

Targeting Anoikis Resistance in Tumor Cells Using Bioactive Natural Compounds

Thesis submitted

**In partial fulfilment of the requirement for the
degree of**

DOCTOR OF PHILOSOPHY

in

Biotechnology

Submitted by

Shweta Gulia

2K20/PHDBT/02

Under the supervision of

Dr. Asmita Das
Associate Professor
Delhi technological University

Dr. Prakash Chandra
Assistant Professor
Delhi Technological University



**To the
Department of Biotechnology**

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daultpur, Main Bawana Road,
Delhi-110042, India

December, 2024

Acknowledgement

I am deeply grateful to all those who have supported and guided me throughout my Ph.D. journey. First and foremost, I would like to extend my sincere gratitude to Prof. Prateek Sharma, Vice Chancellor of DTU, Delhi, and Prof. Jai Prakash Saini, Former Vice Chancellor of DTU, for providing outstanding facilities and infrastructure that were instrumental in my research.

I owe immense gratitude to my supervisor, Dr. Asmita Das, and co-supervisor Dr. Prakash Chandra, for their invaluable guidance, unwavering encouragement, and profound expertise. Their support, patience, and exceptional mentorship have been the cornerstone of my academic and research journey.

I am also deeply thankful to Prof. Yasha Hasija, Head and DRC Chairperson, Department of Biotechnology, and Prof. Pravir Kumar, Former Head and DRC Chairman, Department of Biotechnology, DTU, Delhi, for their insightful suggestions and valuable advice, which significantly enriched the quality of my work.

My heartfelt thanks extend to the members of the DRC and SRC, including Prof. Jai Gopal Sharma, Dr. Navneeta Bharadvaja, Dr. Smita Rastogi, and Dr. Kriti Bhandari, for their continuous encouragement, constructive feedback, and invaluable guidance throughout this journey. I am also grateful to the technical staff, particularly Mr. Chhail Bihari Singh, Mr. Jitender Singh, Mr. Lalit, and all the staff members of the Department of Biotechnology, whose consistent support has been indispensable.

I deeply appreciate the camaraderie and collaboration of my fellow Ph.D. scholars Dr. Ritu, Himanshi, Simran Singh, Monika Yadav, and Yuvraj Sharma. Their engaging discussions and support, especially during challenging deadlines, have made the past four years a memorable and fulfilling experience.

Finally, I would like to express my heartfelt gratitude to my family, whose unwavering support and love have been my greatest source of strength. I would like to express my deepest gratitude to my father, who is no longer with us. His unwavering love and blessings continue to be a source of strength and guidance in my life, and I feel his presence with me always. My mother, my siblings Aakash and Shikha, and my beloved nephew and niece, Taksheel and Tvesha, have been my pillars of encouragement. I am especially thankful to my elder sister, Shikha Gulia, for her steadfast motivation, which has kept me focused and resilient throughout this challenging yet rewarding journey. In the end, I would like to express my heartfelt gratitude and dedicate this thesis to my father who is no longer with us, but forever in my heart, and my beloved Maasi, Mrs. Raj Bala, who have departed for their heavenly abode. Though they are no longer with us, their love and blessings continue to inspire and guide me, leaving an everlasting imprint on my life.

Shweta Gulia
2K20/PHDBT/02



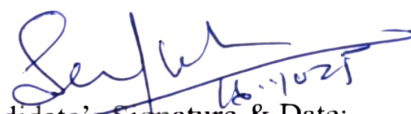
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-
110042

CANDIDATE'S DECLARATION

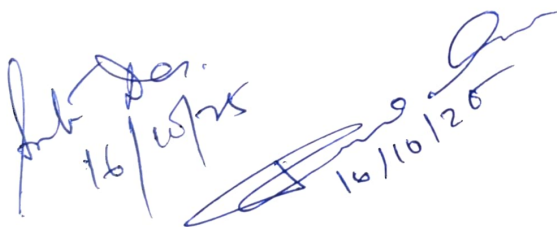
I, **Shweta Gulia, 2K20/PHD/BT/02**, hereby certify that the work which is being presented in the thesis entitled "**Targeting Anoikis Resistance In Tumor Cells Using Bioactive Natural Compounds**" in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 26 August 2020 to 31st December 2024 under the supervision of **Dr. Asmita Das** and Co Supervision of **Dr. Prakash Chandra**, Department of Biotechnology, Delhi Technological University.

The matter presented in the thesis has not been submitted by me for the award of any other.

Place: Delhi


Candidate's Signature & Date: 16/10/25

Shweta Gulia (2K20/PHDBT/02)


16/10/25

Signature of Supervisor(s)


Signature of External Examiner



DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-
110042

CERTIFICATE BY THE SUPERVISORS

Certified that **Shweta Gulia (2K20/PHDBT/02)** has carried out their search work presented in this thesis entitled **“Targeting Anoikis Resistance In Tumor Cells Using Bioactive Natural Compounds”** for the award of **Doctor of Philosophy** from Department of Biotechnology, Delhi Technological University, Delhi, under our supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Place: Delhi

Date: 16/10/25

Dr. Asmita Das
Associate Professor

Department of Biotechnology
Delhi Technological University

Dr. Prakash Chandra
Assistant Professor

Department of Biotechnology
Delhi Technological University

Prof. Yasha Hasija
Professor HoD

Department of Biotechnology
Delhi Technological University

ABSTRACT

Cancer has remained one of the greatest health threats globally, contributing to significant mortality, and, in most occasions, life-altering consequences for the patients. Against this background, even with the numerous therapies and drugs applied in such situations, no single treatment works for this lethal disease. One of the primary obstacles that present challenges in cancer treatment and research is the fact that cancer cells become resistant to cell death mechanisms, treatment therapies, drugs, or any other means of treatment that induce cell death.

