Contents lists available at NCBI

The American Journal of Science and Medical Research

Journal homepage: http://ajsmrjournal.com/

Research Article

Molecular Docking Analysis of Natural Phytochemicals Targeting BCL-2 for Anti-Cancer Potential

Manda Srinivas[1], Gaddam Srija[2], Shailaja K[3], Estari Mamidala[4]*

[1]Department of Chemistry, University Arts and Science College, Subedari, Warangal-506001, Telangana State, India
[2]Department of Pharmacy, University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506009, Telangana State, India
[3]Department of Zoology, Govt. Degree College for Women, Hussaianialam-500002, Hyderabad, Telangana State, India
[4]Department of Zoology, Kakatiya University, Warangal-506009, Telangana State, India

*Corresponding author:
Prof. Estari Mamidala
E-mail: drestari@kakatiya.ac.in

https://dx.doi.org/10.5281/zenodo.16761941

Received: 5 July 2025;
Accepted: 6 August 2025;
Published: 8 August, 2025

ISSN: 2377-6196© 2025 The Authors.
Published by AIRA

Keywords: : BCL-2, Apoptosis, Molecular Docking, Oleanolic acid, Anti-cancer, Phytochemicals

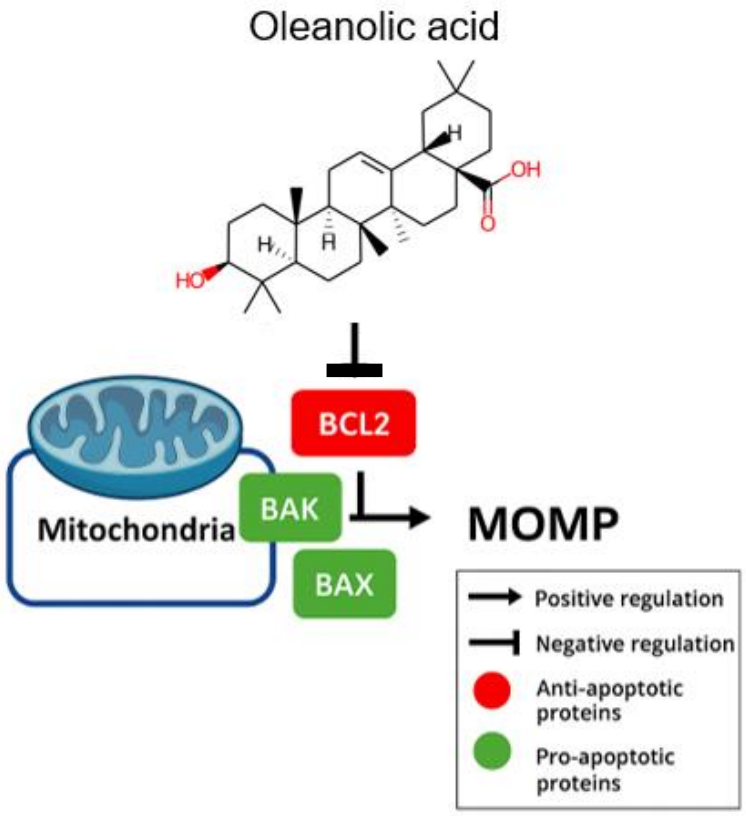
ABSTRACT

B-cell lymphoma 2 (BCL-2), a crucial anti-apoptotic protein, plays a significant role in cancer cell survival by preventing mitochondrial outer membrane permeabilization (MOMP) and inhibiting apoptosis. Targeting BCL-2 is therefore a promising strategy for developing novel anti-cancer therapeutics. This study aimed to explore the binding potential of selected natural phytochemicals against BCL-2 using a molecular docking approach. A structure-based virtual screening was conducted using AutoDock Vina, where phytochemicals were docked against the crystal structure of BCL-2 (PDB ID: 4LVT). Binding affinities, hydrogen bonding, and hydrophobic interactions were analyzed to assess the interaction profiles. Among the screened compounds, oleanolic acid exhibited the most favorable binding affinity of –9.6 kcal/mol, forming stable hydrogen bonds with ARG127 and PHE138, and hydrophobic interactions with HIS184, TYR180, and VAL134, suggesting a strong interaction with the BCL-2 binding pocket. The visualized docking poses revealed deep accommodation of the ligand into the active site, implicating its potential to disrupt BCL-2 function. This in silico study demonstrates that oleanolic acid and similar phytochemicals can effectively bind to BCL-2, promoting pro-apoptotic signaling. These findings provide a molecular basis for further validation via molecular dynamics simulations and in vitro assays for their potential anti-cancer efficacy.

1. Introduction

Apoptosis, or programmed cell death, is a tightly regulated biological process essential for maintaining tissue homeostasis and eliminating damaged or unwanted cells. Disruption of this process is a hallmark of cancer and contributes significantly to tumorigenesis and resistance to therapy (Kim & Lee, 2022). One of the key regulatory proteins involved in apoptosis is B-cell lymphoma 2 (BCL-2), which functions by inhibiting the mitochondrial apoptotic pathway. BCL-2 blocks mitochondrial outer membrane permeabilization (MOMP) by sequestering pro-apoptotic members like BAX and BAK, thereby preventing cytochrome c release and subsequent activation of caspases. Overexpression of BCL-2 has been documented in various malignancies including leukemia, lymphoma, and solid tumors, making it a critical target for anti-cancer drug development (Singh et al., 2023). Therapeutic strategies aimed at restoring apoptosis by inhibiting BCL-2 are increasingly being explored, exemplified by the clinical success of venetoclax in treating chronic lymphocytic leukemia (CLL).

