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CONTENTS

Molecular docking analysis of curcumin–glucose conjugate as potential modulators of breast cancer stemness via β-catenin inhibition

ROHIT PUJARI[1], MUMTESH SAXENA[2] and SHIV KUMAR DUBEY[1*]

[1]Department of Biochemistry, College of Basic Sciences and Humanities, [2]Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar-263145 (U.S. Nagar, Uttarakhand)
[*]Corresponding author's email id: shivdub@gmail.com

ABSTRACT: Cancer remains a leading cause of mortality worldwide, with breast cancer being one of the most prevalent and aggressive malignancies in women. A subpopulation of breast cancer cells, known as breast cancer stem cells (BCSCs), drives tumor initiation, progression, metastasis, and therapeutic resistance due to their self-renewal and pluripotency. The Wnt/β-catenin signaling pathway is crucial for maintaining BCSC properties, with β-catenin serving as a central effector that promotes stemness, survival, and tumor relapse. Conventional therapies often fail to eliminate BCSCs, resulting in poor prognosis and frequent recurrence. Natural compounds like curcumin exhibit anticancer activity with minimal side effects, yet their clinical utility is limited by low bioavailability and efficacy. To overcome these challenges, we designed a curcumin-glucose bioconjugate aimed at inhibiting β-catenin (PDB ID: 7AFW) in BCSCs. Molecular docking studies of curcumin, glucose, selected bioconjugate, and a known β-catenin binder (R9Q) revealed that the curcumin-glucose conjugate effectively interacts with key residues of β-catenin, suggesting potential inhibition of signaling. By targeting β-catenin, the conjugate is expected to disrupt BCSC self-renewal and survival, thereby impacting the breast cancer cell population and reducing tumor progression and relapse. These *in silico* findings provide a foundation for further *in vitro* and *in vivo* studies to validate the curcumin-glucose bioconjugate as a promising therapeutic strategy against breast cancer stem cells.

Key words: β-Catenin, breast cancer stem cells, curcumin conjugates, molecular docking, Wnt signaling pathway

Throughout history, natural products have been a rich source of a variety of bioactive chemicals, many of which have significant medicinal potential. Among these, curcumin, has attracted substantial interest for its extensive pharmacological activities and low toxicity profile (Gairola *et al.,* 2022). It regulates multiple molecular targets and exhibits anti-inflammatory, antioxidant, antimicrobial, and anticancer activities, making it a promising natural agent for modulating complex disease pathways (Tabanelli *et al.,* 2021). Additionally, it is crucial for the detection and prognosis of cancer as well as for increasing the chemotherapy-responsiveness and sensitivity of cancer cells (Zoi *et al.,* 2024). However, despite its therapeutic versatility, the clinical translation of curcumin remains limited due to its extremely poor bioavailability, quick metabolism, poor water solubility, and instability under physiological conditions (Tomeh*et al.,* 2019; Bucevic Popovic *et al.,* 2024).

Cancer, characterized by uncontrolled cellular proliferation, continues to be a major global health challenge. Breast cancer is the most commonly diagnosed malignancy and a leading cause of cancer-related mortality among women worldwide (WHO, 2025). In 2020 alone, approximately 2.4 million new cases and 685,000 deaths were recorded, with projections indicating a rise to 2.64 million cases and 1.7 million annual deaths by 2030 ((International Agency for Research on Cancer, 2022). Despite significant progress in diagnostic and therapeutic approaches, breast cancer continues to pose treatment challenges due to high rates of relapse, metastasis, and resistance to standard therapies.

Breast cancer stem cells (BCSCs) consist of subpopulation of cells with self-renewal and differentiation abilities. It serves as a key driver of tumor recurrence and therapeutic resistance by maintaining tumor heterogeneity and promoting regrowth (Song and Farzaneh, 2021). BCSCs

contribute to malignancy, metastatic progression, and treatment failure, making them critical therapeutic targets (Boesch *et al.,* 2016). Among the regulatory pathways governing BCSC stemness, the Wnt/β-catenin signaling plays an important role. Dysregulation of this pathway promotes tumor progression, epithelial–mesenchymal transition (EMT), immune modulation, and resistance to chemotherapy (Mani *et al.,* 2008).