Genetic alterations in normal and neoplastic cells render them resistant to these adverse conditions, thereby leading to their survival, growth, and progression in the body. Normal cells need to communicate with neighbouring cells, adhere to the extracellular matrix, and timely cell death to maintain cellular homeostasis. In contrast, cancer cells circumvent these mechanisms, thereby enabling themselves to survive in conditions that would normally activate death processes and mechanisms. Anoikis is a specialized form of apoptotic cell death resulting from the loss of cell-cell or extracellular-matrix contact. Tumor cells that become resistant to anoikis are termed as anoikis-resistant cancer cells. In such cells, the overexpression and activity of anti-apoptotic proteins inhibit the release of cytochrome c into the cytosol and the consequent activation of the caspase cascade, two events central to apoptosis. Consequently, these anoikis-resistant cells proceed through cell proliferation after escaping cell death and thereby gain the ability to migrate into distant organs for establishment of secondary tumors, hence initiating metastasis. In fact, it was derived from the literature review for this study that key processes, such as epithelial- mesenchymal transition and autophagy, act as major negative regulators of anoikis. These processes facilitate the enhanced migration potential of cancer cells by epithelial-to- mesenchymal transformation and support cell survival without attachment to the ECM or cell neighbours. As such, our research focused on identifying molecular pathways involved in anoikis that would lead to the discovery of common genes between anoikis, apoptosis, EMT, autophagy, and metastasis. Thus, this thesis aims to study the process of anoikis and screen the inhibitors that targeted proteins having a negative regulatory effect on this cellular process. We screened a set of 102 natural compounds with previously described anti-tumor properties in order to identify the modulators of anti-apoptotic genes regulating this process of anoikis. A combination of the in-silico methods such as molecular docking and molecular dynamics simulations, differential gene expression analysis, and nanoparticle-based drug delivery was employed to treat anti-tumor activity of one among the several screened bioactive compounds against a cancer cell line. Molecular docking and molecular dynamics simulations enabled us to study interactions occurring between targeted anti-apoptotic (anoikis resistance associated proteins) protein and their ligands by evaluating their binding affinities and behavior in a simulated biological environment. These computational tools have thus served in the screening of hundreds of natural compounds and identification of genes that could be relevant, using data obtained from experiments validated in wet lab environment procured from online databases. Accordingly, these bioactive compounds might be tailored and further therapeutically applied to target anoikis in cell death resistant tumor cells, which would imply a new approach in cancer therapy. As a result, we found out that there are few proteins that are common among anoikis associated pathways of autophagy, apoptosis and EMT. We also screened genes involved in metastasis and core anoikis. Bcl-2, Bax, BIRC3 and IKK α were proteins common among all these pathways, we further studied Bcl-2 and its interaction with natural compounds indicating Dioscin interacts with Bcl-2 by direct binding and hence can be further investigated for cancer therapy. In another approach, we procured genes that were differentially expressed in anoikis resistance, circulating tumor cells (CTC) and metastatic and benign prostate cancer. We

extracted common genes in these three data sets and checked how the levels of gene expression

of these genes were changed by a set of natural compounds in combination and alone. We employed GEO2R, MS Excel and certain mathematical codes in this methodology. 8 genes out of 53 DEGs including MYLIP, ST7L, SSTR1, ZSCAN12, SKIL, ANXA2, KIF5C, and CAV2 were regulated by all four compounds named nitidine chloride, bilobalide, CDCS (Chenodeoxycholic acid) and gastrodin. This observation suggests potential synergistic effects of natural compounds and alteration of gene expression by multiple compounds proves beneficial in preventing anoikis resistance. Following this we studied the effect of natural compound on differentially expressed anoikis resistance genes at different time intervals. We acquired GEO Dataset GSE158788 which had the gene expression profile of tumor associated genes at different time intervals treated with Gallic acid. We matched this data with the expression of our differentially expressed anoikis resistance genes and found out each of the genes showed fold change in gene expression at 2 hours, 4 hours, 6 hour and 9 hours. Following this, to study the drug delivery of natural compound having potent drug capabilities we conjugated gallic acid with gold nanoparticle. We synthesized gold nanoparticle by citrate reduction method. Gold nanoparticle has been known for its anti-cancerous properties, anti-oxidant property and non-toxic nature. Nanoparticle-gallic acid conjugation was studied by FTIR, UV-Visible spectrophotometry and Size distribution by intensity by Dynamic light scattering. Drug release was studied in PBS and changes in size of the nanoparticle, changes in absorption spectra depicted successful conjugation and drug release. Cytotoxic effect of gallic acid, gold nanoparticle and gallic acid loaded gold nanoparticle was studied by performing MTT Assay. We used Colon cancer cell lines (Col 320) for cell viability assay and studying cytotoxicity by the 3 components (AuNP, GA-AuNP, and GA). After treatment of cells with the different dosage concentrations of AuNP, GA-AuNP and GA at 12 hours, 24 hours and 48 hours and measured absorbance at 570 nm using a microplate reader. The GA-AuNP complex consistently demonstrates superior efficacy in inhibiting cell viability, as evidenced by the lowest IC₅₀ values across all time points. At 12 hours, GA-AuNP (30.27 µg/mL) significantly outperforms Gallic Acid (57.21 µg/mL) and Gold Nanoparticles (70.63 µg/mL). This trend continues at 24 hours, with GA-AuNP (19.23 µg/mL) remaining more effective than Gallic Acid (42.53 µg/mL) and Gold Nanoparticles (59.57 µg/mL). At 48 hours, GA-AuNP (18.35 µg/mL) maintains its superior efficacy compared to Gallic Acid (35.12 µg/mL) and Gold Nanoparticles (48.29 µg/mL). These results highlight the enhanced potential of the GA-AuNP complex in inhibiting cell viability over time. The study has verified potential for targeting anoikis resistance in cancer cells using natural compounds, focusing on the molecular pathways and genes involved in apoptosis, EMT, autophagy, and metastasis. This study elucidates the complex interaction between autophagy, apoptosis, and anoikis in an effort to realize their important roles in cancer prognosis. Common genes and proteins in their pathways could thus be targeted to regulate anoikis, an important process involved in cancer progression. Hence, computational methods using bioactive natural compounds would be a promising way for modulating these regulatory molecules. Dioscin, nitidine chloride, gastrodin, CDCA, bilobalide, and gallic acid were found to have the maximum potential compounds for altering pathways and genes related to anoikis. Additionally, gold nanoparticles offer an effective platform for drug delivery with drug loading and release. Gallic acid showed toxicity towards cancer cells and it showed enhanced efficacy when loaded in gold nanoparticles, as evinced by MTT Assay results. These results point toward a new and effective avenue in cancer therapy by targeting anoikis resistance with natural compounds, particularly in combination and in conjunction with advanced drug delivery systems. Future studies in regard to this shall be based on in vivo studies and clinical trials, with new discovery of other natural compounds and nanoparticles targeted for more infiltration of cancer therapy.