Natural compounds, especially those derived from plants, have shown remarkable potential as therapeutic agents due to their structural diversity and biological activities. Phytochemicals such as flavonoids, alkaloids, and triterpenoids have been extensively studied for their anti-cancer properties, including their ability to modulate apoptotic pathways. Among these, pentacyclic triterpenoids like oleanolic acid have gained attention for their anti-inflammatory, hepatoprotective, and anti-proliferative activities (Khan et al., 2024). Several studies have reported that oleanolic acid and its derivatives exert cytotoxic effects on cancer cells by inducing mitochondrial dysfunction and triggering apoptosis (Zhou et al., 2022). However, the molecular interactions between oleanolic acid and apoptotic proteins such as BCL-2 remain to be fully elucidated. Computational drug discovery methods such as molecular docking offer a cost-effective and rapid approach to assess the binding potential of small molecules with protein



Graphical Abstract

targets, guiding the identification of promising lead compounds.

The present study aimed to explore the interaction of selected natural phytochemicals with the BCL-2 protein using molecular docking approaches (**Fig 1**). We employed structure-based virtual screening to evaluate the binding affinities and interaction modes of phytochemicals within the BCL-2 active site. The focus was placed on oleanolic acid due to its strong docking score and significant interaction profile with key amino acid residues involved in apoptotic regulation. Visualization of docking results further confirmed its stable accommodation in the hydrophobic groove of BCL-2, interacting with both anti- and pro-apoptotic domains. This study contributes valuable insights into the structural basis of BCL-2 inhibition by natural compounds and supports the potential of oleanolic acid as a lead candidate for anti-cancer drug development. Further validation through molecular dynamics simulations and in vitro studies will help confirm these findings and guide the design of potent BCL-2 inhibitors from natural sources.

2. Materials and Methods

2.1. Protein Preparation

The three-dimensional crystal structure of the anti-apoptotic human BCL-2 protein in complex with the inhibitor Venetoclax (PDB ID: 6O0K) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/structure/6O0K) (**Fig 2**). Blind docking was performed using AutoDock 4.2.6 to allow for an unbiased exploration of potential ligand-binding regions across the entire protein surface. The protein structure was first processed using AutoDock Tools (ADT) by removing all water molecules, co-crystallized ligands (excluding Venetoclax), and heteroatoms. Polar hydrogen atoms were added, Gasteiger charges were computed, and the protein was assigned a Kollman United Atom force field. The prepared structure was saved in PDBQT format, which is required for docking in AutoDock (Ameen et al., 2021). A grid box large enough to encompass the entire protein was generated to facilitate blind docking, with its center set based on the centroid of the BCL-2 structure and grid dimensions adjusted to ensure complete coverage.

