In Silico Screening of *Sargassum cinereum*-Associated Bioactive Compounds: Molecular Docking and Toxicity Prediction for Potential Cardioprotective Applications

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Abstract. Cardiovascular disease remains a major cause of mortality, and excessive cardiomyocyte apoptosis contributes to ischaemia-reperfusion injury and adverse cardiac remodelling. Caspase-3 is a principal executioner protease in apoptotic signalling, although predicted ligand binding alone cannot establish cardioprotection. This study comparatively evaluated gallic acid (PubChem CID 370), phloroglucinol (CID 359), and fucosterol (CID 5281328), selected compounds associated with brown algae, against human caspase-3 (PDB ID 6CKZ). The peptide-derived covalent inhibitor Ac-DW3-KE was used as a crystallographic structural reference to define the binding region. Ligands were prepared using Open Babel and AutoDockTools 1.5.7, docked using AutoDock Vina 1.2.5, and inspected using BIOVIA Discovery Studio Visualizer 2021.Toxicity was predicted using pkCSM and ProTox-III. Ac-DW3-KE produced a docking score of −7.3 kcal/mol. Among the test ligands, fucosterol showed the most favourable score (−7.1 kcal/mol), followed by gallic acid (−5.0 kcal/mol) and phloroglucinol (−4.0 kcal/mol). The 0.2 kcal/mol difference between Ac-DW3-KE and fucosterol was small but not a potency comparison because conventional Vina docking does not model covalent-bond formation or reaction energetics. pkCSM predicted no Ames mutagenicity, hERG I inhibition, hepatotoxicity, or skin sensitisation for the three compounds; however, fucosterol was predicted to inhibit hERG II. ProTox-III predicted LD_{50} values of 1,190, 200, and 890 mg/kg and toxicity classes 4, 3, and 4 for gallic acid, phloroglucinol, and fucosterol, respectively. Fucosterol therefore warrants prioritised experimental evaluation, together with focused cardiac-safety testing, before any cardioprotective or caspase-3-inhibitory conclusion is made.

Keywords: *Sargassum cinereum*, cardioprotection, caspase-3, molecular docking, gallic acid, phloroglucinol, fucosterol, and toxicity prediction.

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1 Introduction

Cardiovascular disease remains the leading global cause of death and disability, with ischaemic heart disease accounting for a substantial proportion of the burden [1,2]. Although timely reperfusion limits infarct size, restoration of blood flow can itself intensify oxidative stress, calcium overload, mitochondrial dysfunction, inflammation, and cell death. Loss of viable cardiomyocytes reduces contractile reserve and contributes to ventricular remodelling and heart failure [3,4]. These processes motivate the search for compounds that can modulate injury-associated cell death signalling without impairing physiological cellular functions.

Cardiac cell death is mechanistically heterogeneous. Necrosis, apoptosis, necroptosis, pyroptosis, ferroptosis, and autophagy-associated processes can coexist and interact during ischaemia-reperfusion and chronic cardiac remodelling [3,5,6]. In the intrinsic apoptotic pathway, mitochondrial outer-membrane permeabilisation promotes cytochrome-c release, apoptosome assembly, and initiator caspase-9 activation. The extrinsic pathway begins with death-receptor signalling and caspase-8 activation. Both pathways converge on executioner caspases, particularly caspase-3 and caspase-7 [7,8]. Caspase-3 cleaves numerous structural, signalling, and DNA repair substrates, producing the characteristic biochemical and morphological features of apoptosis. Increased cleaved caspase-3 has been observed in experimental myocardial injury, and genetic or pharmacological modulation of caspase-dependent signalling can reduce damage in selected models [4,9]. Nevertheless, apoptosis is not the sole determinant of cardiomyocyte loss, and caspase-3 participates in context-dependent non-apoptotic processes. Binding to caspase-3 is therefore only one mechanistic hypothesis; it cannot demonstrate reduced infarction, improved contractility, or clinical cardioprotection [10].

Marine brown algae synthesize structurally diverse phenolics, phlorotannins, sterols, carotenoids, terpenoids, fatty acids, and sulfated polysaccharides [11,12]. Species of Sargassum have attracted interest because their extracts and isolated constituents show antioxidant, anti-inflammatory, metabolic, and cytoprotective effects in preclinical systems [13,14]. Such activities are biologically relevant to cardiovascular injury, but indirect antioxidant or anti-inflammatory evidence must be distinguished from the direct protection of cardiomyocytes or demonstrable modulation of caspase-3.

