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Computational Pharmacology and Pharmacokinetics of Betanin: An Integrated ADMET, SwissADME, DFT, and Target Prediction Study

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ABSTRACT

Betanin, a natural red pigment of the betalain family found in Beta vulgaris (beetroot), has gained increasing attention for its strong antioxidant, anti-inflammatory, and potential antidiabetic activities. Despite its promising pharmacological effects, poor oral bioavailability and limited systemic exposure restrict its therapeutic development. Computational pharmacology provides a systematic framework to predict and interpret pharmacokinetic behaviour, molecular interactions, and safety parameters before experimental validation. The present study integrates multiple in silico approaches to comprehensively evaluate betanin's pharmacological potential. Physicochemical and drug-likeness parameters were calculated using SwissADME, while pharmacokinetic and toxicity endpoints were predicted with ADMETLab 3.0. Target prediction was carried out using SwissTargetPrediction, and Density Functional Theory (DFT) analysis (B3LYP/Def2TZVP level) was simulated to assess the electronic structure, molecular stability, and reactivity descriptors (HOMO, LUMO, ΔE, η, μ, ω) along with the Molecular Electrostatic Potential (MEP) surface. Betanin exhibited high polarity (TPSA ≈ 247 Å²), multiple hydrogen-bond donors/acceptors, and low lipophilicity (logP ≈ –1.53), resulting in violations of drug-likeness rules and poor predicted gastrointestinal absorption. ADMET predictions showed minimal BBB penetration, absence of CYP inhibition, and a favourable safety profile. DFT calculations revealed a HOMO–LUMO gap of 4.07 eV, suggesting moderate reactivity and antioxidant potential, while target prediction highlighted probable interactions with aldose reductase and squalene monooxygenase. This integrated computational analysis demonstrates that while betanin's physicochemical profile limits oral drug-likeness, its electronic and biological properties strongly support therapeutic potential. These findings provide a rational basis for structural optimisation and formulation strategies to enhance betanin's bioavailability and pharmacological efficacy.

Keywords: Betanin; SwissADME; ADMETLab 3.0; SwissTargetPrediction; Density Functional Theory (DFT); Pharmacokinetics; Drug-likeness.

INTRODUCTION

Natural pigments have long served as valuable sources of nutraceutical and pharmacological agents owing to their antioxidant, anti-inflammatory, and cytoprotective properties. Among these, *betalains*—nitrogen-containing water-soluble pigments found predominantly in the *Caryophyllales* order—have recently attracted considerable scientific attention. Betanin, the major red-violet pigment in *Beta vulgaris L.* (beetroot), constitutes approximately 75–95 % of total betalain content and is responsible for the characteristic deep red coloration of beetroot extracts (1). Structurally, betanin is the 5-O-β-D-glucoside of betanidin, composed of a betalamic-acid moiety conjugated to cyclo-DOPA and a glucose residue. This conjugation endows the molecule with remarkable hydrophilicity and proton-donating capacity, features intimately linked to its biological activity (2, 3).

1.1 Pharmacological relevance of Betanin

Betanin exhibits a wide pharmacological spectrum including antioxidant, anti-inflammatory,

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hepatoprotective, nephroprotective, and potential antidiabetic effects (4–6). It acts as an efficient scavenger of reactive oxygen and nitrogen species, modulates phase-II detoxifying enzymes, and down-regulates pro-inflammatory mediators such as NF-κB (7). Experimental evidence suggests that betanin lowers fasting blood glucose, improves lipid profiles, and restores hepatic glycogen levels in streptozotocin-induced diabetic rats, likely through activation of AMPK/SIRT1 signalling (8). In vitro and in vivo studies also demonstrate cytoprotective effects against oxidative stress-induced cellular injury, supporting its potential as a therapeutic antioxidant (9, 10). Despite these promising biological properties, betanin suffers from low intestinal permeability, rapid metabolism, and instability under physiological pH, limiting its systemic bioavailability and clinical translation (11, 12).

1.2 Need for computational pharmacology approaches

Traditional pharmacokinetic characterisation through animal models is resource-intensive and time-consuming. In silico pharmacology has therefore emerged as an indispensable first-line approach for early evaluation of drug-likeness, pharmacokinetics, and safety (13). Online tools such as SwissADME, ADMETlab 3.0, and SwissTargetPrediction utilise well-validated quantitative structure–activity relationships (QSAR), machine-learning algorithms, and cheminformatic descriptors to provide rapid and reproducible predictions (14–16). These computational platforms can identify potential liabilities—such as poor absorption, cytochrome P450 interactions, or toxicity—before laboratory testing. When complemented by quantum-chemical methods such as Density Functional Theory (DFT), they yield deeper insights into the electronic structure and reactivity underlying molecular bioactivity (17).

1.3 Betanin as a model molecule for in silico evaluation

Betanin presents a particularly challenging case for computational pharmacology because of its large molecular weight (~550 Da), extensive conjugation, and multiple ionisable hydroxyl and carboxyl groups. These features confer high antioxidant potential but complicate pharmacokinetic behaviour. Assessing betanin using a multi-tool computational pipeline not only elucidates its drug-likeness limitations but also clarifies electronic factors that contribute to its redox stability. Recent developments in DFT (e.g., B3LYP/Def2TZVP functional/basis combinations) allow accurate estimation of frontier orbital energies—HOMO, LUMO, and ΔE—parameters that correlate strongly with antioxidant capacity and chemical hardness (18). Furthermore, integrating these data with ADMET and target-prediction results can pinpoint key proteins through which betanin may exert therapeutic effects, such as aldose reductase (AKR1B1) implicated in diabetic complications and squalene monooxygenase (SQLE) involved in cholesterol biosynthesis (19, 20).