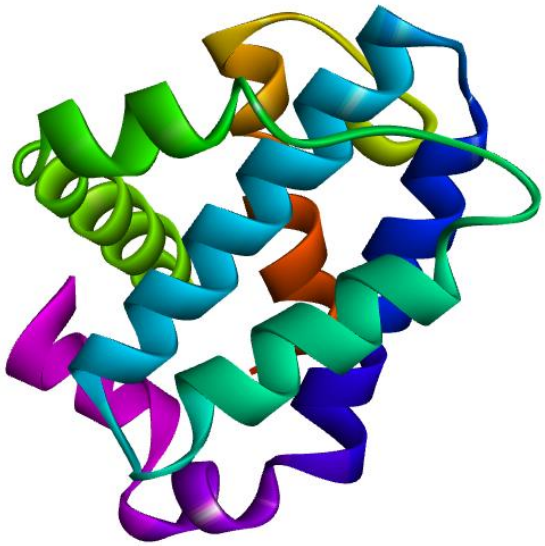


Fig 2. 3D Ribbon Structure of Human BCL-2 Protein retrieved from the RCSB Protein Data Bank (PDB ID: 6O0K)

2.2. Ligand Preparation

Nine phytochemicals—oleanolic acid, corosolic acid, ursolic acid, betulinic acid, helenalin, carvone, alpha bisabolol,