Sargassum cinereum occurs in tropical coastal habitats and has been subjected to pharmacognostic and preliminary phytochemical examinations [15]. The brown algal composition varies substantially with taxonomic identity, collection site, season, maturity, extraction solvent, and analytical platform. Consequently, a constituent reported in another Sargassum species cannot automatically be assigned to a particular S. cinereum sample. Because the present study does not report chromatographic or spectrometric confirmation of the investigated material, gallic acid, phloroglucinol, and fucosterol are described conservatively as selected compounds associated with brown algae rather than as compounds isolated from the study sample.

Gallic acid is a trihydroxybenzoic acid with antioxidant and anti-inflammatory activities. Preclinical cardiovascular studies have described reductions in oxidative injury and cardiac biomarkers; however, its effects depend on the dose, tissue, and disease context, and gallic acid can also promote apoptosis in malignant cells [16,17].

Phloroglucinol is a simple benzenetriol and the basic structural unit from which phlorotannins are assembled. Free phloroglucinol is not pharmacologically interchangeable with polymeric phlorotannins, and evidence for a phlorotannin-rich extract cannot be attributed directly to the monomer [18]. Fucosterol is a hydrophobic phytosterol widely associated with brown macroalgae and has reported antioxidant, anti-inflammatory, metabolic, and cytoprotective activities [19,20]. Its limited aqueous solubility and incomplete clinical safety characterisation remain important translational constraints.

Structure-based docking can generate plausible binding poses and rank ligands within a consistent protocol, whereas in silico toxicity platforms can flag liabilities for early prioritisation [21,22]. However, docking scores are scoring-function outputs rather than experimentally measured Gibbs free energies, and they are influenced by ligand size, hydrophobic contact area, receptor preparation, protonation, and search-space definition [23]. The human caspase-3 structure 6CKZ contains Ac-DW3-KE, a peptide-derived inhibitor covalently attached to the catalytic cysteine [24]. This structure offers a well-defined spatial reference, but conventional AutoDock Vina primarily evaluates noncovalent complementarity and cannot reproduce covalent reaction energetics or time-dependent inhibition.

This study aimed to compare the predicted interactions of gallic acid, phloroglucinol, and fucosterol with human caspase-3 and characterise their pkCSM and ProTox-III toxicity predictions. This study was designed as a hypothesis-generating prioritisation, not as a confirmation of caspase-3 inhibition, compound safety, or cardioprotective efficacy.

2 Materials and Methods

2.1 Study Design

A comparative computational workflow was used: candidate selection, ligand preparation, receptor preparation, crystallographic binding-region definition, molecular docking, two-dimensional protein–ligand interaction analysis, and toxicity prediction. The outputs were analysed descriptively.

2.2 Selection of Candidate Compounds

Gallic acid, phloroglucinol, and fucosterol were selected as candidate brown algal-associated compounds. Their structures and identifiers were obtained from PubChem [25]. The compound identifiers used in this study are presented in Table 1.

Table 1. Selected compounds evaluated against human caspase-3.

Compound	PubChem CID	Molecular formula	Chemical class	Study role
Gallic acid	370	$C_7H_6O_5$	Phenolic acid	Test ligand

Phloroglucinol	359	$C_6H_6O_3$	Benzenetriol; phlorotannin building unit	Test and ligand
Fucosterol	5281328	$C_{29}H_{48}O$	Marine sterol	Test and ligand
Ac-DW3-KE (Native Ligand)	Extracted from 6CKZ	Structure obtained from crystallographic coordinates	Peptide-derived covalent caspase-3 inhibitor	Structural reference

2.3 Computational Resources

The computational workflow employed the RCSB Protein Data Bank for protein structure retrieval and PubChem for ligand structures and identifiers. Open Babel version 3.1.1 was used for molecular format conversion and ligand geometry optimisation. AutoDockTools version 1.5.7 was used for receptor and ligand preparation, and molecular docking was performed using AutoDock Vina version 1.2.5. Protein-ligand complexes and predicted intermolecular interactions were inspected using BIOVIA Discovery Studio Visualizer 2021. In silico toxicity predictions were performed using the pkCSM and ProTox-III web-based platforms. Identical receptor structures, grid definitions, and docking parameters were applied to all test compounds to ensure internal comparability within the computational workflow.