Table of content

Acknowledgement.....	2
Declaration	3
Certificate.....	4
Abstract.....	5
List of Tables	10
List of figures.....	11
List of Abbreviations... ..	15
CHAPTER 1 INTRODUCTION	17
Chapter 2 Review of Literature.....	28
2.1 Anoikis Resistance in Cancer Progression.....	28
2.2 Molecular Mechanisms of Anoikis Resistance	28
2.3 Role of EMT in Anoikis Resistance	29
2.4 Interplay Between Autophagy, Apoptosis, and Anoikis	29
2.5 Therapeutic Targeting of Anoikis Resistance.....	34
2.6 Computational Approaches in Cancer Research	34
Chapter 3 Finding key targets of anoikis resistance	36
3.1 Rationale of the study	36
3.1 Methodology	37
3.1.1 Assortment and analysis of data	37
3.1.2 Gene expression profiling.....	37
3.1.3 Data collection for differential gene expression analysis	37
3.1.4 Identification of total differentially expressed genes	37
3.1.5 Identification of common differentially expressed genes.....	37
3.1.6 Enrichment analysis	38
3.2 Results and discussion	38
3.2.1 Intersection and cross talks between key genes in multiple cancer related pathway.....	38
3.2.2 Study of anoikis resistance related genes for understanding the role of genes for regulation of anoikis	41
3.2.3 Biological process affected by common DEGs and their influence on anoikis in cancer progression	45
3.2.4 Biological pathways associated with anoikis and anoikis resistance and their roles.....	48
Chapter 4 Identification of natural compounds for modulation of anoikis-resistance mediators	50
4.1 Rationale of the study	50

4.2 Methodology	51
4.2.1 Screening phytochemicals against the tumor-associated molecule-Ligand and protein preparation	51
4.2.2 Molecular docking and MD Simulation, MM/PBSA and MM/GBSA calculation	52
4.2.3 Differential gene expression of Dioscin	53
4.2.4 Studying the impact of bioactive natural compounds on differentially expressed genes.....	53
4.2.5 Screening of bioactive natural compounds	53
4.2.6 Analysis of combinatorial natural compound formulation	53
4.3 Results and discussion	54
4.3.1 Targeting Bcl-2 as a key pivot for apoptosis and anoikis	54
4.3.2 Molecular dynamics Simulation for structural and binding dynamics analysis	61
4.3.3 MM/GBSA and MM/PBSA for energy calculations for the protein-ligand complex	63
4.3.4 Root-mean square deviation (RMSD) and RMSF analysis for studying conformational changes of the protein-ligand complexes.....	64
4.3.5 Radius of gyration.....	65
4.3.6 Hydrogen bond formations between Bcl-2 complexes	66
4.3.7 Statistical analysis of gene expression microarray data to assess the effect of Dioscin on common genes among the differentially expressed genes across various tumor samples	68
4.3.8 Effect of natural compounds on gene expression regulation of the differentially expressed genes in anoikis resistance	70
4.3.9 Combinatorial effect of multiple natural compounds on anoikis regulation	75
CHAPTER 5 In-vitro analysis of natural compound mediated impact on anoikis-resistance cell line	80
5.1 Rationale of the study	80
5.2 Methodology	81
5.2.1 Studying effect of natural compounds on anoikis resistance-associated genes at different time intervals	81
5.2.2 Gold nanoparticle synthesis	81
5.2.3 Drug-Nanoparticle conjugation	81
5.2.4 Drug-release over time	82
5.2.5 MTT Assay	82
5.3 Result and Discussion	83
5.3.1 Natural compound effecting anoikis resistance-associated genes at different time intervals	83
5.3.2 Gallic acid and stage specific regulation of cell death.....	85
5.3.3 Gold Nanoparticle for drug delivery- Formation, Size and stability of gold nanoparticles and GA-AuNP	87

5.3.4 SEM Analysis	88
5.3.5 Gallic acid loading dynamics	89
5.3.6 Analysis of size distribution by intensity using Dynamic light scattering	91
5.3.7 Fourier transform infrared spectroscopy (FTIR) Analysis.....	94
5.3.8 Drug Release Profiles.....	96
5.3.9 Drug release kinetics.....	97
5.3.10 Time and dose dependent effect of gallic acid- In vitro study.....	99
5.3.10.1 Time and dose-dependent effect of AuNP.....	99
5.3.10.2 GA-AuNP exhibits a dose- and time-dependent effect.....	100
Chapter 6 Conclusion	103
References.....	107

List of Tables

TABLE 2.1 EXAMPLES OF GENES AND SIGNALLING PATHWAYS THAT ARE COMMON AMONG AUTOPHAGY, ANOIKIS AND APOPTOSIS	30
TABLE 4.1 BIOACTIVE COMPOUNDS WITH TOP DOCKING SCORE	54
TABLE-4.2(A) HYDROPHOBIC INTERACTIONS BETWEEN BCL-2 AND ABT-737(CONTROL)	56
TABLE 4.2(B) HYDROGEN BONDS BETWEEN BCL-2 AND ABT-737 (CONTROL)	57
TABLE 4.2 (C) π - STACKING INTERACTIONS BETWEEN BCL-2 AND ABT-737 (CONTROL)	58
TABLE 4.3 (A) HYDROPHOBIC INTERACTIONS BETWEEN BCL-2 AND DIOSCIN	59
TABLE 4.3(B) HYDROGEN BONDS BETWEEN BCL-2 AND DIOSCIN	60
TABLE 4.3(C) π - STACKING INTERACTIONS BETWEEN BCL-2 AND DIOSCIN	61
TABLE 4.4(A) BINDING ENERGY CALCULATIONS OF BCL-2 AND DIOSCIN COMPLEX	64
TABLE 4.4(B) BINDING ENERGY CALCULATIONS OF BCL-2 AND ABT-737 COMPLEX	64
TABLE 4.5(A): GENE EXPRESSION REGULATION BY DIOSCIN	70
TABLE 4.6(A) CHANGE IN GENE EXPRESSION OF THE DOWNREGULATED GENES BY NATURAL COMPOUNDS	74
TABLE 4.6(B) CHANGE IN GENE EXPRESSION OF UP-REGULATED GENES BY NATURAL COMPOUNDS	75
TABLE 5.1 EFFECT ON EXPRESSION LEVELS (LOGFC VALUE) OF 53 COMMON GENES BY GALLIC ACID AT DIFFERENT TIME POINTS	85
TABLE 5.2 CUMULATIVE RELEASE PROFILE OF GALLIC ACID (GA) LOADED ON GOLD NANOPARTICLES (AUNP) IN PBS OVER TIME	99
TABLE 5.3 HALF MAXIMAL INHIBITORY CONCENTRATION (IC ₅₀)	102