Curcumin demonstrates potent inhibitory effects on the Wnt/β-catenin pathway in breast cancer cells. It downregulates key regulators such as disheveled (Dvl), β-catenin, slug, and cyclin D1, thereby preventing β-catenin nuclear translocation and suppressing oncogenic transcriptional programs (Wang *et al.,* 2018; Wang *et al.,* 2021). In hormone receptor-negative models, curcumin further activates GSK3β (Glycogen synthase kinase-3 beta) enhancing β-catenin degradation and reducing metastatic potential (Mishra *et al.,* 2019; Farghadani and Naidu, 2021). Although curcumin effectively modulates Wnt signaling, its therapeutic impact is severely constrained by its poor pharmacokinetics and limited bioavailability.

To address these limitations, present study explores the development of curcumin–glucose conjugate designed and hypothesized to enhance bioavailability and potentiate anticancer activity. Conjugation of curcumin's phenolic hydroxyl groups with glucose diesters is hypothesized to improve solubility, metabolic stability, and cellular uptake, potentially generating synergistic therapeutic effects (Bertoncini-Silva *et al.,* 2024). Earlier investigations have shown that curcumin bioconjugates with different ligands have better antibacterial and antiproliferative properties than native curcumin (Dubey *et al.,* 2008). Glucose, a key metabolic regulator, provides a crucial function in BCSC energy homeostasis and influences oncogenic pathways, including Wnt/β-catenin making it an ideal ligand for bioconjugation (Parvathy *et al.,* 2009; Ediriweera and Jayasena, 2023).

In the present research, molecular docking was done to examine the interactions of curcumin–glucose conjugate with essential proteins of the Wnt/β-catenin pathway. These results offer insight on how the bioconjugate impede BCSC-driven breast tumour growth and interfere with β-catenin-mediated stemness.

MATERIALS AND METHODS

Protein Structure Preparation

The key active site residues of β-catenin were identified using the UniProt Knowledgebase (UniProtKB) under the UniProt ID: P35222. The crystal structure of β-catenin (PDB ID: 7AFW) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org). The selection of this specific PDB entry was based on multiple quality parameters, including high structural resolution, acceptable R-value (a measure of the model's agreement with experimental data), and comprehensive sequence coverage, ensuring accurate representation of the

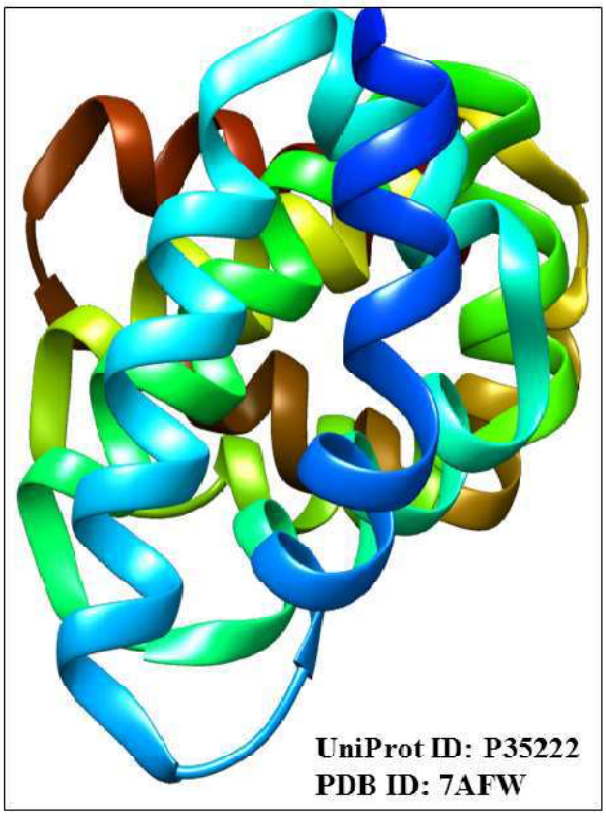

Fig. 1: The 3D structure of β-Catenin representing characteristic armadillo repeat domain as an elongated alpha-helical superhelix