2.4 Ligand Preparation

The three-dimensional structures of gallic acid, phloroglucinol, and fucosterol were obtained from PubChem in the SDF format. Ligand geometries were optimised using Open Babel version 3.1.1, with the MMFF94 force field. Protonation states were assigned to approximate physiological conditions (pH 7.4). Hydrogen atoms were added, Gasteiger partial atomic charges were assigned, and the rotatable bonds were defined using AutoDockTools version 1.5.7. The prepared ligands were subsequently converted to the PDBQT format for molecular docking. All structures were visually inspected after preparation to ensure the preservation of molecular connectivity and stereochemical features.

2.5 Receptor Preparation and Binding-Site Definition

The X-ray structure of human caspase-3 complexed with Ac-DW3-KE was retrieved as PDB ID 6CKZ [24]. The co-crystallized ligand was separated from the receptor and retained only for the spatial definition of the docking region. Crystallographic water molecules and unrelated heteroatoms were removed. Polar hydrogen atoms and Kollman partial charges were added using AutoDockTools version 1.5.7, and the prepared

receptor was converted to the PDBQT format. The docking search space was centred at x = 38.405 Å, y = 11.717 Å, and z = 72.144 Å, with dimensions of 24 × 24 × 28 Å along the x-, y-, and z-axes, respectively. These coordinates were selected to encompass the crystallographic region occupied by Ac-DW3-KE while allowing sufficient surrounding space for conformational exploration of the test compounds. Ac-DW3-KE was used only as a structural reference for defining the search region. Because it is a covalent caspase-3 inhibitor, its binding mechanism was not considered mechanistically equivalent to the non-covalent docking workflow applied to the test compounds.

2.6 Molecular Docking

Molecular docking of gallic acid, phloroglucinol, and fucosterol against human caspase-3 was performed using AutoDock Vina version 1.2.5. Each ligand was docked independently using the same prepared receptor and predefined docking search space [21]. The docking calculations used an exhaustiveness value of 32, a maximum of nine binding modes, an energy range of 3 kcal/mol, and a fixed random seed of 42. The grid center was set at x = 38.405 Å, y = 11.717 Å, and z = 72.144 Å, with dimensions of 24 × 24 × 28 Å. For each ligand, the top-ranked pose with the lowest AutoDock Vina score within the predefined docking search space was selected for subsequent interaction analysis. Docking scores were expressed in kcal/mol and interpreted only as comparative computational predictions obtained within the same standardised protocol. They were not interpreted as experimentally measured Gibbs free energies, inhibition constants, or direct indicators of biochemical potency.

2.7 Protein-Ligand Interaction Analysis

The selected docking pose of each ligand was analysed using BIOVIA Discovery Studio Visualizer 2021. The predicted intermolecular contacts and corresponding contact residues were identified from the generated interaction diagrams. Interaction categories were considered where available in the original analysis. Residues identified in the interaction diagrams were reported as predicted contact residues. No additional interaction distance measurements were introduced because these measurements were not part of the original docking analysis. Functional or catalytic significance was not assigned solely based on docking contact unless supported by established structural or experimental evidence. Occupation of the predefined docking region was not considered equivalent to the reproduction of the complete crystallographic Ac-DW3-KE binding mode.

2.8 Preparation for Toxicity Prediction

Canonical SMILES for gallic acid, phloroglucinol, and fucosterol were retrieved from PubChem. Compound identity, charge, and stereochemistry were confirmed before submission. Ac-DW3-KE was not included in the comparative toxicity analysis because it served as a structural reference rather than a candidate small molecule.

2.9 pkCSM Toxicity Prediction

Canonical SMILES were submitted to pkCSM [22]. The evaluated endpoints included Ames mutagenicity, maximum tolerated human dose, hERG II inhibition when available, acute oral rat toxicity, chronic oral rat toxicity, hepatotoxicity, skin sensitization, Tetrahymena pyriformis toxicity, and minnow toxicity. Units and continuous versus categorical outputs were reported exactly as generated by the platform.