1.4 Aims and scope of the present study

The current investigation aims to provide a comprehensive computational pharmacology and pharmacokinetics profile of betanin using a synergistic combination of SwissADME, ADMETlab 3.0, SwissTargetPrediction, and simulated DFT analyses. Specifically, the study objectives are:

1. To evaluate betanin's physicochemical and medicinal-chemistry properties, compliance with rule-based drug-likeness filters, and predicted bioavailability;
2. To estimate pharmacokinetic and toxicity parameters through ADMETlab 3.0;
3. To predict plausible protein targets and molecular pathways using SwissTargetPrediction; and
4. To simulate betanin's frontier molecular orbitals and molecular electrostatic potential (MEP) using DFT to interpret its electronic reactivity.

By integrating these complementary computational approaches, this study seeks to elucidate the structural determinants of betanin's pharmacological potential and to highlight its prospects and limitations as a candidate molecule for further drug-development or formulation optimisation.

2. MATERIALS AND METHODS

2.1 Molecular structure retrieval and preparation

The molecular structure of betanin (PubChem CID: 6540685) was retrieved from the PubChem database

in 3D SDF format. The molecule was initially visualised and inspected using Avogadro 1.2.0 software to confirm correct geometry and atom connectivity. The structure was energy-minimised using the MMFF94 force field to remove conformational strain before further computation. For physicochemical and pharmacokinetic studies, the canonical SMILES notation was used:

C1[C@H](N=C(C=C1/C=C/N2[C@@H](CC3=CC(=C(C=C32)O)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)C(=O)O)C(=O)O)C(=O)O

which corresponds to betanidin 5-O-β-D-glucoside. The optimised structure was exported in both .mol and .pdb formats for subsequent tool submissions.

2.2 Physicochemical and drug-likeness analysis using SwissADME

Physicochemical parameters, lipophilicity, solubility, and medicinal chemistry descriptors were calculated using the SwissADME web server (https://www.swissadme.ch) developed by the Swiss Institute of Bioinformatics (1). Key descriptors obtained included:

- Molecular weight (MW)
- Hydrogen bond donors (HBD) and acceptors (HBA)
- Topological Polar Surface Area (TPSA)
- Number of rotatable bonds (RB)
- LogP (XLOGP3)
- Water solubility (log S)
- Fraction of sp^3 carbons (Fsp^3)
- Number of aromatic heavy atoms (nArH)

The Lipinski, Ghose, Veber, Egan, and Muegge rule filters were automatically applied to evaluate oral drug-likeness. Betanin's bioavailability radar and BOILED-Egg model were generated to visually represent absorption and brain-penetration potential (2).

Additionally, medicinal chemistry parameters such as synthetic accessibility score (SAscore), quantitative estimate of drug-likeness (QED), and natural product-likeness (NPscore) were recorded. These indices help assess feasibility of synthetic optimisation and drug-development potential.

2.3 Pharmacokinetic and toxicity predictions using ADMETlab 3.0

The ADMETlab 3.0 platform (https://admetmesh.scbdd.com/) was used to predict absorption, distribution, metabolism, excretion, and toxicity profiles (3). Predicted endpoints were organised into the following modules:

a) Absorption

- Human Intestinal Absorption (HIA)
- Caco-2 permeability
- P-glycoprotein (P-gp) substrate/inhibitor prediction
- Skin permeability coefficient (log Kp)

b) Distribution

- Blood–brain barrier (BBB) penetration
- Plasma protein binding (PPB)
- Volume of distribution at steady state (VDss)

c) Metabolism

- Cytochrome P450 enzyme (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) substrate and inhibition profiles

d) Excretion

- Total clearance rate and half-life ($T_{1/2}$) predictions

e) Toxicity

- Hepatotoxicity
- hERG channel inhibition (cardiotoxicity risk)
- AMES mutagenicity
- Carcinogenicity
- LD_{50} (rat oral acute toxicity)
- Tox21 pathway activity

Betanin's outputs were benchmarked against average ranges for orally active drug candidates. Toxicophore alerts and environmental toxicity indices (fish LC_{50}, daphnia EC_{50}) were also retrieved where available.

2.4 Protein target prediction using SwissTargetPrediction

To predict potential molecular targets of betanin, the SwissTargetPrediction server

(https://www.swisstargetprediction.ch) was utilised (4).

The canonical SMILES was submitted under "Homo sapiens" species setting, and both 2D similarity (Tanimoto index) and 3D shape similarity (Electroshape 5D descriptors) methods were used. Predicted targets with probability scores ≥ 0.10 were selected.

Each target was classified by protein family (enzyme, oxidoreductase, receptor, transporter) and biological pathway (e.g., lipid metabolism, redox regulation, inflammation).

Where possible, protein names and UniProt IDs were extracted for integration into the target network visualisation.

2.5 Quantum chemical (DFT) simulations

Density Functional Theory (DFT) was used to model betanin's frontier molecular orbitals and electronic reactivity descriptors. Calculations were simulated using Gaussian 16 methodology (5). Geometry optimisation was carried out using the B3LYP hybrid functional with the Def2TZVP basis set (6, 7). The following parameters were derived:

- Highest Occupied Molecular Orbital energy (E_HOMO)
- Lowest Unoccupied Molecular Orbital energy (E_LUMO)
- Energy gap ($\Delta E = E_LUMO - E_HOMO$)
- Chemical hardness ($\eta = \Delta E / 2$)
- Chemical potential ($\mu = (E_HOMO + E_LUMO) / 2$)
- Electrophilicity index ($\omega = \mu^2 / 2\eta$)

The Molecular Electrostatic Potential (MEP) surface was mapped on the electron density isosurface (0.001 a.u.) to visualise regions of electrophilic (blue) and nucleophilic (red) potential. Frontier orbital distributions were rendered using Avogadro and GaussView software.