List of Figures

FIGURE 3.2 THE COMMON GENES BAX, BCL2, IKKA (CHUK), AND BIRC3 ARE INVOLVED IN CROSS-TALKS BETWEEN NON-CANONICAL AND CANONICAL PATHWAY NF-KB, TNF, AND FAS RECEPTOR PATHWAY. T BARS INDICATE INHIBITION, WHEREAS ARROWS INDICATE ACTIVATION.	39
FIGURE 3.3 NETWORK OF CONNECTIONS BETWEEN COMMON GENES BCL-2, BAX, BIRC3 AND CHUK.....	40
FIGURE 3.4 EXPRESSION LEVELS OF BCL-2 IN DIFFERENT TUMORS AND THEIR PAIRED NORMAL SAMPLES. BRCA-BREAST INVASIVE CARCINOMA, CHOL-CHOLANGIO CARCINOMA, DLBC - DIFFUSE LARGE B CELL LYMPHOMA, ESCA- OESOPHAGEAL CARCINOMA, KICH-KIDNEY CHROMOPHOBE, KIRC-KIDNEY RENAL PAPILLARY CELL CARCINOMA, LAML-ACUTE MYELOID LEUKEMIA, LGG-BRAIN LOWER GRADE GLIOMA PAAD-PANCREATIC ADENOCARCINOMA, PCPG-PHEOCHROMOCYTOMA AND PARAGANGLIOMA, SARC-SARCOMA, SKCM-SKIN CUTANEOUS MELANOMA, STAD-STOMACH ADENOCARCINOMA, THYM-THYMOMA.....	41
FIGURE 3.5 DATASET: GSE100629, ANOIKIS RESISTANT VS NON ANOIKIS RESISTANT PCA CELLS. LEFT GRAPH IS A BOX PLOT SHOWING DISTRIBUTION OF EXPRESSION LEVELS IN TEST CONTROL SAMPLES WHILE GRAPH ON RIGHT SHOWS VOLCANO PLOT ILLUSTRATING DIFFERENTIALLY EXPRESSED GENES BETWEEN TEST AND CONTROL SAMPLES IN THE DATASET. GENES WITH $1 < -\text{LOGFC} < -1$ AND $P\text{-VALUE} < 0.05$ WERE CONSIDERED STATISTICALLY SIGNIFICANT.....	42
FIGURE 3.6: DIFFERENTIAL GENE EXPRESSION ANALYSIS OF GSE106363 DATASET, LEFT PANEL: BOXPLOT. REPRESENTED ARE GENE EXPRESSION LEVELS FOR TEST, GREEN, AND CONTROL, PURPLE, SAMPLES FOR THE SIX DIFFERENT GSM IDENTIFIERS (GSM2836597 THROUGH GSM2836600). BOXES CORRESPOND TO THE INTERQUARTILE RANGE WITH A BLACK LINE AT THE MEDIAN AND WHISKERS EXTENDING TO 1.5 TIMES THE IQR VALUES.	42
FIGURE 3.7 DATASET: GSE46602, PROSTATE CANCER CELLS AND ADJACENT NORMAL PROSTATE CELL. THE BOX PLOT ON LEFT REPRESENTS THE DISTRIBUTION OF GENE EXPRESSION LEVELS IN TEST AND CONTROL SAMPLES FOR THIS DATASET. VOLCANO PLOT ON RIGHT SHOWS DEGS BETWEEN TEST AND CONTROL SAMPLES IN THIS DATASET. GENES WITH $1 < -\text{LOGFC} < -1$ AND $P\text{VALUE} < 0.05$ ARE CONSIDERED STATISTICALLY SIGNIFICANT.....	43
FIGURE 3.8 WORK FLOW DEMONSTRATING BRIEF METHODOLOGY AND STEPS INVOLVED IN PROCESSING OF DATA AND CLASSIFYING DIFFERENTIALLY EXPRESSED GENES.....	44
FIGURE 3.9 THE DATA PROCESSING AND FILTERING OF ALL 3 DATASETS IN PCA. THE TOTAL NUMBER OF UPREGULATED GENES WAS 2927 AND THE TOTAL NUMBER OF DOWNREGULATED GENES THAT WERE OBTAINED WAS 3831, FROM ALL THREE DATASETS TOGETHER. WHILE, THE TOTAL NUMBER OF GENES THAT WERE COMMON AND DOWNREGULATED WERE 34, TOTAL COMMON UPREGULATED GENES WERE 19.	44
FIGURE 3.10 FOLLOWING FIGURE DEMONSTRATE THE GENE ENRICHMENT ANALYSIS OF 19 COMMON UPREGULATED GENES AND 34 COMMON DOWNREGULATED GENES RELATING TO BIOLOGICAL PROCESSES.....	46
FIGURE 3.11 INVOLVEMENT OF THE DEGS IN PATHWAYS LIKE EMT-ASSOCIATED PATHWAYS,	