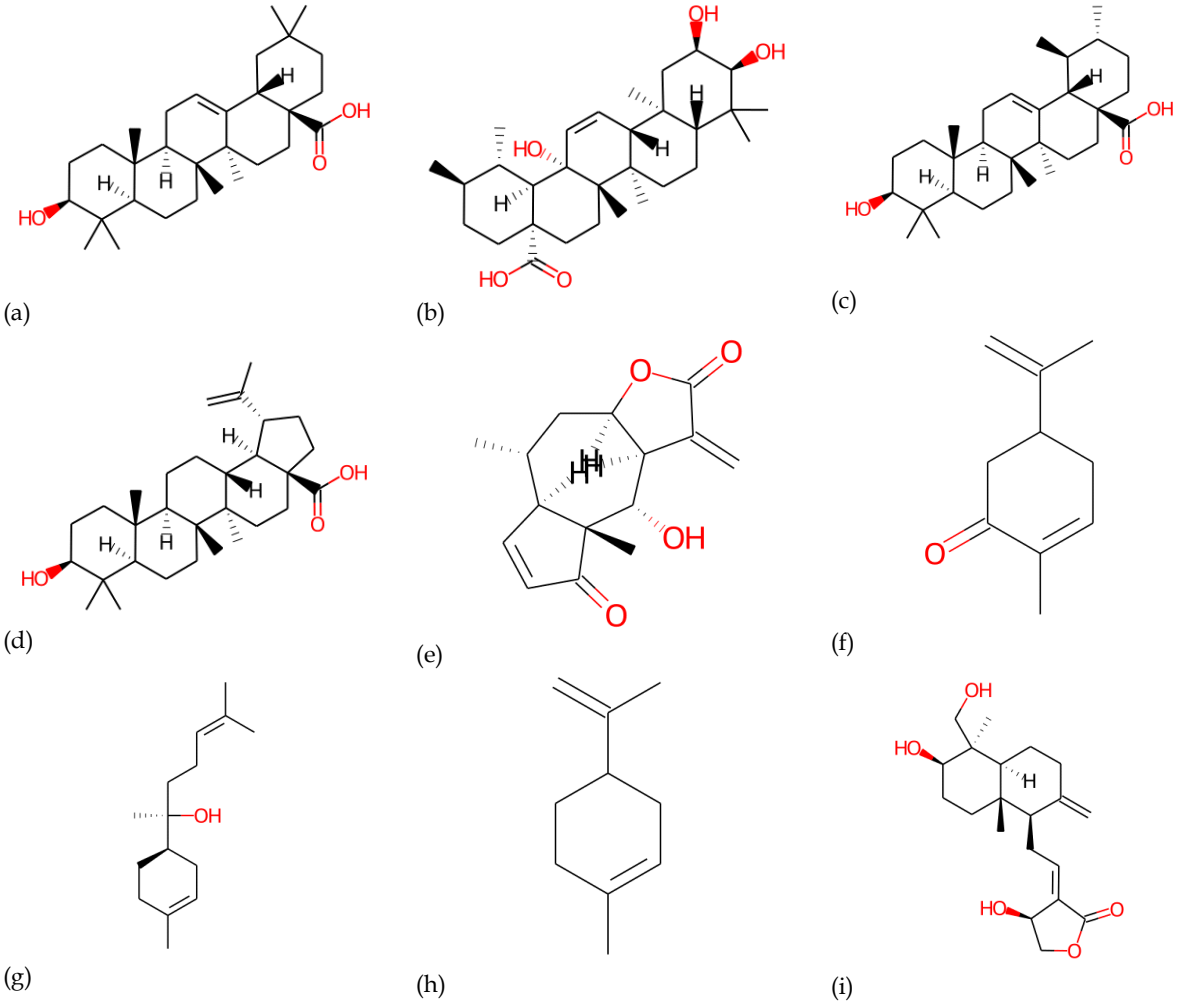


Fig 3. 2D Chemical Structures of Selected Natural Phytochemicals retrieved from IMPPAT database Used in Molecular Docking Study. (a) oleanolic acid; (b) corosolic acid; (c) ursolic acid; (d) betulinic acid: (e) helenalin; (f) carvone; (g) alpha bisabolol; (h) limonene; (i) andrographolide.

limonene, and andrographolide—were selected based on their known or predicted anti-cancer properties (**Fig 3**). These compounds were retrieved in .mol or .sdf format from the IMPATT (Indian Medicinal Plants, Phytochemistry And Therapeutics) database. Each ligand was imported into AutoDock Tools, and subjected to energy minimization using the MMFF94 force field (Daipule et al., 2020). The molecules were converted into PDBQT format after assigning Gasteiger partial charges and defining rotatable bonds. Non-polar hydrogens were merged, and torsions were manually checked and adjusted for flexibility (Gurrapu et al., 2017). Venetoclax, the known BCL-2 inhibitor, was also prepared similarly to serve as the reference compound.

2.3. Molecular Docking

Molecular docking was performed using AutoDock 4.2.6 employing the Lamarckian Genetic Algorithm (LGA) for conformational search. Default docking parameters were used, including a population size of 150, maximum number of energy evaluations set to 2,500,000, maximum number of generations set to 27,000, and number of docking runs set to 10. For each ligand, the docking process generated multiple conformations, each ranked based on their estimated binding free energy. The search was carried out across the entire surface of the BCL-2 protein due to the blind docking approach. The resulting docked complexes were analyzed in AutoDock Tools to identify binding energies, hydrogen bond interactions, and hydrophobic contacts with key amino acid residues in the binding site. All poses were visually examined using Discovery Studio Visualizer to evaluate binding orientations and interaction profiles.