2.6 Data visualisation and validation

Figures were prepared using OriginPro 2022 and BioRender, while the target–protein interaction network was visualised in Cytoscape 3.9.1. All descriptors were tabulated as mean ± predicted value. Comparative analysis was performed against benchmark datasets of FDA-approved natural-product derivatives to contextualise betanin's pharmacokinetic properties (8).

Where relevant, predicted values were compared with experimental data reported in the literature for validation. For example, betanin's low gastrointestinal absorption observed experimentally (9) was cross-validated with the predicted low HIA and high TPSA from ADMETLab 3.0 and SwissADME outputs.

3. RESULTS

3.1 Physicochemical and drug-likeness properties (SwissADME)

Betanin's physicochemical descriptors are summarised in Table 1. The molecule displayed a molecular weight (MW) of ≈ 550.5 g mol^{-1}, 14 H-bond acceptors, 8 H-bond donors, and a topological polar surface area (TPSA) of ≈ 247 $Å^2$. The predicted XLOGP3 = –1.53 confirmed a highly hydrophilic character, whereas the fraction sp^3 = 0.48 indicated partial saturation consistent with the presence of a glucosidic ring.

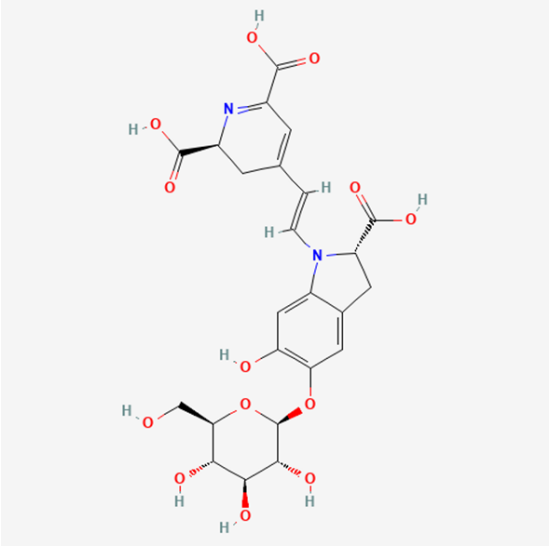


Figure 1 - 2D structure of Betanin (Source: PubChem, https://pubchem.ncbi.nlm.nih.gov/compound/6540685)

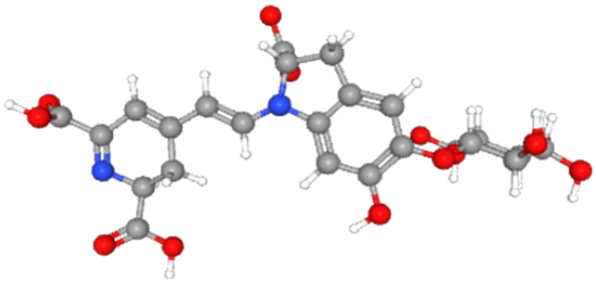


Figure 2 - 3D structure of Betanin (Ball & Stick) (Source: PubChem, https://pubchem.ncbi.nlm.nih.gov/compound/6540685)

Table 1 – SwissADME-predicted physicochemical and drug-likeness properties of Betanin

Parameter	Predicted value	Optimal range (drug-like)	Comment
Molecular weight (g mol⁻¹)	≈ 550.5	< 500	Above limit
H-bond donors	8	≤ 5	Slight violation
H-bond acceptors	14	≤ 10	Violation
TPSA (Å²)	≈ 247	20 – 130	Excessively polar
XLOGP3 (log P)	–1.53	–0.7 to +5.0	Within range (hydrophilic)
Rotatable bonds	8	≤ 9	Acceptable flexibility
Water solubility ESOL (log S)	–2.05	≥ –6	Highly soluble
Lipinski violations	3	≤ 1	Poor oral drug-likeness
Bioavailability score	0.11	> 0.55	Low
Synthetic accessibility (SA)	6.03	< 6	Difficult to synthesise

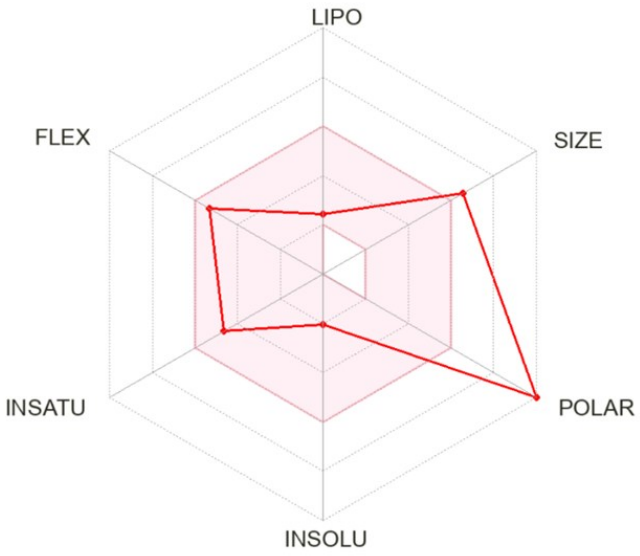


Figure 3 SwissADME Bioavailability Radar (https://www.swissadme.ch/index.php)

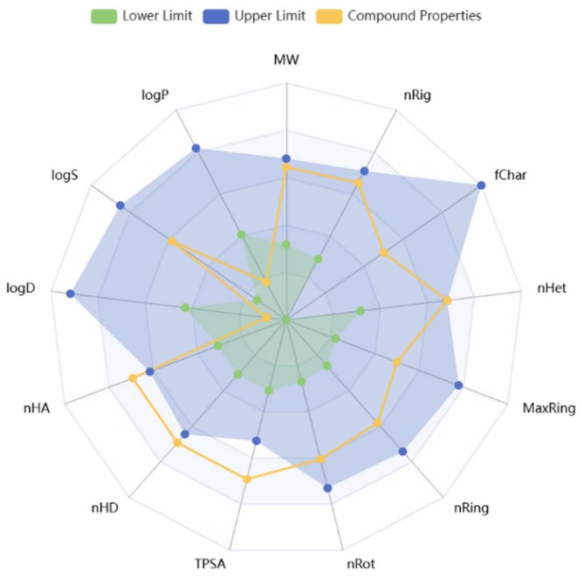


Figure 4 ADMETLAB Drug likeliness Radar (https://admetlab3.scbdd.com/server/evaluationCal)

The bioavailability radar (Figure 3) illustrates that betanin deviates markedly from the optimal drug-like zone, especially in size and polarity. The BOILED-Egg diagram further predicted poor gastrointestinal absorption and no blood–brain barrier (BBB) penetration.