functional domains in protein. Protein preparation of β- catenin was done via chimera1.17.3 (Gairola and Dubey, 2025). The processed file was saved in PDB format for use in further molecular docking as depicted in Figure 1.

Ligand Structure Preparation

Ligand preparation is a critical step in molecular docking, as it ensures the generation of energetically stable structures with correct spatial orientation and stereochemistry. Glucose was chosen as a ligand for conjugation with curcumin because of its pivotal role in cellular metabolism and its capacity to regulate critical biochemical pathways implicated in cancer, such as glycolysis and oxidative phosphorylation. The curcumin–glucose bioconjugate is hypothesized to exhibit synergistic antiproliferative effects and improved bioavailability, combining the bioactivities of both constituents. The diester was designed using ChemDraw Ultra 8.0, and its three-dimensional geometry was energy-minimized using Chem3D with the MM2 force field to obtain a stable conformation suitable for *in silico* docking studies. This careful ligand preparation increases the likelihood of identifying biologically relevant interactions with target proteins during virtual screening.

Molecular Docking

Molecular docking of β-catenin (PDB ID: 7AFW) with curcumin and its diester with glucose molecule was performed using AutoDock Vina integrated within the PyRx platform**.** Ligands were treated as flexible molecules, while the protein was considered rigid. Docking grid parameters were defined to encompass the entire binding pocket, and both protein and ligand structures were converted to the required PDBQT format for compatibility with AutoDock Vina. The dimensions for grid center, in angstrom, are X:35.4682, Y:38.0439, Z:48.4857, exhaustiveness value is 8, and the box coordinates values are X:70.1643, Y:30.7945 & Z:22.3801. Curcumin and glucose were taken from PUBCHEM database in ready-to-dock formats (curcumin: PubChem CID 969516; glucose: PubChem CID 5793). The curcumin– glucose conjugate was drawn in ChemDraw (CDX format), converted to SDF, and subsequently converted to PDB format using Open babel software. Prepared structures were loaded in PyRx software, designating the protein as macromolecule. Ligands were energy-minimized, converted to PDBQT, and docked against β-catenin. Discovery Studio software was used to show protein–ligand interactions in order to pinpoint important contacts and binding residues (Gairola *et al.,* 2025).R9Q, a known binder of β-catenin, was selected as the reference ligand because its interaction with β-catenin has been experimentally validated by X-ray crystallography and is deposited in the RCSB Protein Data Bank with resolved ligand coordinates. The ligand binds at a biologically relevant site on β-catenin, confirming a specific and reproducible interaction. R9Q is suitable for use as a reference compound in structural, mechanistic, and inhibitor-design studies.

ADMET and Toxicity Prediction

The ADMET (Absorption, Distribution, Metabolism, Excretion & Toxicity) characteristics of curcumin, glucose, and the curcumin-glucose diester were evaluated using contemporary computational platforms, including the Toxicity Estimation Software Tool (T.E.S.T), AdmetSAR, and SwissADME. SMILES (Simplified Molecular Input Line Entry System) notations of the chemical structures, generated using ChemDraw Ultra, were employed for ADMET profiling in AdmetSAR. Updated resources like AdmetSAR 2.0 (Cheng *et al.,* 2019), SwissADME (Daina *et al.,* 2017) and T.E.S.T. was employed to generate a detailed toxicity prediction probability, and classification of active and inactive toxicity types. Lipinski violations and toxicity class predictions were made using SWISS ADME software. The acute oral toxicity predictions and water solubility predictions were made using T.E.S.T. software. These analyses ensured that the bioconjugate exhibited acceptable drug-likeness and safety parameters prior to further *in-silico* evaluation.

