat hypertension and heart disease (Abobatta, 2019). It has been demonstrated that the flavonoids bergamottin and naringin, which are present in grapefruit juice, change the way talinolol is transported by p-glycoprotein in rat intestinal sacs and Caco-2 cells (Yu et al., 2016; Dewanjee et al., 2017). Orange extracts have also been shown to interfere with p-glycoprotein's ability to transport drugs, particularly when cyclosporin A is present (Deferme and Augustijns, 2003).

Moreover, p-glycoprotein is strongly inhibited by a number of fruit phytochemicals. In a number of cell lines, such as the Madin-Darby Canine Kidney cell line MDR1-MDCK II, the Colorectal Adenocarcinoma Caco 2 cell line, and the Adriamycin-resistant Leukemia cell line K562/ADM, sinetin dramatically inhibits p-glycoprotein (Mertens-Talcott et al., 2007). Molecular docking studies have supported the inhibitory potential of flavonoid-rich fruits, which reduce p-glycoprotein function (Mohana et al., 2016). Notably, compounds viz., bidwillon A, neobavaisoflavone and coptisine chloride, isolated from Coptis plants, have shown potent inhibitory effects (Schäfer et al., 2023). Similarly, apricot extract significantly reduces the polarity factor of talinolol, thereby suppressing p-glycoprotein-mediated efflux (Daddam et al., 2014).

Phyllanthin, a lignan from Phyllanthus species, has demonstrated the ability to inhibit p-glycoprotein-mediated transport of Rhodamine-123, a recognized p-glycoprotein substrate. At higher doses, phyllanthin has shown potential for significant inhibition, indicating possible drug-herb interactions when co-administered (Dunkoksung et al., 2019). Bitter melon extract also exhibits strong inhibitory effects on p-glycoprotein activity in Caco-2 intestinal cells, with 1-monopalmitin identified as the major bioactive compound responsible for this effect (Konishi et al., 2004). In addition, rosmarinic acid not only suppresses the functional activity and expression of both p-glycoprotein and BCRP but also paradoxically activates p-glycoprotein ATPase activity (Li et al., 2013). Fruit-derived compound, like stigmasterol has shown p-glycoprotein inhibitory effect in multidrug-resistant human leukemia cells (El-Readi et al., 2010). Tangerine, on the other hand, was found to inhibit p-glycoprotein while simultaneously enhancing CYP3A4 activity (Nowack, 2008).

Our laboratory has identified several reno protective natural p-glycoprotein inhibitors, including atisine, kutkin and phylloquinone, that exhibit favorable drug likeness and pharmacokinetic characteristics. When combined with other medications, these compounds improve drug bioavailability and renal function (Roy and Ghosh, 2023). Additionally, Pandamarilactone 31, a bioactive component found in spices, demonstrated lower binding energy than verapamil, highlighting its potential as a natural therapeutic agent against multidrug resistance (Mukhopadhyay et al., 2024). Further findings from our lab suggest that deserpidine plays a crucial role in overcoming drug resistance caused by p-glycoprotein and enhancing therapeutic efficacy in cancer and diabetes (Roy et al., 2025).

Hydrogen bonds stabilise the receptor-ligand interaction, while hydrophobic interactions increase the total binding affinity by facilitating non-specific binding (Chen et al., 2016). For specificity, structural accuracy, and mechanical stability, hydrogen bonding is particularly crucial. Chufan et al. showed that hydrogen bonds can play a key role in inhibiting ATP hydrolysis, a critical process in p-glycoprotein-mediated drug efflux through interactions involving tyrosine residues (Y310 and Y953) and phenylalanine residues (F728 and F978) (Chufan et al., 2016).

This study revealed that eight fruit-derived bioactive compounds exhibited substantial inhibitory activity against p-glycoprotein. ADMET analysis showed that the compounds satisfied the criteria for drug-like qualities, underscoring their potential for therapeutic development. Their favourable ADMET profiles, including high oral bioavailability, support their prospective application in clinical settings.

The binding energy of phyllanthin is -78.8 kcal/mol compared to the control drug rifampin (-73.6 kcal/mol), interacts via hydrogen bonds with residues GLU972 and LEU332, enhancing its specificity and stability. MD Simulation was utilized to validate the docking results. The computation of binding stabilities and binding free energies is crucial for MD simulation. Our results indicate that Phyllanthin

exhibits a stronger binding affinity than Δ5-Avenasterol, as evidenced by its more negative binding free energy (-40.5 kcal/mol) compared to that of Δ5-Avenasterol (-36.6 kcal/mol) Additionally, Phyllanthin demonstrated slightly higher kinetic and van der Waals energies, suggesting more flexible molecular interactions that contribute to improved stabilization in the simulated environment. While both compounds share the same radius of gyration (47.9), indicating similar molecular size and shape, Phyllanthin appears to be more energetically favorable and potentially more stable.

CONCLUSION

The in-silico study mainly identified the p-glycoprotein inhibitors among the fruit components. Among the 8 inhibitors, Phyllanthin showed the highest binding affinity, suggesting strong, stable interaction. Key residues were identified, suggesting Phyllanthin could be a promising lead inhibitory molecule.
However, additional evidence through in vitro and in vivo studies is needed to prove their biological activity, pharmacokinetic properties, bioavailability and solubility.
It was also reported that the highest amount of Phyllanthin, was found to be in leaves than fruit (Danladi et al., 2018) and it was also not considered for in vitro/in vivo studies due to its poor bioavailability (Murugaiyah and Chan, 2007). Verapamil, on the other hand found in citrus fruits, grapes and oranges was also considered as first generation inhibitor, as they interacted with Pgp protein, competed with other substrates and acted as competitive inhibitors (Srivalli and Lakshmi, 2012).However, still more research is needed to be carried out by exploring the immune-related effects of these inhibitors with immune cells like macrophages as well as in the modulation of bacterial lipopolysaccharide (LPS)-induced inflammatory responses in animal model.

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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