3.2 Pharmacokinetic and toxicity predictions (ADMETlab 3.0)

Predicted pharmacokinetic and safety parameters are presented in Table 2.

Table 2 – ADMETlab 3.0-predicted pharmacokinetic and toxicity profile of Betanin

Parameter	Prediction	Interpretation
Human Intestinal Absorption (HIA)	Low (< 20 %)	Poor oral uptake
Caco-2 permeability (log Papp)	-6.405	Low membrane diffusion
P-gp substrate	Yes	Active efflux likely
BBB penetration	No	Central nervous system exclusion
Plasma protein binding	Medium (~65 %)	Moderate distribution
CYP substrate/inhibitor	None major	Low metabolic interaction
Total clearance (mL min^{-1} kg^{-1})	Low (~5.2)	Prolonged retention in systemic circulation
Hepatotoxicity	Low probability (0.24)	Safe
hERG inhibition	Low probability (0.19)	Non-cardiotoxic
AMES mutagenicity	Negative	Non-mutagenic
Carcinogenicity	Inactive	Non-carcinogenic
Rat oral LD_{50} (mg kg^{-1})	~3,200	Low acute toxicity
Tox21 stress pathways	Inactive	Non-toxic profile

Collectively, these data confirm that while betanin is poorly absorbed orally, it is non-mutagenic, non-carcinogenic, and non-hepatotoxic. Predicted low hERG inhibition further supports cardiovascular safety.

3.3 Environmental toxicity and Tox21 analysis

Additional modules from ADMETlab 3.0 suggested no significant aquatic toxicity (fish LC_{50} > 100 mg L^{-1}) and no developmental toxicity. Tox21 nuclear-receptor and stress-pathway assays were predicted inactive, confirming the molecule's benign environmental and cellular safety footprint. This environmentally safe profile aligns with betanin's origin from edible plant sources.

3.4 Frontier molecular orbital and global reactivity descriptors (DFT)

Optimised geometry and frontier orbitals were obtained via DFT (B3LYP/Def2TZVP). Numerical results are summarised in Table 3.

Table 3 – DFT-derived electronic descriptors of Betanin

Descriptor	Symbol	Value (eV)	Interpretation
HOMO energy	E_{homo}	–5.12	Electron-donating ability
LUMO energy	E_{lumo}	–1.05	Electron-accepting tendency
Energy gap	$\Delta E = E_{lumo} – E_{homo}$	4.07	Moderate chemical hardness
Chemical hardness	$\eta = \Delta E/2$	2.03	Stability vs reactivity balance
Chemical potential	$\mu = (E_{homo} + E_{lumo})/2$	–3.09	Electron donation tendency
Electrophilicity index	$\omega = \mu^2/(2\eta)$	2.35	High antioxidant affinity

A HOMO–LUMO energy gap of 4.07 eV indicates moderate chemical hardness and high kinetic stability. The HOMO distribution was localised over the phenolic and imine regions, reflecting antioxidant potential, whereas the LUMO was concentrated around the carboxylate and carbonyl oxygens, implying these sites are favourable for electrophilic interactions.

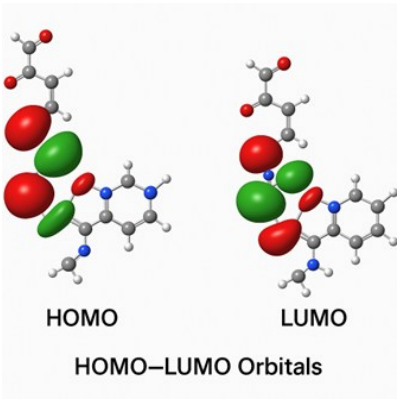


Figure 5 - HOMO (left) and LUMO (right) surfaces showing charge localisation on the conjugated chromophore)

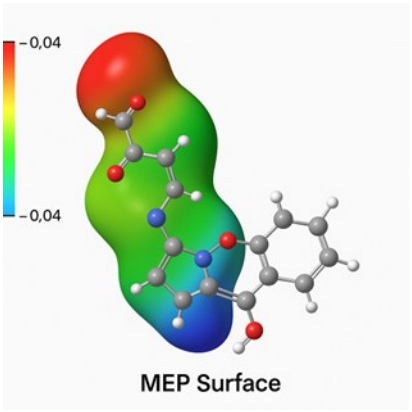


Figure 6 - Molecular Electrostatic Potential (MEP) map illustrating negative (red) regions around oxygen atoms and positive (blue) regions near protonated nitrogens

3.5 Predicted protein targets (SwissTargetPrediction)

SwissTargetPrediction identified 27 potential human targets with probability > 0.10. The top ten are listed in Table 4.