RESULTS AND DISCUSSION

The crystal structure of β-catenin (PDB ID: 7AFW) was selected from 46 available structures based on

its highest resolution of 1.81 Å. The protein, used for docking studies, has a molecular weight of 18.45 kDa and contains 1,314 atoms. The structure consists of 167 deposited residues which were utilized for analysis.The actual molecular weight of the protein is approximately 85 kDa, as reported in UniProt (UniProt ID: P35222), which represents the collective mass including associated water molecules. However, for this study, β-catenin crystal structures were obtained and utilized from the RCSB Protein Data Bank.The structure consists of one unique protein chain, providing a well-defined and consistent binding pocket for ligand interactions. The high structural integrity of this model ensures reliable molecular docking and accurate interaction profiling with curcumin and its designed bioconjugate.

Molecular Docking

Molecular docking investigations were undertaken *in silico* to investigate the binding interactions of curcumin, glucose, and their diester bioconjugate with β-catenin (PDB ID: 7AFW). The crystal structure of β-catenin was retrieved in PDB format, and ligand analogs of curcumin were designed using ChemDraw 3D chemical structure editor by modifying side chains to generate novel variants.

Table 3: ADMET profile of curcumin, glucose and their bioconjugate based on predictive *in-silico* model

ADMET	Value		
	CUR	Glucose	CUR-GLC-DI
Human Intestinal Absorption	+	-	+
Blood Brain Barrier	-	-	+
Bioavailability score	+	+	+
CYP1A2 inhibitor	-	-	+
CLp	-	-	+
Carcinogenicity	-	-	-
Neurotoxicity	-	-	-
Skin corrosion	-	-	-
Hepatotoxicity	-	-	-
skin sensitization	-	-	-
Nephrotoxicity	-	-	-

Note: "- "Represent absence or less risk of the respective toxicity or poor pharmacokinetic outcome

The potential of ligands as β-catenin inhibitors was evaluated by ranking them according to binding free energy and analysing important interactions with the active site residues. The known binder R9Q exhibited the highest binding affinity (-7.8 kcal/mol), interacting with Phe110, Ser107, and Asp106 residue. Curcumin demonstrated binding affinity of -6.3 kcal/mol, engaging Asn147, Phe110, and Tyr111 residues. The curcumin analog (GLC+CUR+GLC) demonstrated higher affinity (-7.7 kcal/mol), which is comparable to that of its known binder, while retaining interactions with critical key residues such

Table 1: Comparative docking simulation result of Curcumin, its bioconjugate with glucose, glucose and known binder with ²-Catenin

Name of the ligand	Residues	Binding Free Energy (kcal/mol)
Curcumin	Asn147, Phe110, Asp106, Tyr111, Asn77, Arg42, Ser107, Lys37, His76	-6.3
Cur-Glc-bioconjugate	Lys37, Try111, His76, Ser107, Asp106, Phe110, Lys149, His33	-7.7
Glucose	Ile153, Phe110, Thr114, His80, His76, Lys149, Asn118	-5.3
R9Q (Known Binder)	Phe110, Ser107, Phe150, Leu109, Asp106, Lys145, Thr146, Asn147	-7.8

Table 2: *In silico* prediction of acute oral toxicity, plasma protein binding, water solubility and toxicity class of curcumin, glucose and their bioconjugate

Name	Lipinski violations (count)	Predicted acute oral toxicity (log(1/mol/kg))	LogS (Water solubility)	Toxicity class
Curcumin	Accepted (0 violation)	2.147	-4.031	III
Glucose	Accepted (0 violation)	1.703	-4.25	IV
CUR-GLC-Biconjugate	Accepted (0 violation)	1.492	-3.017	III