TRK-SIGNALLING (NEUROTROPHIC FACTOR-MEDIATED TRK RECEPTOR SIGNALLING), TGF- B) SIGNALLING, MTOR (MAMMALIAN TARGET OF RAPAMYCIN) SIGNALLING, PI3K/AKT (CLASS I PI3K SIGNALLING.....	49
FIGURE 4.1 DOCKED STRUCTURE OF BCL-2 IN COMPLEX WITH ABT-737, ILLUSTRATING KEY INTERACTIONS 2D (LEFT AND 3D (RIGHT) IDENTIFIED USING BIOVIA DISCOVERY STUDIO.	57.
FIGURE 4.2 DIOSCIN AND BCL-2 COMPLEX AFTER MOLECULAR DOCKING, PERTINENT INTERACTIONS BY AMINO ACID RESIDUES WITH LIGAND ATOMS.....	60
FIGURE 4.3 3D STRUCTURE SHOWING BCL-2 AND DIOSCIN COMPLEX BOUND TO EACH OTHER	61
FIGURE 4.4 RESIDUES INTERACTIONS OF NATURAL COMPOUND DIOSCIN AND ANTIAPOPTOTIC PROTEIN BCL-2 DURING MDS. PROTEIN IS REPRESENTED BY BLUE COLOUR AND LIGAND IS REPRESENTED IN YELLOW COLOUR	62
FIGURE 4.5 RESIDUES INTERACTIONS OF THE REFERENCE MOLECULE (CONTROL) ABT-737 AND ANTIAPOPTOTIC PROTEIN BCL-2 DURING MDS. PROTEIN IS REPRESENTED BY BLUE COLOUR AND LIGAND IS REPRESENTED IN YELLOW COLOUR.....	62
FIGURE 4.6 RMSD GRAPH REPRESENTING THE COMPARATIVE ANALYSIS OF ABT-737 AND DIOSCIN WITH RESPECT TO THEIR BINDING TO THE BCL-2 PROTEIN DURING MD SIMULATIONS. THE MD SIMULATION'S ENTIRE RUNNING TIME IS DEPICTED ON THE X-AXIS IN NANOSECONDS. THE RMSD VALUES ARE DISPLAYED ON THE Y-AXIS IN ANGSTROMS (Å).	64
FIGURE 4.7 RMSF GRAPH REPRESENTING THE COMPARATIVE ANALYSIS OF DIOSCIN AND ABT- 737 CONCERNING THEIR INTERACTIONS WITH THE BCL-2 PROTEIN DURING MOLECULAR DYNAMICS (MD) SIMULATIONS. THE RMSF VALUES FOR BOTH COMPOUNDS ARE CALCULATED, PROVIDING VALUABLE INSIGHTS INTO THEIR DYNAMIC BEHAVIOUR AND FLEXIBILITY AT THE ATOMIC LEVEL WITHIN THE BINDING SITE	65
FIGURE 4.8 THE RESULTS OF THE RADIUS OF GYRATION (RG) ANALYSIS FOR THE SCREENED BCL-2 SYSTEMS DURING THE 100 NANOSECOND MOLECULAR DYNAMICS (MD) SIMULATION ARE SHOWN IN THIS FIGURE. THE Y-AXIS SHOWS THE RG VALUES IN NANOMETRES (NM), WHILE THE X-AXIS SHOWS THE SIMULATION TIME IN NANOSECONDS (NS).....	66
FIGURE 4.9 A) H-BONDS FORMED BETWEEN ABT-737(CONTROL) AND BCL-2 PROTEIN FORMED DURING ENTIRE MD SIMULATION. PERCENTAGE OF H-BOND IS REPRESENTED ON THE Y- AXIS WHILE AMINO ACID RESIDUE INVOLVED IN THE H-BOND FORMATION IS REPRESENTED ON THE X-AXIS. ASPARAGINE (ASN140) AND GLYCINE (GLY142) DISPLAYED HIGHEST PERCENTAGE(44.5% AND 50.25% RESPECTIVELY) OF H-BOND FORMATION DURING MD SIMULATION.....	67
FIGURE 4.9 B) H-BONDS FORMED BETWEEN DIOSCIN AND BCL-2 PROTEIN FORMED DURING ENTIRE MD SIMULATION. % OF H-BOND IS REPRESENTED ON THE Y-AXIS WHILE AMINO ACID RESIDUE INVOLVED IN THE H-BOND FORMATION IS REPRESENTED ON THE X-AXIS. AROMATIC AMINO ACID TRYPTOPHAN REPRESENTS HIGHEST PERCENTAGE (97.4%) OF H- BONDS FORMED DURING MD SIMULATION, WHILE ARGININE (ARG143) BEING THE SECOND HIGHEST (22.6%) OF IT	67
FIGURE 4.10 DOT PLOT (LEFT) AND BOX PLOT (RIGHT) ILLUSTRATING THE NORMALIZATION	

AND CATEGORIZATION OF DATA POINTS, REPRESENTING DIFFERENTIALLY EXPRESSED GENES	69
FIGURE 4.11 EFFECT OF GASTRODIN, NITIDINE CHLORIDE, CDCA AND BILOBALIDE ON COMMON DIFFERENTIALLY EXPRESSED GENES. GASTRODIN ALONE REGULATED THE HIGHEST NUMBER OF GENES WHILE BILOBALIDE ALONE REGULATED MINIMUM NUMBER OF GENES. THERE WERE 5 GENES WHICH WERE NOT REGULATED BY ANY COMPOUND ALONE.	71
FIGURE 4.12 EACH BAR IN THE GRAPH REPRESENTS THE FOLD CHANGE IN GENE EXPRESSION LEVELS UPON TREATMENT WITH THE RESPECTIVE NATURAL COMPOUND. POSITIVE VALUES INDICATE UPREGULATION, WHILE NEGATIVE VALUES SIGNIFY DOWNREGULATION. THE DIFFERENTIAL EXPRESSION PATTERNS HIGHLIGHTED IN THIS GRAPH UNDERScore THE POTENTIAL OF THESE BIOACTIVE NATURAL COMPOUNDS IN MODULATING ANOIKIS RESISTANCE, THEREBY A PROMISING AVENUE FOR THERAPEUTIC INTERVENTION IN CANCER TREATMENT	71
FIGURE 4.13 THE VENN DIAGRAM ILLUSTRATES HOW VARIOUS BIOACTIVE NATURAL COMPOUNDS REGULATE THE GENE EXPRESSION OF 53 COMMON DEGS. AMONG ALL THE GENES, EIGHT GENES WERE REGULATED BY ALL 4 COMPOUNDS.	77
FIGURE 4.14 EFFECT OF THE 4 NATURAL COMPOUNDS ON DIFFERENT PATHWAYS AND TUMOR MARKERS. THIS DIAGRAM SUMMARIZES GENES REGULATED BY GASTRODIN, NITIDINE CHLORIDE, CDCA AND BILOBALIDE, AND THE BIOLOGICAL PATHWAYS AND PROCESS THEY ARE ASSOCIATED WITH.	78
FIGURE 5.1 DEMONSTRATION OF WORK FLOW FOR ANALYSATION OF EFFECT BIOACTIVE NATURAL COMPOUND ON 53 COMMON DEGS.....	83
FIGURE 5.2 EACH BAR IN THE GRAPH REPRESENTS THE FOLD CHANGE IN GENE EXPRESSION LEVELS AT EACH TIME POINT COMPARED TO THE UNTREATED GENES. POSITIVE VALUES INDICATE UPREGULATION, WHILE NEGATIVE VALUES SIGNIFY DOWNREGULATION. THE DIFFERENTIAL EXPRESSION PATTERNS ACROSS THESE TIME POINTS UNDERScore THE REGULATORY INFLUENCE OF GALLIC ACID ON ANOIKIS RESISTANCE-ASSOCIATED GENES PROVIDING VALUABLE INSIGHTS INTO ITS THERAPEUTIC POTENTIAL IN OVERCOMING CANCER CELL RESISTANCE.....	86
FIGURE 5.3(A) ABSORBANCE SPECTRA (GAUSSIAN CURVE) OF GOLD NANOPARTICLES, GOLD NANOPARTICLE TYPICALLY ABSORBS AT 400-500 NM	88
FIGURE 5.3(B)SCANNING ELECTRON MICROSCOPE (SEM) IMAGE REVEALING THE SURFACE MORPHOLOGY AND SIZE OF GOLD NANOPARTICLES SYNTHESIZED THROUGH THE CITRATE REDUCTION METHOD	89
FIGURE 5.4 UV-VIS ANALYSIS OF GALLIC ACID-GOLD NANOPARTICLE COMPLEX. COMPARED TO 0.5 MM GALLIC ACID SOLUTION (212-250 NM), THE SUPERNATANT SHOWED: SMALLER ABSORPTION AT 280-300 NM AND ANOTHER PEAK BETWEEN 320-370 NM	90
FIGURE 5.5 THE TIME-DEPENDENT DRUG LOADING PROFILE OF GALLIC ACID ONTO GOLD NANOPARTICLES. THE GRAPH SHOWN PROVIDES THE TIME COURSE INVARIANCES OF GALLIC ACID DRUG LOADING ONTO GOLD NANOPARTICLES. DIFFERENT COLOURED LINES ARE OBTAINED WITH THE CUMULATIVE AMOUNT OF GALLIC ACID LOADED AT VARIOUS TIMES MEASURED AGAINST THE DIFFERENT TIME POINTS IN HOURS, MEASURED EVERY 2	