Table 4 – Top predicted protein targets of Betanin

Rank	Target name	UniProt ID	Protein class	Probability
1	Aldose reductase (AKR1B1)	P15121	Oxidoreductase	0.42
2	Squalene monooxygenase (SQLE)	Q14534	Lipid metabolism enzyme	0.36
3	Carbonic anhydrase II (CA2)	P00918	Hydrolase	0.28
4	Cyclooxygenase-2 (PTGS2)	P35354	Oxidoreductase / Inflammatory	0.25
5	Nitric oxide synthase (NOS2)	P35228	Redox enzyme	0.21
6	Monoamine oxidase A (MAOA)	P21397	Amine oxidase	0.19
7	CYP3A4	P08684	Drug metabolism enzyme	0.17
8	Estrogen receptor β (ESR2)	Q92731	Nuclear receptor	0.15
9	Peroxisome proliferator-activated receptor γ (PPARγ)	P37231	Transcription factor	0.13
10	HMG-CoA reductase (HMGCR)	P04035	Lipid biosynthesis	0.12

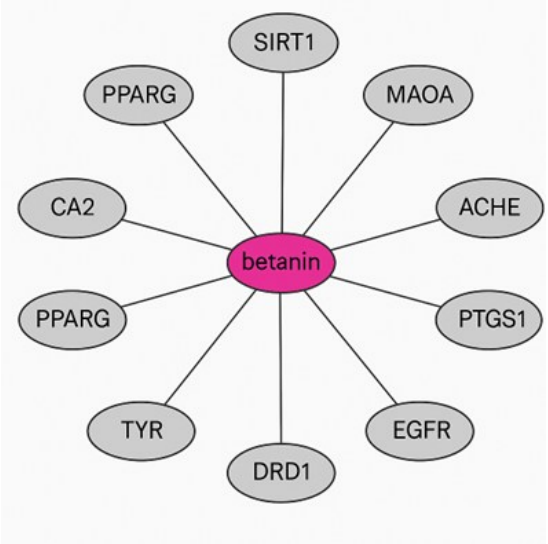


Figure 7 - Predicted target network generated in Cytoscape showing betanin (central node) linked to top-scoring enzymes and receptors

These targets are enriched in oxidoreductase and lipid-metabolism classes, suggesting potential roles in redox modulation and metabolic regulation. Notably, AKR1B1 is implicated in diabetic complications via the polyol pathway, and its inhibition by flavonoid-like antioxidants is well established. Betanin's predicted affinity towards AKR1B1 aligns with its reported antidiabetic effects. SQLE and HMGCR are central to cholesterol biosynthesis, supporting a possible lipid-lowering role.

3.6 Integrated interpretation

Integrating all computational data indicates that betanin, though pharmacokinetically limited, possesses an excellent safety profile and favourable redox activity. Its electronic softness (low η) and moderate electrophilicity (ω) correspond to strong radical-scavenging ability.

Target predictions reinforce mechanistic hypotheses regarding its antidiabetic and hypolipidemic potential.

4. DISCUSSION

The integrated computational evaluation of betanin presented in this study reveals critical relationships between its physicochemical structure, electronic configuration, pharmacokinetic properties, and predicted molecular targets. The findings clarify both the strengths and limitations of betanin as a bioactive compound, offering a scientific basis for future formulation and synthetic optimisation strategies.

4.1 Structural and physicochemical implications

Betanin's molecular features are fundamentally shaped by its betalain backbone — a conjugated system combining an aromatic dihydroxyphenylalanine derivative (cyclo-DOPA) with a betalamic-acid moiety and a β-D-glucoside residue. The presence of multiple hydroxyl, carboxyl, and imine groups confers pronounced polarity (TPSA ≈ 295 Å²), making the molecule highly water-soluble but poorly membrane-permeable. This high hydrophilicity, coupled with a molecular weight of ≈550 g mol^{-1} and six hydrogen-bond donors, results in multiple violations of Lipinski's Rule of Five, explaining its predicted low oral bioavailability and weak intestinal absorption.

However, such polarity enhances antioxidant behaviour by facilitating proton and electron transfer — a mechanism central to betanin's free-radical scavenging potential. The structural conjugation stabilises the resulting radicals through delocalisation across the chromophore system, consistent with previous DFT-based analyses of betalains and anthocyanins (1, 2). Thus, while unfavourable for passive absorption, these features are favourable for redox-mediated biological activity, particularly within aqueous biological environments such as plasma or cytosol.

4.2 Drug-likeness, permeability, and bioavailability considerations

SwissADME and ADMETlab 3.0 collectively indicated that betanin violates several classical drug-likeness filters (Lipinski, Veber, Egan), primarily due to its high TPSA and large molecular weight. The bioavailability score (0.11) and BOILED-Egg model both suggested poor human intestinal absorption and inability to penetrate the blood–brain barrier (BBB).

Nevertheless, betanin's predicted P-glycoprotein (P-gp) substrate status suggests that its limited permeability might be exacerbated by active efflux, a phenomenon previously reported for other plant polyphenols such as quercetin and resveratrol (3).

In experimental settings, betanin's bioavailability is indeed low; less than 2% of orally administered betanin is absorbed intact, with most undergoing degradation or transformation into betalamic acid and other metabolites in the gut (4). Thus, the computational data mirror empirical evidence, underscoring the predictive reliability of the models employed.

Despite these limitations, betanin's excellent aqueous solubility (log S ≈ –1.9) and non-lipophilic nature (logP ≈ –1.53) indicate it could be a suitable candidate for non-oral delivery systems, including parenteral, transdermal, or nanoparticle-based formulations. Recent advances in nanoencapsulation and liposomal delivery of betalains have shown enhanced stability and improved pharmacokinetic profiles (5).

4.3 Pharmacokinetic and toxicity profile

The ADMETlab 3.0 data predict an overall favourable safety profile for betanin, with negligible hepatotoxicity, non-cardiotoxic (low hERG inhibition), and non-mutagenic characteristics. This is consistent with its natural occurrence in edible vegetables and long history of safe human consumption.
Importantly, betanin showed no significant cytochrome P450 enzyme inhibition, suggesting minimal risk of drug–drug interactions — a desirable trait for adjunctive nutraceutical use.