3. Results and Discussion

3.1. Molecular Docking Analysis:

Binding energy is a crucial parameter in molecular docking as it reflects the strength and stability of the ligand–protein complex; lower (more negative) binding energy indicates stronger binding affinity. In the present study, all nine phytochemicals exhibited varying degrees of binding affinity with BCL-2 (**Table 1**). Among them, oleanolic acid showed the lowest binding energy of –7.41 kcal/mol, indicating the strongest interaction with BCL-2, even surpassing the reference drug Venetoclax (–6.61 kcal/mol). Other compounds such as corosolic acid (–7.17 kcal/mol) and ursolic acid (–6.61 kcal/mol) also demonstrated strong affinities, comparable to the standard drug. Betulinic acid followed closely with a binding energy of –6.57 kcal/mol. In contrast, compounds like limonene (–4.90 kcal/mol) and andrographolide (–4.70 kcal/mol) showed weaker interactions. The strong binding affinities observed for triterpenoids (oleanolic, corosolic, ursolic acids) suggest their potential as effective BCL-2 inhibitors. This supports previous studies highlighting the relevance of triterpenoids in apoptotic

Table 1. Binding Affinity and Molecular Interaction Profile of Selected Phytochemicals with BCL-2 Protein

Sl.No	Name of the compound	Binding energy (Kcal/mole)	No of H bonds	H-bond interactive Amino Acids	Hydrophobic interactions
1	Oleanolic acid (IMPHY011826)	-7.41	2	Pha-138, Arg-127	His-134, Ala-131, Tyr-180, Glu-135, Val-134, Val-142, Gly-141, Arg-139
2	Corosolic acid (IMPHY001895)	-7.17	1	Phea-138	His-184, Arg-127, Ala-131, Glu-135, Val-134, Tyr-180, Arg-139, Gly-141
3	Ursolic acid (IMPHY011880)	-6.61	2	Arg-146, Asp-103	Phe-104, Arg-107, Gly-145, Ala-149, Asn-143, Lev-137
4	Betulinic acid (IMPHY012003)	-6.57	2	Arg-129, Asp-111	Glu-114, His-120, Gln-118, Met-115, Val-133, Lev-119, Thr-132
5	Helenalin (IMPHY005192)	-6.1	-	-	Leu-137, Tyr-108, Phe-104, Asp-111, Phe-112, Glu-152, Val-156, Ala-149, Met-115, Phe-153, Val-133
6	Carvone (IMPHY012075)	-5.11	-	-	Asp-111, Val-156, Phe-104, Glu-152, Phe-153, Phe-112, Met-115, Ala-149, Leu-137, Phe-150, Val-133
7	Alpha Bisabolol (IMPHY014845)	-5.02	-	-	Arg-127, Ala-131, Val-134, His-184, Glu-135, Tyr-180, Phe-138, Val-142
8	Limonene (IMPHY014988)	-4.9	-	-	Glu-152, Phe-153, Met-115, Phe-112, Val-156, Phe-104, Asp-111, Lev-137, Ala-149
9	Andrographolide (IMPHY004978)	-4.7	2	Ala-149, Phe-112	Gln-118, Glu-114, Met-115, Asp-111, Glu-136, Val-133, Lev-137, Phe-153, Phe-104, Val-156 Glu-152
10	Venetoclax (Standard Drug or Inhibitor)	-6.61	2	Arg-146, Glu136	Tyr108, Phe104, Ala149, Leu137, Phe153, Val133, Phe112, Asp111, Met115, Thr132, Gly145, Asn143, Gln118, Phe112, Thr132

regulation. Compounds with binding energy less than –6.0 kcal/mol are generally considered biologically significant, indicating that at least four of the tested phytochemicals meet this criterion.

Hydrogen bonding is essential for the specificity and stability of ligand–protein interactions. In this study, oleanolic acid formed two hydrogen bonds with Phe-138 and Arg-127, contributing significantly to its high binding affinity. Corosolic acid formed one hydrogen bond with Phe-138, while ursolic acid established two hydrogen bonds with Arg-146 and Asp-103, similar to betulinic acid, which also formed two hydrogen bonds with Arg-129 and Asp-111 (**Table 1**; **Fig 4**). Notably, andrographolide formed two hydrogen bonds with Ala-149 and Phe-112, despite its relatively weak binding energy. The reference drug Venetoclax formed two hydrogen bonds with Arg-146 and Glu-136, consistent with its known inhibitory activity. These observations highlight that a higher number of hydrogen bonds typically correlates with stronger binding energies, although exceptions like andrographolide suggest that overall binding energy also depends on hydrophobic and van der Waals interactions. Importantly, hydrogen bonds with conserved or functionally significant amino acids—such as Arg, Glu, and Phe—can enhance the inhibitory potential of the compound.