HOURS. THE INITIAL LOADING COULD HAVE BEEN DEPENDENT ON CERTAIN ASPECTS SUCH AS AVAILABILITY OF ADSORPTION SURFACE AREA AND AFFINITY OF GALLIC ACID TOWARDS AUNP SURFACES. IN ALL CASES, THE CUMULATIVE GALLIC ACID LOAD INCREASED WITH TIME TO REACH A MAXIMUM OF 1.900 ML, AN INDICATION OF EFFECTIVE AUNP ADSORPTION ABILITY FOR DRUG MOLECULES.	90
FIGURE 5.6(A) SIZE DISTRIBUTION OF AUNPS AMONG VARIOUS RECORDS. MOST OF THE NANOPARTICLES CORRESPOND TO A SIZE RANGE OF 10 NM TO 100 NM. THE PEAKS OF INTENSITY REPRESENT THE RELATIVE ABUNDANCE OF PARTICLES AROUND GIVEN SIZE RANGES.	91
FIGURE 5.6(B) SIZE DISTRIBUTION BY INTENSITY OF GALLIC ACID-LOADED GOLD NANOPARTICLES. THE DLS GRAPH SHOWS THE SIZE DISTRIBUTION BY INTENSITY FOR GALLIC ACID-LOADED GOLD NANOPARTICLES (RECORDS 2023: B1, 2024: B2, AND 2025: B3). THE PEAKS IN SIZE DISTRIBUTION VARY, INDICATING DIFFERENT RANGES OF NANOPARTICLE SIZES COMPARED TO FREE GOLD NANOPARTICLES. THIS DISTRIBUTION SUGGESTS SUCCESSFUL LOADING OF GALLIC ACID ONTO THE GOLD NANOPARTICLES, AFFECTING THEIR SIZE CHARACTERISTICS.	92
FIGURE 5.7 FTIR SPECTRA FOR CITRATE-CAPPED GOLD NANOPARTICLES, GALLIC ACID, AND GALLIC ACID-LOADED GOLD NANOPARTICLES. CHARACTERISTIC PEAKS FOR GALLIC ACID WERE OBSERVED, WHICH THEN SHIFTED OR VARIED IN INTENSITY UPON LOADING ONTO GOLD NANOPARTICLES, THUS PROVING THAT GALLIC ACID HAS INTERACTED AND BOUND WITH THE NANOPARTICLE SURFACE. THE STRETCHING PEAKS OF PHENOLIC O-H AND C=O IN GA-AUNPS FURTHER PROVED THE CONJUGATION OF GALLIC ACID ONTO THE GOLD NANOPARTICLES.	95
FIGURE 5.8 LINEAR REGRESSION ANALYSIS OF THE CUMULATIVE RELEASE OF GALLIC ACID FROM GOLD NANOPARTICLES (GA-AUNPS) WITH TIME. A GRAPH TO SHOW VERY STRONG LINEARITY, AS INDICATED BY $R^2 = 1$. THE RELATED GRAPH SHOWS A CONSISTENT AND CONTROLLED DRUG RELEASE PROFILE. THE SLOPE OF THE LINEAR REGRESSION (211.73913) DEPICTS THE RATE AT WHICH GALLIC ACID RELEASE OCCURRED, AND THE MINIMAL RESIDUAL SUM OF SQUARES (2.40445E-26) FURTHER CHECKS ACCURACY OF THE MODEL FIT	98
FIGURE 5.9(A) TIME- AND DOSE-DEPENDENT CYTOTOXIC EFFECTS OF GALLIC ACID ON CANCER CELL VIABILITY AS STUDIED BY MTT ASSAY.	99
FIGURE 5.9(B) THIS GRAPH ILLUSTRATES THE TIME- AND DOSE-DEPENDENT EFFECTS OF GOLD NANOPARTICLES ON THE VIABILITY OF CANCER CELLS AT 12, 24, AND 48 HOURS. THE VIABILITY DECREASES PROGRESSIVELY WITH INCREASING CONCENTRATIONS OF GALLIC ACID, DEMONSTRATING ITS POTENT CYTOTOXIC EFFECTS ON THE CELLS.	100
FIGURE 5.9(C) THIS GRAPH DEPICTS THE PERCENTAGE OF VIABLE CANCER CELLS FOLLOWING TREATMENT WITH VARIOUS CONCENTRATIONS OF GALLIC ACID LOADED NANOPARTICLE OVER 12, 24, AND 48 HOURS. THE DATA SHOW A SIGNIFICANT REDUCTION IN CELL VIABILITY WITH INCREASING CONCENTRATIONS OF GALLIC ACID AND LONGER EXPOSURE TIMES, HIGHLIGHTING THE POTENT CYTOTOXIC EFFECTS OF GALLIC ACID.	101