· Class I represents extremely toxic substances with LD_{50} values ≤ 5 mg/kg, while Class II includes highly toxic compounds with LD_{50} values between 5 and 50 mg/kg. Class III corresponds to moderately toxic compounds (LD_{50} : 50–300 mg/kg), Class IV to slightly toxic compounds (LD_{50} : 300–2,000 mg/kg), and Class V to practically non-toxic compounds with LD_{50} values greater than 2,000 mg/kg.

as Arg200, Pro238, Ala272, and Leu275. Glucose displayed the lowest affinity (-5.3 kcal/mol), interacting with Thr201, Asn261, and Gln165 residues. Notably, the curcumin–glucose conjugate exhibited stable interactions and favorable binding with β-catenin, suggesting its potential as a lead compound to inhibit Wnt/β-catenin signaling (Table 1, Figure 2a, 2b, 2c, 2d).

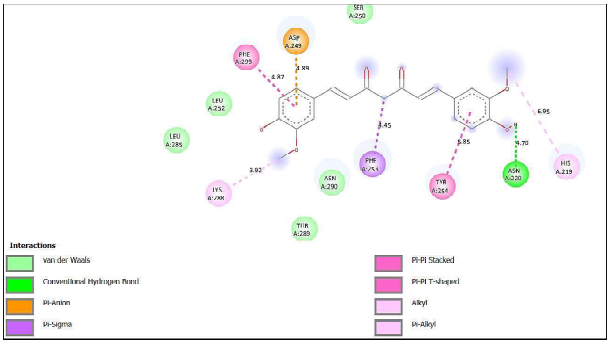

Fig. 2a: 2D interaction of curcumin with β-Catenin

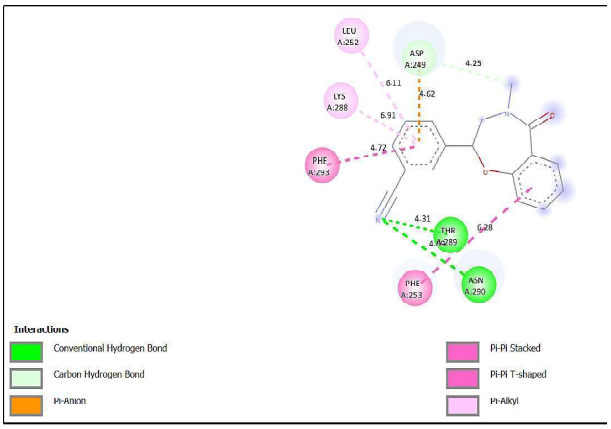

Fig 2b: 2D interaction of known binder with β-Catenin

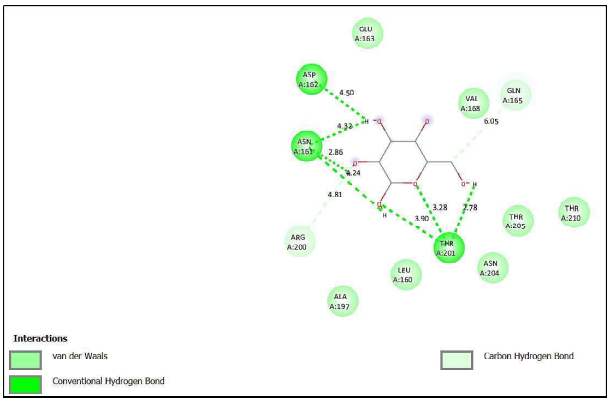

Fig 2c: 2D interaction of glucose with β-Catenin

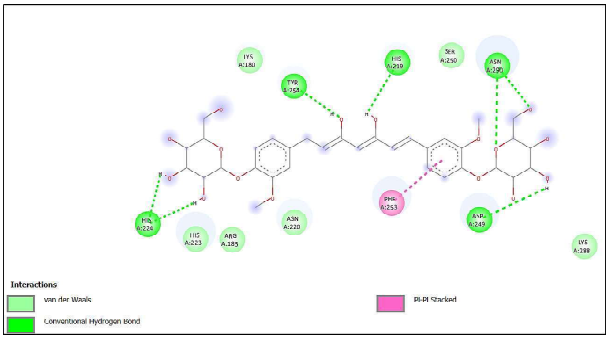

Fig 2d: 2D interaction of curcumin-glucose-bioconjugate with β-Catenin

ADMET Predictions

CONCLUSION

Curcumin and its diester bioconjugate with glucose have the ability to bind with β-catenin, a crucial regulator of Wntsignaling, in an efficient manner, according to the molecular docking studies. Curcumin showed strong interaction with key residues, while its conjugate maintained highest binding potential and preserved crucial contacts within the β-catenin binding site, suggesting their potential as effective modulators. Although glucose alone displayed lower affinity, its integration into bioconjugate contributed to enhanced binding characteristics. Notably, these conjugates retained interactions with core active site residues, including Phe110, Tyr111, and Lys37, indicating stable ligand-protein complexes. The incorporation of glucose, despite its relatively moderate individual binding, appears to contribute synergistically within the conjugate framework, aiming to enhance the overall binding efficacy and therapeutic potential of the curcumin conjugates. The known binder R9Q displayed the strongest interaction (-7.8 kcal/mol), validating the docking approach and providing a benchmark for comparison. Overall, these computational findings support the therapeutic promise of curcumin conjugate as potential inhibitors of β-catenin in Wnt signaling. However, extensive validation through clinical trials and wet-lab experiments is essential to confirm their bioactivity, pharmacological safety, and overall efficacy. Computational studies offer a compelling