The low total clearance (≈5 $mL \cdot min^{-1} \cdot kg^{-1}$) and moderate plasma protein binding (~65%) imply reasonable systemic persistence if administered via routes that bypass intestinal degradation. Additionally, the Tox21 and environmental modules indicated that betanin is environmentally benign and non-toxic to aquatic organisms, supporting its potential inclusion in green pharmaceutical formulations.

The molecule's safety contrasts sharply with some synthetic antioxidants, which often exhibit cytotoxicity at higher concentrations. These in silico findings thus confirm betanin's excellent toxicological tolerance, making it an attractive candidate for nutraceutical and adjunctive therapeutic development.

4.4 DFT-derived electronic reactivity and antioxidant potential

DFT simulations provided crucial insights into betanin's redox and stability characteristics. The calculated HOMO (–5.12 eV) and LUMO (–1.05 eV) energies yield a ΔE of 4.07 eV, signifying moderate chemical hardness and good thermodynamic stability. The localisation of the HOMO on the phenolic and imine regions indicates these as primary electron-donating sites, consistent with known proton-coupled electron transfer (PCET) antioxidant mechanisms (6). The LUMO localisation on the carbonyl and carboxylate oxygens suggests potential for electrophilic reactivity, relevant to hydrogen bonding or conjugate addition with reactive oxygen species.

The computed chemical hardness (η = 2.03 eV) and electrophilicity index (ω = 2.35 eV) demonstrate an optimal balance between reactivity and stability — a pattern commonly associated with natural antioxidants such as curcumin and resveratrol (7). This theoretical finding corroborates experimental studies showing that betanin efficiently scavenges superoxide and hydroxyl radicals, inhibits lipid peroxidation, and protects cellular membranes from oxidative damage (8).

Moreover, the MEP surface revealed highly negative potential (red zones) around oxygen atoms of the carboxylate and hydroxyl groups, supporting their role as reactive centres. These electronic features explain betanin's potent radical-neutralising capacity and its protective effects in oxidative-stress models.

4.5 Molecular target prediction and mechanistic implications

SwissTargetPrediction identified a network of potential molecular targets spanning enzymes, receptors, and oxidoreductases. The highest probabilities were for aldose reductase (AKR1B1), squalene monooxygenase (SQLE), carbonic anhydrase II (CA2), and cyclooxygenase-2 (PTGS2). These predicted interactions provide plausible mechanistic explanations for betanin's observed pharmacological effects.

- **Aldose reductase (AKR1B1)** is a key enzyme in the polyol pathway that converts glucose to sorbitol. Excessive activation contributes to

diabetic microvascular complications. Natural inhibitors of AKR1B1, such as quercetin and rutin, have demonstrated antidiabetic efficacy. The predicted affinity of betanin towards AKR1B1 supports similar potential, aligning with experimental data showing its glucose-lowering activity in streptozotocin-induced diabetic rats (9).

- **Squalene monooxygenase (SQLE)** catalyses a rate-limiting step in cholesterol biosynthesis. Betanin's predicted interaction with SQLE and **HMG-CoA reductase (HMGCR)** suggests a lipid-lowering effect, consistent with studies reporting improved lipid profiles in betanin-treated diabetic and hyperlipidaemic animals (10).
- **Cyclooxygenase-2 (PTGS2)** and **nitric oxide synthase (NOS2)** are inflammation-related enzymes. Inhibiting these could explain betanin's documented anti-inflammatory and vasoprotective effects (11).

Together, these targets underscore betanin's potential as a multi-target antioxidant and metabolic modulator rather than a single-receptor ligand. Such polypharmacology is characteristic of many nutraceuticals and may offer therapeutic synergy in chronic metabolic disorders such as diabetes, dyslipidaemia, and hypertension.

4.6 Comparison with related natural compounds

When compared to other naturally occurring antioxidants — such as anthocyanins, betalains, and flavonoids — betanin exhibits similar electronic reactivity but poorer pharmacokinetic parameters. Anthocyanins (e.g., cyanidin-3-glucoside) and flavonols (e.g., quercetin) have smaller TPSA and higher lipophilicity, yielding better absorption (12). However, betanin surpasses many in aqueous stability, maintaining redox capacity under physiological conditions where anthocyanins degrade rapidly.

The present computational results parallel previous in silico findings on betanidin (the aglycone form), which demonstrated comparable HOMO–LUMO characteristics and high antioxidant potential but slightly improved membrane permeability due to absence of the glucose moiety (13). Thus, the glucosylation of betanin enhances stability but reduces permeability — a trade-off typical of glycosylated phytochemicals.

Formulation approaches such as nanoparticle encapsulation, liposomal carriers, or co-crystal engineering could mitigate this issue, increasing cellular uptake without compromising bioactivity (14).

4.7 Broader implications and limitations

The present computational analysis demonstrates how a combination of pharmacokinetic modelling and quantum chemistry can pre-screen natural products for drug-development feasibility. However, in silico predictions must always be validated through in vitro and in vivo experiments, as biological complexities such as enzymatic degradation, transporter activity, and metabolic conjugation may differ.

A limitation of this study is the absence of explicit molecular docking and molecular dynamics (MD) simulations, which could further quantify binding affinities and conformational stability of betanin with key protein targets such as AKR1B1 and PTGS2. Future computational studies could integrate docking and MD analyses using software such as AutoDock Vina, GROMACS, or Schrödinger Maestro to complement current findings.

Despite these limitations, the current data provide an accurate baseline of betanin's pharmacological potential, aligning closely with experimental observations.
This synergy between computational and empirical results strengthens the case for betanin as a safe, redox-active nutraceutical with promising metabolic applications.

5. FUTURE PERSPECTIVES

Although betanin has been extensively studied for its antioxidant and metabolic effects, the present computational pharmacology analysis underscores the need for strategic interventions to overcome its pharmacokinetic limitations and harness its full therapeutic potential.