List of Abbreviations

Bcl2	B Cell Lymphoma 2
Bax	Bcl2 2 Associated X Protein
BIRC3	Baculoviral IAP Repeat Containing 3
CDCP1	Cub Domain Containing Protein 1
CHUK	Conserved Helix Loop Helix Ubiquitous Kinase
EMT	Epithelial To mesenchymal transition
FLICE	Fibroblast Growth Factor
IKKB	Ik Kinase B
MAPK	Mitogen Activated Protein Kinase
Mcl	Myeloid Cell Leukemia Sequence 1
PI3/AKT	Phosphoinositide 3 Kinase/Protein Kinase
DEG	Differentially Expressed Genes
PCA	Prostate Cancer
ECM	Extracellular Matrix
MTT	3 (4,5 Dimethylthiazol 2 Yl) 2,5 Diphenyltetrazolium Bromide
AuNP	Gold Nanoparticle
CTC	Circulating Tumor Cells
PC3	Prostate Cancer Cell Line
GEO	Gene Expression Omnibus
FunRich	Functional Enrichment Analysis Tool
GEO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
GEPIA2	Gene Expression Profiling Interactive Analysis
TCGA	The Cancer Genome Atlas
Apaf	Apoptotic Protease Activating Factor 1
FasL	Fas (Factor associated suicide) Ligand
FADD	Fass Associated Death Domain Protein
TLR	Toll Like Receptor
TRAF	Tnf Receptor Associated Factor
mTOR	Mechanistic Target of Rapamycin
SSTR1	Somastatin Receptor 1
CAV2	Caveolin 2
RhoA	Ras Homolog Family Member A
RMSD	Root Mean Square Deviation
DMSO	Dimethyl Sulfoxide
RMSF	Root Mean Square Fluctuation
DMEM	Dulbacco's Modified Eagle Medium
CID	Compound Identifier
DLS	Dynamic Light Scattering

FTIR	Fourier Transform Infrared Spectroscopy
MDS	Molecular dynamics Simulation
MM/GBSA	Molecular Mechanics Generalized Vorn Surface Are
MM/PBSA	Molecular Mechanics Poisson Boltzmann Surface Area
MCF 7	Michigan Cancer Foundation 7
PBS	Phosphate Buffered Saline
IC50	Minimal Inhibitory Concentration
CDC42	Cell Division Cycle 42
Trk	Tropomyacin Receptor Kinase
ATG	Autophagy Related
CIAPS	Cellular inhibitor of apoptosis

CHAPTER 1 INTRODUCTION

Cancer is linked to a higher risk of death, poor health, and costly healthcare. It is essential to comprehend cancer's onset and progression in order to design strategies that can be used to improve cancer patients' health and well-being (Miller et al., 2019). The cellular environment, signaling pathways, immune responses, and chemicals involved in tumor development and regression must all be studied in order to design effective cancer therapies. It is well-known that interactions between malignant and nonmalignant cells create a tumor microenvironment, which influences cancer genesis and progression (Arneth, 2020). The tumor microenvironment is made up of immune cells, blood vessels, ECM (extracellular matrix), lymphocytes, fibroblasts, signaling chemicals, and bone marrow-derived inflammatory cells (Spill et al., 2016) (Del Prete et al., 2017). Endothelial cells are the initial component of the tumor microenvironment, and they hold a crucial role in immune system defense and tumor formation (Baghban et al., 2020). The angiogenic vessels formed in tumors are generally produced from endothelial progenitor cells or branch outwards from preexisting capillaries. In this way, endothelial cells offer nutritional support for the development and growth of tumors. Cells of the immune system such as lymphocytes, macrophages, and granulocytes make up the other significant components of the immune system (Hanahan & Weinberg, 2011) (Del Prete et al., 2017). These immune cells participate in a wide range of immunological responses and activities, including inflammatory reactions that the tumor regulates to help it survive (Denk & Greten, 2022). Cancer stem cells are produced by angiogenesis and the presence of immune cells in the tumor microenvironment, and they also form a complicated cellular signaling environment (Jahanban-Esfahlan et al., 2019). Additionally, cancer stem cells function as tumor-initiating cells, accelerating the spread of cancerous cells and causing treatment resistance by a variety of intrinsic mechanisms, including genetic and epigenetic alterations (Arneth, 2020) (Bayne et al., 2012). Dysregulated autophagy, apoptosis, and anoikis are often intrinsic outcomes that assist cancer growth within the tumor microenvironment (Ferraresi et al., 2020) (Safa, 2022) (Nepali & Kyprianou, 2023). Autophagy is a cellular process in which cellular components are degraded and recycled (X. Li et al., 2020). It is a critical process for cellular homeostasis and is required for cell survival, growth, development, and general health (Poillet-Perez et al., 2021). Perturbations in autophagy can occur due to reasons such as a mutation in autophagy-related (ATG) genes like the gene ATG2, ATG5, ATG9, and UVRAG (UV radiation resistance-associated gene protein) due to microsatellite instability caused by disturbed DNA mismatch repair (X. Li et al., 2020). In addition, during cell stress, the number of autophagosomes tends to increase, which leads to upregulated autophagy (X. Yin et al., 2019). Upregulated autophagy results in enhanced cell growth rates resulting in tumor expansion. Conditions like nutrition deprivation and tumor therapies (e.g. chemotherapy) lead to stress-induced autophagy which can lead to cancer tumor cell dormancy, multiple drug resistance, etc. during tumor therapies (White & DiPaola, 2009) (M. Liu et al., 2018). Stress-induced autophagy gives rise to cancer cell dormancy, it comes with the possibility of resumed tumor cell growth later once the stress factor has been removed from the microenvironment of the cells. Therefore, autophagy mediators are central in tumor therapy because they can work in different manners, they can suppress tumor formation or promote it depending on the tumor microenvironment and cellular environment (Yun & Lee, 2018) (T. Luo et al., 2016). When the autophagic and apoptotic pathways are inactivated irregularly the cells can become immortal, for instance, the gene ATG5 along with ATG12 has a role in autophagosome formation (X. Li et al., 2020). The formation of autophagosomes heavily relies on the ATG5 and ATG12 genes, which are integral in assembling a significant protein complex (Walczak & Martens, 2013). The ATG5 gene codes for the Atg5 protein, while the ATG12 gene produces the Atg12 protein (Badadani, 2012). By combining the Atg12 and Atg5 proteins, a critical component of