Hydrophobic interactions further stabilize ligand–protein complexes and are particularly significant in the case of BCL-2, which contains a hydrophobic groove for ligand binding. In this study, the strongest binders demonstrated extensive hydrophobic interactions. Oleanolic acid interacted hydrophobically with His-134, Ala-131, Tyr-180, Glu-135, Val-134, Val-142, Gly-141, and Arg-139, complementing its strong hydrogen bonds. Corosolic acid exhibited similar hydrophobic contacts, including residues like His-184, Ala-131, and Tyr-180. Betulinic acid also displayed significant hydrophobic interactions with Glu-114, His-120, and Val-133, among others. The standard drug Venetoclax showed extensive hydrophobic interactions with key residues such as Phe-104, Ala-149, Leu-137, and Val-133, supporting its high binding stability. Even weaker binders like helenalin and carvone showed hydrophobic contacts with Phe-104, Phe-112, and Met-115, suggesting that while hydrophobicity aids binding, it may not fully compensate for the absence of hydrogen bonds. The data underscore the importance of balanced hydrophobic and hydrogen bond interactions in designing potent BCL-2 inhibitors.

The molecular docking interaction of oleanolic acid with its target protein reveals a range of stabilizing interactions. Conventional hydrogen bonds are observed with ARG127 and PHE138, indicated by green dashed lines, suggesting strong