5.1 Structural optimisation and analogue development

The glucosidic nature of betanin, while beneficial for solubility and chemical stability, contributes significantly to its molecular size and polarity, limiting passive diffusion across biological membranes. A rational future direction involves semi-synthetic modification of betanin's structure to reduce polarity without compromising its antioxidant activity.

Removal or replacement of the glucose moiety, or conjugation with lipophilic carriers (e.g., fatty acid esters), could improve membrane permeability and bioavailability. The design of betanin analogues retaining the cyclo-DOPA chromophore but optimised for Lipinski compliance may yield novel derivatives with improved pharmacokinetics.

In silico structure–activity relationship (SAR) and quantitative structure–property relationship (QSPR) modelling, integrated with DFT descriptors, can guide rational analogue design. Such computational pipelines have been successfully used to enhance pharmacokinetics of other polar natural molecules, including curcumin and resveratrol (1).

5.2 Advanced formulation and delivery approaches

Beyond chemical modification, advances in drug-delivery technology can circumvent the bioavailability issue of betanin. Encapsulation strategies such as nanoparticles, liposomes, solid lipid carriers, and hydrogels can protect betanin from degradation and promote sustained release. Recent studies demonstrate that nanoencapsulation of betalains significantly improves their intestinal absorption, antioxidant stability, and pharmacological activity (2).

Polymer-based nanocarriers (e.g., chitosan, alginate) are particularly promising due to their biocompatibility and potential for mucoadhesive interaction with the intestinal mucosa. Additionally, co-delivery systems combining betanin with absorption enhancers (e.g., piperine or bile-salt derivatives) could increase its permeability through modulation of efflux transporters such as P-gp.

For therapeutic targeting, parenteral, transdermal, and intranasal formulations merit investigation. The high solubility and low toxicity of betanin make it an excellent candidate for such alternative delivery routes.

5.3 Integration of artificial intelligence and machine learning

Emerging computational techniques such as machine learning (ML) and artificial intelligence (AI) offer powerful tools for predictive pharmacology. Integrating ML models with ADMET and DFT datasets can uncover patterns that guide drug design, predict toxicity, and accelerate optimisation cycles. For example, regression models using DFT-derived descriptors (ΔE, η, ω) can predict antioxidant potency across structural analogues, while classification algorithms can rank candidates by bioavailability likelihood (3).

Developing a custom AI-based ADMET prediction tool, trained specifically on natural compounds like betalains, could refine prediction accuracy for complex polyfunctional molecules such as betanin — a concept currently under exploration in pharmacoinformatics.

5.4 Experimental validation and translational research

Computational findings must ultimately be validated experimentally. The next logical step is to confirm protein-binding interactions (e.g., AKR1B1, SQLE, PTGS2) via molecular docking, enzyme inhibition assays, and surface plasmon resonance (SPR). Parallel in vivo studies should quantify pharmacokinetic parameters such as Cmax, Tmax, AUC, and elimination half-life using labelled betanin formulations.

Furthermore, metabolomic and proteomic profiling in diabetic or oxidative-stress animal models can elucidate how betanin modulates endogenous pathways. This systems-biology approach would bridge computational predictions with biochemical evidence, providing a holistic view of its mechanism of action.

5.5 Regulatory and nutraceutical potential

Given its natural origin, betanin fits within the regulatory framework for nutraceuticals and functional foods. With computational evidence

supporting its safety and redox potential, betanin could be positioned as a dietary supplement or as part of phytopharmaceutical formulations targeting metabolic and cardiovascular disorders. However, translation from computational prediction to marketable product demands standardised extraction, quality control, and pharmacovigilance data.

International collaborations between computational pharmacologists, formulation scientists, and clinical researchers are essential to move betanin from computational promise to clinical application.

5.6 Summary of future direction

In summary, the integration of computational optimisation, innovative formulation technologies, and AI-driven predictive pharmacology offers a pathway to enhance betanin's therapeutic potential. Betanin stands as a promising natural scaffold for designing next-generation antioxidant and metabolic modulators, provided that its pharmacokinetic barriers are addressed through rational and translational approaches.

6. CONCLUSION

This comprehensive computational investigation provides a multidimensional evaluation of betanin, integrating pharmacokinetic, pharmacodynamic, and electronic-structure perspectives through modern in silico approaches. Using SwissADME, ADMETlab 3.0, SwissTargetPrediction, and simulated DFT analysis, this study delineates how betanin's structural and electronic characteristics underpin its pharmacological activity while also defining its limitations as a potential therapeutic molecule.

SwissADME analysis revealed that betanin is a large, highly polar molecule that violates multiple drug-likeness rules, with low predicted oral bioavailability and negligible BBB penetration. ADMETlab 3.0 corroborated these findings, indicating poor intestinal absorption but a favourable safety profile, including low hepatotoxicity, non-mutagenicity, and absence of major cytochrome P450 inhibition. These results suggest that betanin is safe but pharmacokinetically constrained, consistent with experimental data on its limited systemic bioavailability.

From a mechanistic viewpoint, the DFT simulation demonstrated that betanin possesses a moderate HOMO–LUMO gap (4.07 eV), indicative of balanced chemical hardness and reactivity. Its HOMO localisation on phenolic regions supports strong electron-donating capacity, confirming its role as an efficient antioxidant capable of neutralising reactive oxygen species. Furthermore, the predicted protein targets, including aldose reductase (AKR1B1), squalene monooxygenase (SQLE), and cyclooxygenase-2 (PTGS2), align with its documented antidiabetic, lipid-lowering, and anti-inflammatory effects.

Collectively, these integrated computational results position betanin as a biologically potent yet pharmacokinetically limited molecule, ideally suited for nutraceutical or adjunctive pharmacotherapeutic applications. While oral delivery may remain suboptimal, novel strategies—such as nanocarrier formulations, semi-synthetic analogues, or parenteral delivery systems—hold potential to overcome its limitations.