foundation, but further biological evaluation is crucial to establish whether or not bioconjugate is a viable candidate for natural, targeted cancer chemotherapy.

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REFERENCES

Bertoncini-Silva, C., Vlad, A., Ricciarelli, R., Giacomo Fassini, P., Suen, V. M. M., and Zingg, J. M. (2024). Enhancing the bioavailability and bioactivity of curcumin for disease prevention and treatment. *Antioxidants*, 13(3): 331.

Boesch, M., Sopper, S., Zeimet, A. G., Reimer, D., Gastl, G., Ludewig, B., and Wolf, D. (2016). Heterogeneity of cancer stem cells: Rationale for targeting the stem cell niche. *Biochimica et Biophysica Acta (BBA) – Reviews on Cancer,* 1866(2): 276–289.

Bučević Popović, V., Karahmet Farhat, E., Banjari, I., Jeličić Kadić, A., and Puljak, L. (2024). Bioavailability of oral curcumin in systematic reviews: A methodological study. *Pharmaceuticals*, 17(2): 164.

Cheng, F., Li, W., Zhou, Y., Yu, Y., Shen, J., and Tang, Y. (2019). AdmetSAR 2.0: An improved tool for ADMET prediction. *Nucleic Acids Research,* 47(W1), W322–W330.

Daina, A., Michielin, O. and Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness. *Scientific Reports,* 7: 42717.

Dubey, S. K., Sharma, A. K., Narain, U., Misra, K., and Pati, U. (2008). Design, synthesis and characterization of some bioactive conjugates of curcumin with glycine, glutamic acid, valine and demethylenatedpiperic acid and study of their antimicrobial and antiproliferative properties. *European Journal of Medicinal Chemistry,* 43(9): 1837–1846.

Ediriweera, M. K., and Jayasena, S. (2023). The role of reprogrammed glucose metabolism in cancer. *Metabolites,* 13(3):345.

Farghadani, R., and Naidu, R. (2021). Curcumin: Modulator of key molecular signaling pathways in hormone-independent breast cancer. *Cancers,* 13(14): 3427.

Gairola, K., and Dubey, S. K. (2025). In-silico analysis of curcumin conjugates targeting the Wnt signaling pathway in Breast Cancer Stem Cells. *Pantnagar Journal of Research* 23(1): 1-7.