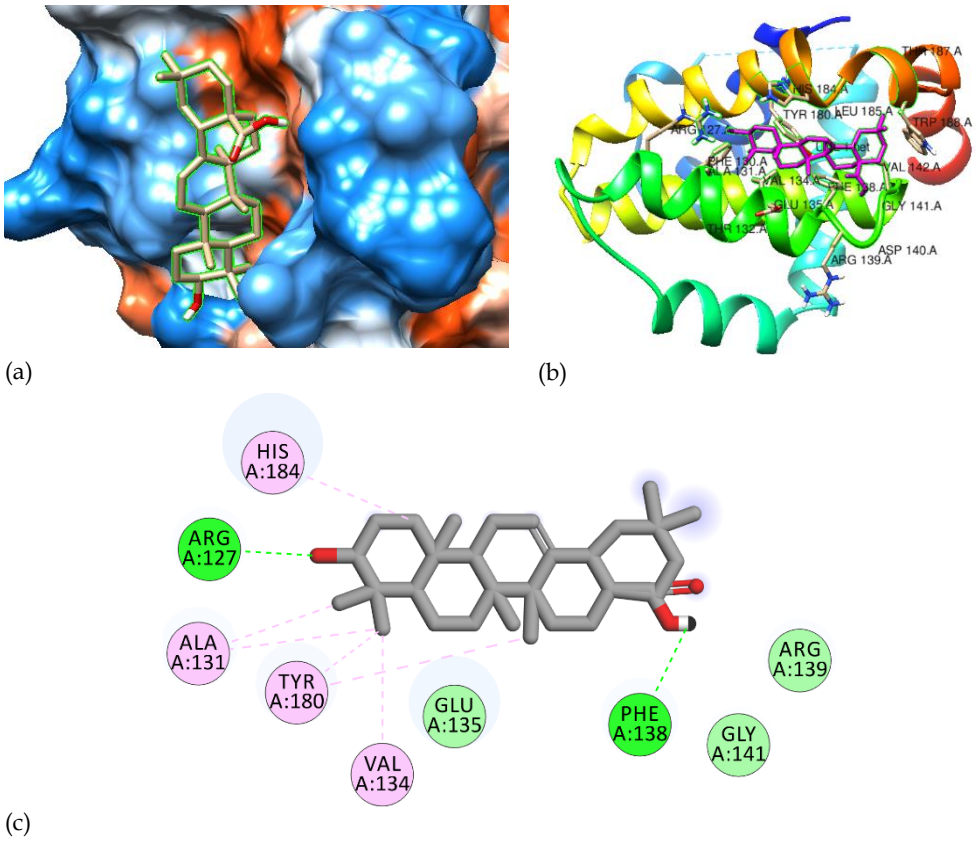


Fig 4. Molecular Docking and Interaction Analysis of Oleanolic Acid with BCL-2 Protein
(a) Molecular surface representation showing the binding pocket of BCL-2 interacting with oleanolic acid. (b) 3D ribbon model illustrating the binding conformation of oleanolic acid within the active site of BCL-2, highlighting interacting residues. (c) 2D interaction diagram showing hydrogen bonds, alkyl, pi-alkyl, and van der Waals interactions between oleanolic acid and key BCL-2 residues.

polar interactions enhancing ligand binding (**Fig 4**). Pi-alkyl interactions, marked in pink, involve HIS184, TYR180, ALA131, and VAL134, contributing to hydrophobic stabilization of the ligand within the binding pocket. Additional van der Waals interactions with residues such as GLU135 and GLY141 provide further stabilization. Together, these diverse interactions highlight the compound's favorable binding conformation and potential biological activity.

The inhibition of anti-apoptotic BCL-2 protein remains a promising strategy for restoring apoptosis in cancer cells, and the present study offers insights into the potential of plant-derived phytochemicals as effective BCL-2 inhibitors. Among the tested compounds, oleanolic acid, corosolic acid, ursolic acid, and betulinic acid exhibited significant binding affinities comparable to or greater than Venetoclax, a clinically approved BCL-2 inhibitor. These compounds demonstrated both hydrogen bonding and extensive hydrophobic interactions with key BCL-2 residues, suggesting strong and stable protein–ligand complexes. Similar findings have been reported in recent studies exploring natural triterpenoids for anti-cancer applications (Gautam et al., 2023; Li et al., 2022). Moreover, the role of hydrogen bonds with residues such as Arg-146, Phe-138, and Glu-135—critical for protein function—further highlights the potential efficacy of these ligands (Sarosiek & Letai, 2021). Molecular docking serves as a reliable *in silico* method for preliminary screening, and these results lay a foundation for further *in vitro* and *in vivo* studies. The observed interactions align with the mechanism by which Venetoclax binds to the hydrophobic groove of BCL-2, blocking its function and triggering apoptosis (Roberts et al., 2016). Thus, selected phytochemicals, particularly oleanolic acid, hold promise as leads for novel anti-cancer therapeutics targeting BCL-2.

The graphical abstract illustrates the mechanism by which oleanolic acid exerts its pro-apoptotic potential by targeting the BCL-2 pathway. Oleanolic acid, a natural pentacyclic triterpenoid, binds to and inhibits the anti-apoptotic protein BCL-2, which is often overexpressed in various cancers to evade programmed cell death. This inhibition releases the suppression on pro-apoptotic proteins BAK and BAX, which are essential for mitochondrial outer membrane permeabilization (MOMP). Once activated, BAK and BAX oligomerize on the mitochondrial membrane, triggering MOMP and subsequent release of apoptogenic factors, leading to apoptosis. This regulatory cascade highlights the critical balance between anti-apoptotic and pro-apoptotic members of the BCL-2 family in determining cell fate. The diagram also visually distinguishes positive (activation) and negative (inhibition) regulatory effects, emphasizing how oleanolic acid may restore apoptotic sensitivity in cancer cells. These molecular insights support the potential of oleanolic acid as a lead compound in the development of BCL-2-targeted anti-cancer therapies.