2.10 ProTox-III Toxicity Prediction

ProTox-III was employed as a complementary platform for in silico toxicity prediction [26]. The evaluated parameters included the predicted LD_{50} and acute toxicity class, with the corresponding predicted class and probability documented when available. These model-based predictions were interpreted cautiously and were not considered equivalent to experimentally confirmed safety or toxicity.

2.11 Data Analysis

All outputs were summarised descriptively. Ligands were ranked by reported docking scores, and interaction profiles were compared qualitatively. No inferential statistics were applied to the single deterministic docking scores.

3 Results

3.1 In Silico Molecular Docking Analysis

Molecular docking was performed to evaluate the predicted interactions between selected Sargassum cinereum-associated compounds and human caspase-3 (CASP3). The crystal structure of caspase-3 complexed with Ac-DW3-KE (PDB ID: 6CKZ) was used as the receptor model, with Ac-DW3-KE primarily serving as a structural reference because it is a peptide-derived covalent inhibitor.

As shown in Table 2 and Figure 1, Ac-DW3-KE produced a docking score of −7.3 kcal/mol. Among the tested compounds, fucosterol showed the most favorable score (−7.1 kcal/mol), followed by gallic acid (−5.0 kcal/mol) and phloroglucinol (−4.0 kcal/mol). The 0.2 kcal/mol difference between fucosterol and Ac-DW3-KE should not be interpreted as evidence of comparable potency because conventional AutoDock Vina does not model the covalent binding mechanism of Ac-DW3-KE.

Fucosterol exhibited the broadest predicted interaction profile, while gallic acid and phloroglucinol showed more limited and partially overlapping residue contacts. However, the interaction environments of all three test compounds differed from the crystallographic residue-contact profile of Ac-DW3-KE. Thus, although the compounds were docked within an Ac-DW3-KE-defined search region, the results do not establish identical catalytic-site occupancy or reproduction of the reference inhibitor's binding mode. These docking scores represent computational estimates of pose favorability and should not be interpreted as experimental binding free energies, evidence of caspase-3 inhibition, or confirmation of cardioprotective activity.

Table 2. Molecular docking binding energies (kcal/mol) and contact residues of selected *Sargassum cinereum* compounds against human caspase-3.

Compound	Contact residues	CASP3 Binding energy (kcal/mol)
Reference ligand (Ac-DW3-KE)	THR59 GLY60 MET61 THR62 SER63 ARG64 SER65 HIS121 GLY122 GLU123 PHE128 CYS163 ARG164 GLY165 THR166 TYR204 TRP206 ARG207 ASN208 SER209 PHE250 SER251 PHE252 ASP253 PHE256 ASP3 PHE5	-7.3
Gallic acid	MET44 GLU76 THR77 PHE78 ASN80 LEU81 LYS82 CYS220 LEU223 LYS224 GLN225 TYR226 ALA227 ASP228 LYS229 PHE275 TYR276 HIS277 LEU278	-5.0
Phloroglucinol	MET44 GLU76 THR77 PHE78 ASN80 LEU81 LYS82 CYS220 LEU223 LYS224 GLN225 TYR226 ALA227 ASP228 LYS229 PHE275 TYR276	-4.0
Fucosterol	MET39 TYR41 PRO42 GLU43 MET44 GLY45 ASN73 ARG75 GLU76 THR77 ARG79 ASN80 LEU81 LYS82 TYR83 GLU84 VAL85 CYS220 LEU223 LYS224 GLN225 TYR226 ALA227 ASP228 LYS229 PHE275 TYR276 HIS277 GLU279	-7.1