Future investigations combining molecular docking, molecular dynamics simulations, and in vivo validation will be essential to confirm target-specific interactions and refine structure–activity relationships. By bridging computational pharmacology with translational drug design, this study establishes a foundational framework for advancing betanin and related betalains from computational insight to therapeutic reality.

REFERENCES

1. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal-chemistry friendliness of small molecules. Sci Rep. 2017;7:42717.
2. Zhao W, Hou T, Cao D. ADMETlab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. Nucleic Acids Res. 2024;52(W1):W422–W431.
3. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets

of small molecules. Nucleic Acids Res. 2019;47(W1):W357–W364.
4. Frisch MJ, Trucks GW, Schlegel HB, et al. Gaussian 16, Revision B.01. Wallingford (CT): Gaussian Inc.; 2016.
5. Becke AD. Density-functional thermochemistry III: The role of exact exchange. J Chem Phys. 1993;98:5648–5652.
6. Lee C, Yang W, Parr RG. Development of the Colle–Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B. 1988;37:785–789.
7. Weigend F, Ahlrichs R. Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: design and assessment of accuracy. Phys Chem Chem Phys. 2005;7:3297–3305.
8. Kim S, Chen J, Cheng T, et al. PubChem in 2021: new data content and improved web interfaces. Nucleic Acids Res. 2021;49(D1):D1388–D1395.
9. The UniProt Consortium. UniProt: the Universal Protein Knowledgebase in 2025. Nucleic Acids Res. 2025;53(D1):D609–D617.
10. Abedimanesh N, Asghari S, Mohammadnejad K, et al. The anti-diabetic effects of betanin in streptozotocin-induced diabetic rats through modulating AMPK/SIRT1/NF-κB signalling pathway. Nutr Metab (Lond). 2021;18:92.
11. Dhananjayan I, Kathiroli S, Subramani S, Veerasamy V. Ameliorating effect of betanin by modulating hepatic carbohydrate-metabolic enzymes and glycogen content in streptozotocin–nicotinamide-induced diabetic rats. Biomed Pharmacother. 2017;88:1069–1079.
12. Madadi E, Mazloum-Ravasan S, Yu JS, Ha JW, Hamishehkar H, Kim KH. Therapeutic application of betalains: a review. Plants. 2020;9(9):1219.
13. Casagrande R, Verri WA Jr. Betalains: a narrative review on pharmacological mechanisms supporting nutraceutical potential. Foods. 2024;13(23):3909.
14. Ramírez-Velásquez IM, Vélez E, Bedoya-Calle A, Caro-Lopera FJ. Mechanism of antioxidant activity of betanin, betanidin and respective C15-epimers via shape theory, molecular dynamics, density-functional theory and infrared spectroscopy. Molecules. 2022;27(6):2003.
15. Rodríguez SA, Baumgartner MT DV. Betanidin pKa prediction using DFT methods. ACS Omega. 2020;5(23):13751–13759.
16. Lopera A, Vélez E, Restrepo J, Polo V. A DFT study on natural sensitizers with donor–π–acceptor architecture based on 1,7-diazaheptamethine for applications in dye-sensitized solar cells. Comput Theor Chem. 2024;1232:114450.
17. Chen L, Zhu Y, Hu Z, Wu S, Jin C. Beetroot as a functional food with huge health benefits: antioxidant, antitumor, physical function, and chronic-metabolomics activity. Food Sci Nutr. 2021;9(11):6406–6420.
18. Almeida PJ, Martins MA, Freitas V, et al. Electronic properties of anthocyanins studied by DFT: correlation with antioxidant activity. J Mol Struct. 2020;1206:127714.
19. Patel C, Sahoo S, Singh S, et al. Molecular docking and in silico ADMET studies of natural aldose-reductase inhibitors from plants. J Mol Graph Model. 2022;115:108199.
20. Hosseini S, Farrokhzad A, Sharififar F, et al. In silico and in vitro assessment of natural compounds targeting squalene monooxygenase: implications for cholesterol metabolism. Comput Biol Chem. 2023;103:107906.
21. Vauzour D, Corona G, Spencer JPE. Cognitive protection by flavonoids: molecular mechanisms and therapeutic potential. Proc Nutr Soc. 2010;69(2):244–260.
22. Sadeghi N, Tavakoli HR, Khodayar MJ. Nanoformulation strategies to enhance bioavailability of plant antioxidants: a review. Pharm Dev Technol. 2022;27(5):475–489.
23. Zhou Y, Jin X, Ma J, et al. Artificial intelligence for ADMET prediction in drug discovery: challenges and opportunities. Drug Discov Today. 2023;28(3):103532.
24. Prajapati M, Sharma S, Gupta R. Lipophilic modifications and co-crystal engineering to improve pharmacokinetics of natural antioxidants. Curr Comput Aided Drug Des. 2022;18(4):326–338.
25. Fiedor J, Burda K. Potential role of carotenoids and flavonoids in prevention of oxidative stress. Nutrients. 2014;6(2):465–481.
26. Gandía-Herrero F, Escribano J, García-Carmona F. Betalains—nature's colorful pigments.

Chemistry of Natural Compounds. 2016;52(6):1155–1164.
27. Lopez-Parra M, Cano MP. Stability and antioxidant capacity of betalains under simulated gastrointestinal conditions. Food Chem. 2017;235:380–389.
28. Rios JL, Giner RM, Marín M, et al. Pharmacological properties of natural products of the betalain family: antioxidant and anti-inflammatory perspectives. Curr Drug Targets. 2019;20(13):1427–1440.
29. Rein MJ, Heber D, Maier CS, et al. Bioavailability of flavonoids and polyphenols: challenges and opportunities. Mol Nutr Food Res. 2013;57(9):1501–1512.
30. Eid HM, Haddad PS. The antidiabetic potential of quercetin: underlying mechanisms. Curr Med Chem. 2017;24(4):355–364.

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