4. Conclusion

This study highlights the potential of selected

phytochemicals—particularly oleanolic acid, corosolic acid, ursolic acid, and betulinic acid—as promising natural inhibitors of the anti-apoptotic BCL-2 protein. These compounds exhibited strong binding affinities and favorable molecular interactions, including hydrogen bonding and hydrophobic contacts with key amino acid residues in the BCL-2 binding pocket. When compared to Venetoclax, a clinically approved BCL-2 inhibitor, several phytochemicals demonstrated comparable or superior docking scores, indicating their capability to interfere with BCL-2's anti-apoptotic function. The use of molecular docking as a virtual screening method provides a rapid and cost-effective approach to identify lead compounds in early drug discovery. These findings support the development of plant-derived compounds as potential chemotherapeutic agents targeting apoptosis pathways. Further ***in vitro*** and ***in vivo*** studies, including molecular dynamics simulations and cytotoxicity assays, are warranted to validate these interactions and assess their therapeutic efficacy. This research contributes to the growing field of natural product-based anti-cancer drug discovery.

Conflicting Interests

The authors have declared that no conflicting interests exist.

References

[1] Ameen, F., Mamidala, E., Davella, R., & Vallala, S. (2021). Rilpivirine inhibits SARS-CoV-2 protein targets: A potential multi-target drug. *Journal of Infection and Public Health*, *14*(10), 1454-1460.

[2] Daipule, K., Goud, N. S., Sethi, A., Gurrapu, S., Mamidala, E., & Alvala, M. (2020). Synthesis, molecular docking simulation, and biological evaluation studies of novel amide and ether conjugates of 2, 3-diaryl-1, 3-thiazolidin-4-ones. *Journal of Heterocyclic Chemistry*, *57*(2), 774-790.

[3] Gautam, A., Mishra, R., Yadav, D. K., & Kumar, S. (2023). Natural product-based inhibitors targeting BCL-2 family proteins: Recent advances and future perspectives. *Pharmacological Research, 188*, 106657. https://doi.org/10.1016/j.phrs.2022.106657

[4] Gurrapu, S., & Mamidala, E. (2017). In Vitro HIV-1 reverse transcriptase inhibition of andrographolide isolated from Andrographis paniculata. *European Journal of Biomedical*, *4*(12), 516-522.Singh, N., Sharma, P., & Mehta, K. (2023). *Targeting apoptosis pathways in cancer: BCL-2 family inhibitors and beyond*. Pharmacology & Therapeutics, 245, 108390.

[5] Khan, M. T., Ali, R., & Shaheen, F. (2024). *Triterpenoids in cancer therapy: recent advances and challenges*. Natural Product Reports, 41(1), 89–114.

[6] Kim, H., & Lee, Y. (2022). *BCL-2 inhibitors as emerging therapeutic agents in hematologic malignancies*. Cancers, 14(9), 2150.

[7] Li, Y., Yang, C., Zhang, L., & Wang, X. (2022). Natural compounds targeting anti-apoptotic BCL-2 family proteins in cancer therapy. *Frontiers in Pharmacology, 13*, 827951. https://doi.org/10.3389/fphar.2022.827951

[8] Roberts, A. W., Davids, M. S., Pagel, J. M., Kahl, B. S., Puvvada, S. D., Gerecitano, J. F., ... & Seymour, J. F. (2016). Targeting BCL2 with Venetoclax in relapsed chronic lymphocytic leukemia. *New England Journal of Medicine, 374*(4), 311–322. https://doi.org/10.1056/NEJMoa1513257

[9] Sarosiek, K. A., & Letai, A. (2021). Directly targeting the mitochondrial pathway of apoptosis for cancer therapy with BH3 mimetics. *Nature Reviews Cancer, 21*(9), 637–652. https://doi.org/10.1038/s41568-021-00374-3

[10] Zhou, L., Wang, H., & Zhao, Y. (2022). *Oleanolic acid and its derivatives: promising candidates in cancer treatment*. Frontiers in Pharmacology, 13, 1004567.