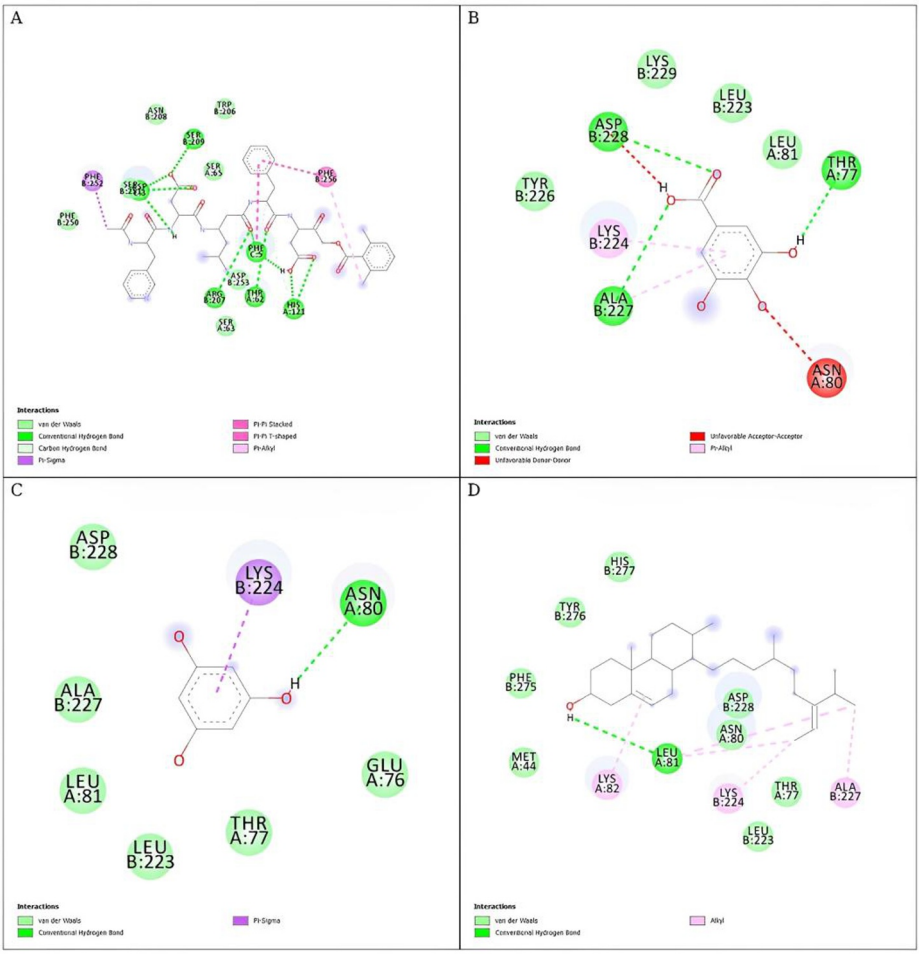


Fig. 1. Molecular docking visualisation of selected *Sargassum cinereum* compounds with human caspase-3 (PDB ID: 6CKZ). (A) Ac-DW3-KE–caspase-3, (B) gallic acid–caspase-3, (C) phloroglucinol–caspase-3, and (D) fucosterol–caspase-3.

3.2 pkCSM Toxicity Predictions

The pkCSM toxicity predictions for gallic acid, phloroglucinol, and fucosterol are listed in Table 3. All three compounds were predicted to be negative for Ames mutagenicity, hERG I inhibition, hepatotoxicity, and skin sensitisation. Gallic acid and phloroglucinol were predicted to be negative for hERG II inhibition, whereas fucosterol was predicted to be positive for hERG II inhibition.

The predicted maximum tolerated human doses were 0.700, 0.107, and −0.653 log mg/kg/day for gallic acid, phloroglucinol, and fucosterol, respectively. The corresponding acute oral rat toxicity values were 2.218, 1.958, and 2.553 mol/kg. For chronic oral

rat toxicity, the predicted lowest observed adverse effect level values were 3.060 log mg/kg body weight/day for gallic acid, 2.149 log mg/kg body weight/day for phloroglucinol, and 0.890 log mg/kg body weight/day for fucosterol.

The predicted Tetrahymena pyriformis toxicity values were 0.285 log µg/L for gallic acid, 0.127 log µg/L for phloroglucinol, and 0.432 log µg/L for fucosterol. The corresponding minnow toxicity predictions were 3.188, 2.734, and −1.711 log mM, respectively. The positive hERG II prediction for fucosterol was the principal toxicity alert identified by pkCSM. This finding is particularly relevant to its proposed evaluation for a cardiovascular application because hERG-channel inhibition may interfere with cardiac repolarization. Nevertheless, this binary computational prediction does not demonstrate experimentally confirmed hERG-channel inhibition. Electrophysiological testing is required to determine whether fucosterol produces concentration-dependent hERG blockade. All pkCSM findings should therefore be interpreted as model-based predictions rather than as observed toxicity or evidence of clinical safety.