Gairola, K., Gururani, S. and Dubey, S.K. (2022). Polyphenols and their effect on the immune system. In: Kesharwani, R.K., Keservani, R.K. and Sharma, A.K. (eds). *Nutraceuticals and Functional Foods in Immunomodulators*. Springer, Singapore, Pp. 121–140.

Gairola, K., Singh, G., Bahuguna, A., Pujari, R., Kesharwani, R. K., and Dubey, S. K. (2025). Computational simulation guided prediction of the inhibitory effect of curcumin, diallyl sulfide and its conjugates on ALDH1A1 to target breast cancer stem cells (BCSCs). *Journal of Biomolecular Structure & Dynamics*, 1–14.

International Agency for Research on Cancer. (2022). Current and future burden of breast cancer: Global statistics for 2020 and 2040. IARC.

Mani, S. A., Guo, W., Liao, M. J., Eaton, E. N., Ayyanan, A., Zhou, A. Y., Brooks, M., Reinhard, F., Zhang, C. C., Shipitsin, M., Campbell, L. L., Polyak, K., Brisken, C., Yang, J., and Weinberg, R. A. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell,* 133(4): 704–715.

Mishra, H., Kesharwani, R. K., Singh, D. V., Tripathi, S., Dubey, S. K., and Misra, K. (2019) Computational simulation of inhibitory effects of curcumin, retinoic acid,

and their conjugates for GSK-3 beta. *Network Modeling Analysis in Health Informatics and Bioinformatics*, 8: 3.

Parvathy, K. S., Negi, P. S., and Srinivas, P. (2009). Antioxidant, antimutagenic and antibacterial activities of curcumin-β-diglucoside. *Food Chemistry,* 115(1): 265–271.

Song, K., and Farzaneh, M. (2021). Signaling pathways governing breast cancer stem cells behavior. *Stem Cell Research & Therapy,* 12: Article 245.

Tabanelli, R., Brogi, S., Benassi, L., Bagnoli, L., and Nesi, M. (2021). Improving curcumin bioavailability: Current strategies and future perspectives. *Pharmaceutics,* 13(10): Article 1715.

Tomeh, M. A., Hadianamrei, R., and Zhao, X. (2019). A review of curcumin and its derivatives as anticancer agents. *International Journal of Molecular Sciences,* 20(5): 1033.

Wang, H., Zhang, K., Liu, J., Yang, J., Tian, Y., Yang, C., Li, Y., Shao, M., Su, W., and Song, N. (2021). Curcumin regulates cancer progression: Focus on ncRNAs and molecular signaling pathways. *Frontiers in Oncology,* 11:Article 660712.

Wang, J. Y., Wang, X., Wang, X. J., Zheng, B. Z., Wang, Y., Wang, X., and Liang, B. (2018). Curcumin inhibits growth via Wnt/β-catenin pathway in non-small-cell lung cancer cells. *European Review for Medical and Pharmacological Sciences,* 22(21): 7492–7499.

World Health Organization. (2025, August 14). Breast cancer: Key facts. WHO. Retrieved from https://www.who.int/news-room/fact-sheets/detail/breast-cancer

Zoi, V., Kyritsis, A. P., Galani, V., Lazari, D., Sioka, C., Voulgaris, S., and Alexiou, G. A. (2024). The role of curcumin in cancer: A focus on the PI3K/Akt pathway. *Cancers,* 16(8): 1554.

Supplementary data: The SMILES entry for curcumin-glucose bioconjugate is [H]C([H])(O)C1([H])OC([H])(OC2CCC(CCC(O)CC(O)CCC3CCC(OC4([H])OC([H])(C([H])([H])O)C([H])(O)C ([H])(O)C4([H])O)C(C3)OC)CC2OC)C([H])(O)C([H]) (O)C1([H])O.

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