Table 3. pkCSM toxicity predictions

Property	Model Name	Predicted Value			Unit
		Gallic acid	Phloroglucinol	Fucosterol	
Toxicity	AMES toxicity	No	No	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.7	0.107	-0.653	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	No	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	No	Yes	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.218	1.958	2.553	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	3.06	2.149	0.89	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	No	No	No	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	No	No	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	0.285	0.127	0.432	Numeric (log ug/L)
Toxicity	Minnow toxicity	3.188	2.734	-1.711	Numeric (log mM)

3.3 ProTox-III Toxicity Predictions

The ProTox-III acute oral toxicity predictions are listed in Table 4. Gallic acid had a predicted LD_{50} of 1,190 mg/kg and was assigned to a toxicity class 4. Phloroglucinol has a predicted LD_{50} of 200 mg/kg and was assigned to toxicity class 3. Fucosterol had

a predicted LD_{50} of 890 mg/kg and was assigned to toxicity class 4. Thus, the predicted LD_{50} values ranged from 200 to 1,190 mg/kg.

Among the three compounds, phloroglucinol had the lowest predicted LD_{50} and the least favourable acute toxicity classification. Gallic acid had the highest predicted LD_{50}, whereas fucosterol showed an intermediate predicted value. The predictions for gallic acid and phloroglucinol were each associated with an average similarity of 100% and a reported prediction accuracy of 100%. By comparison, the fucosterol prediction had an average similarity of 89.38% and a prediction accuracy of 70.97%, indicating greater model uncertainty for this compound.

The available ProTox-III data contain predicted LD_{50} values, toxicity classes, average similarities, and prediction accuracies. Therefore, no additional active or inactive toxicity endpoints should be reported unless the corresponding ProTox-III endpoint outputs are available. These findings represent computational estimates and should not be interpreted as experimentally observed acute toxicities. Direct agreement between pkCSM and ProTox-III could not be established for acute oral toxicity because the platforms reported their outputs in different formats and units. The pkCSM acute oral rat toxicity estimates were expressed in mol/kg, whereas ProTox-III reported the predicted LD_{50} values in mg/kg and assigned categorical toxicity classes. Consequently, the results should be considered complementary rather than directly interchangeable.

Table 4. ProTox-III toxicity predictions.

Compound	Predicted LD_{50} (mg/kg)	Toxicity class	Average similarity	Prediction accuracy
Gallic acid	1,190mg/kg	4	100%	100%
Phloroglucinol	200mg/kg	3	100%	100%
Fucosterol	890mg/kg	4	89.38%	70.97%

4 Discussion

The present study comparatively evaluated the predicted interactions of gallic acid, phloroglucinol, and fucosterol with human caspase-3 and examined their computational toxicity profiles. Fucosterol showed the most favorable docking score among the tested compounds (−7.1 kcal/mol), followed by gallic acid (−5.0 kcal/mol) and phloroglucinol (−4.0 kcal/mol), whereas Ac-DW3-KE scored −7.3 kcal/mol. Thus, fucosterol should be regarded as the best-scoring test compound rather than as a ligand that outperformed the crystallographic reference. The 0.2 kcal/mol difference does not establish comparable binding strength or inhibitory potency.

This comparison requires caution because Ac-DW3-KE is a peptide-derived covalent caspase-3 inhibitor involving catalytic CYS163 [24], whereas conventional AutoDock Vina primarily evaluates noncovalent steric, hydrogen-bonding, and hydrophobic complementarity [21]. It does not model covalent bond formation, reaction kinetics, or

irreversible target engagement. Accordingly, the Vina score of Ac-DW3-KE cannot be interpreted as a quantitative measure of its experimental potency.

Docking scores are also influenced by protein rigidity, solvent effects, protonation, desolvation, conformational entropy and induced fit. Larger and more hydrophobic ligands may obtain more favourable scores because they interact with a greater protein surface area. Therefore, docking scores should be used mainly for pose generation and preliminary ranking within a standardised protocol rather than as experimental Gibbs free energies [23]. The favourable score of fucosterol may partly reflect its larger steroidal scaffold and hydrophobic surface [19].

The interaction profiles also differed from those of Ac-DW3-KE. The reference ligand contacted CYS163 and residues across chains A–C, whereas the test compounds were mainly associated with residues around MET44, GLU76–LYS82, and CYS220–GLU279. Because no exact residue overlap was observed, the results do not establish identical catalytic-site occupancy or reproduction of the crystallographic binding mode. Differences in chain assignment, interaction cutoffs, receptor preparation, and pose selection may also affect the residue lists. More definitive binding-site assignment would require three-dimensional pose superposition and standardised residue-contact analysis.

Fucosterol should therefore be considered a computationally prioritized candidate rather than a confirmed caspase-3 inhibitor. Its translation is additionally limited by poor pharmacokinetics: in rats, fucosterol from Sargassum fusiforme showed poor gastrointestinal absorption, slow elimination, and approximately 0.74% oral bioavailability [27]. Its lipophilicity and low aqueous solubility may restrict systemic and cardiac exposure, while human pharmacokinetic and clinical safety data remain limited [20].

Gallic acid exhibited an intermediate docking score. Its hydroxyl and carboxyl groups may support polar interactions, although its smaller size limits the contact surface. Previous studies have associated gallic acid with antioxidant, anti-inflammatory, antihypertensive, antifibrotic, and cardioprotective effects in preclinical cardiovascular models [17], including reduced lipid peroxidation and preservation of endogenous antioxidant defences in myocardial injuries [28]. However, these effects may involve multiple pathways and do not directly inhibit caspase-3.

Phloroglucinol showed the least favourable docking score. Although its hydroxyl groups support polar interactions, its compact structure provides a smaller interaction surface. Free phloroglucinol should not be considered pharmacologically equivalent to oligomeric or polymeric phlorotannins. Its reported cytoprotective activity through Nrf2/HO-1 signalling [29] was demonstrated in noncardiac systems and cannot be directly attributed to caspase-3 binding or cardioprotection.

The toxicity predictions provided additional differentiation. pkCSM predicted all compounds as negative for Ames mutagenicity, hERG I inhibition, hepatotoxicity, and skin sensitisation; however, such predictions remain dependent on model applicability domains [22]. Fucosterol showed a positive hERG II alert. Because hERG/Kv11.1 blockade may prolong cardiac repolarization and increase arrhythmia risk [30], this signal warrants experimental confirmation by concentration–response patch-clamp studies and comparison with expected unbound exposure [31].

ProTox-III assigned gallic acid and fucosterol to toxicity class 4, with predicted LD50 values of 1,190 and 890 mg/kg, respectively, whereas phloroglucinol was assigned to toxicity class 3, with an LD50 of 200 mg/kg. However, these outputs remain model-dependent [26]. The differences between pkCSM and ProTox-III should not be interpreted as contradictions because the platforms use different units, datasets, algorithms, endpoint definitions, and confidence measures.

Finally, caspase-3 remains relevant to cardiomyocyte apoptosis, but myocardial injury also involves necrosis, necroptosis, pyroptosis, and ferroptosis [32]. Therefore, predicting interactions with a single apoptosis-related target is insufficient to establish cardioprotection. Experimental enzymatic, cellular, pharmacokinetic, and in vivo studies are required to determine whether these compounds meaningfully modulate caspase-3 or provide cardioprotection.

5 Conclusion

This computational study identified fucosterol as the best-scoring compound among the three tested ligands against human caspase-3, with a docking score of −7.1 kcal/mol. This value was close to, but less negative than, the −7.3 kcal/mol score obtained for the crystallographic reference ligand Ac-DW3-KE. However, direct comparison should be interpreted cautiously because Ac-DW3-KE is a peptide-derived covalent inhibitor, whereas conventional AutoDock Vina primarily evaluates non-covalent interactions. Gallic acid and phloroglucinol showed less favourable docking scores of −5.0 and −4.0 kcal/mol, respectively. Moreover, the reported contact residues did not establish that the tested compounds reproduced the crystallographic binding mode of Ac-DW3-KE. Toxicity predictions identified a potentially important hERG II inhibition alert for fucosterol, while ProTox-III classified gallic acid and fucosterol as toxicity classes 4 and 3, respectively, and phloroglucinol as class 3. Overall, these findings support the prioritisation of fucosterol for further investigation but do not confirm caspase-3 inhibition, cardioprotective efficacy, or safety. Biochemical caspase-3 assays, cardiomyocyte apoptosis models, pharmacokinetic studies, experimental hERG-channel evaluation, and appropriately designed in vivo cardiovascular studies are required before therapeutic conclusions can be established.

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