autophagy machinery the Atg12-Atg5 conjugate is formed, which is essential in the elongation and closure of the autophagosomal membrane(Walczak & Martens, 2013)(Walczak & Martens, 2013). The Atg12-Atg5 conjugate works as an E3-like ligase, making it easier for Atg8 family proteins to bind to phosphatidylethanolamine (PE) on the autophagosomal membrane, such as LC3(Microtubule-associated protein 1A/1B-light chain 3) in mammals(Walczak & Martens, 2013)(Y.-K. Lee & Lee, 2016). The formation of autophagosomes is influenced by this step. Upon apoptotic stimulus, Atg5 protein is cleaved and translocated to mitochondria and subsequently, caspase activation and subsequent cell death take place(D. Chen et al., 2012). ATG5 gene activation sensitizes cells to therapy and the silencing of the same can result in resistance to therapy-induced responses(Rouschop et al., 2010). On the contrary, elevated autophagy can support tumor progression by providing nutrition to tumor cells in nutrient deprived circumstances. When the cells are undernourished, increased autophagy enables cells for protein degradation and cellular components to acquire a way to receive or consume macromolecules that are not available in a regulated environment. Thus, in tumor conditions enhanced autophagy can contribute to tumor progression (Badadani, 2012)(Q. Chen et al., 2018)

Anoikis is a mechanism which if not tightly regulated, can support tumor cell survival and proliferation. Anoikis is an apoptotic cell death caused by a cell's lack of adhesion to neighboring cells and extra cellular matrix (ECM) (Ayla & Karahüseyinoğlu, 2019) (Sattari Fard et al., 2023a). Anoikis can be studied experimentally by separating cells from the surrounding cells and cell-matrix. Changes in autophagy and apoptotic pathways enhance cancer spread by disrupting the normal regulation of anoikis. Cells within a simple epithelium can be extruded due to contraction of neighboring cells, and when ECM-detached epithelial cells suffer a number of metabolic abnormalities like lower ATP levels and higher ROS (Reactive oxygen species) levels. Due to the absence of ECM attachment, the ejected cells undergo anoikis. These findings contribute to the physiological context in which anoikis plays a critical role in tissue homeostasis. Therefore, it was understood that anoikis plays the role of providing imperative protection to tumor cells by hindering cells to come in contact and attach to new matrices in unfitting sites after they have lost anchorage and thus preventing dysplastic growth (Corkery et al., 2018)(Schwartz, 2010). EMT (Epithelial to mesenchymal transition) is a major factor that affects anoikis. EMT is characterized by a decline in epithelial markers and a rise in mesenchymal marker proteins such as fibronectin, vimentin, catenin, and E cadherin. Failed function of such mesenchymal markers give rise to cell dedifferentiation, and the cells become phenotypically mesenchymal which is observed by a rise in invasion capability in the cell(Y. N. Kim et al., 2012). EMT abide by a reversible route that has a vital part during wound healing and embryonic development (Barriere et al., 2015). It obliges cells to pass through several checkpoints for cellular regulations. A tumor cell can become anoikis resistant through EMT, which leads to advancement of tumor (Y. N. Kim et al., 2012). EMT can control anoikis indirectly as it regulates the key receptors which mediate interaction from cell to cell. The same key receptors which mediate interaction from cell to cell deliver pro-survival signals in non-tumor cells when they are accurately attached and have maintained contact and signaling with adjoining cells of the cellular matrix(Y. N. Kim et al., 2012). Both EMT and anoikis can result in the activation of signaling pathways that encourage cell death due to the breakdown of cell-to-cell connections and loss of contact with the cellular matrix. However, pro-survival signals can be sent to non-tumor cells when the receptors in charge of mediating cell-cell contacts are correctly attached and maintain contact and signaling with nearby cells and the extracellular matrix(Heerboth et al., 2015). There are several signaling pathways, including those involving receptor tyrosine kinases, integrins, and cell adhesion molecules, are frequently used to transmit these pro-survival signals. These signals sustain cell viability, encourage cell survival,

and prevent apoptosis(RUSSO et al., 2013)(X. Zhong & Rescorla, 2012). The same key receptors which mediate interaction from cell to cell deliver pro-survival signals in non-tumor cells when they are accurately attached and have maintained contact and signaling with adjoining cells of the cellular matrix. Examples of such receptors are discussed herewith. FAK(Focal adhesion kinase) is a non-receptor tyrosine kinase that is essential for cell adhesion and signaling that is mediated by integrins. It serves as a key regulator of cell survival and anti-apoptotic signaling and is activated following integrin interaction with the extracellular matrix. Loss of FAK activation and subsequent induction of apoptosis can result from loss of anchoring to the extracellular matrix, as in the case of anoikis (J. Wang et al., 2022) (J. Y. Kim et al., 2017). Another such is the receptor tyrosine kinase EGFR(epidermal growth factor receptor), which is essential for cell survival, migration, and proliferation. It can be triggered by ligands secreted by nearby cells, such as EGF (epidermal growth factor). Activation of the EGFR can help cells survive and prevent them from going through an apoptosis that is brought on by the loss of anchoring through its downstream signaling pathways(Inge et al., 2011). A crucial modulator of integrin signaling, integrin-linked kinase (ILK) is a serine/threonine protein kinase that interacts with integrins. By activating several signaling pathways, such as the PI3K/AKT(phosphatidyl inositol-3 kinase) and NF- κ B(Nuclear factor kappa light chain enhancer of activated B cell) pathways, it helps cells survive and prevents apoptosis. Apoptosis can be induced when integrin-ILK signaling is disrupted, which happens during anoikis (Zoppi et al., 2018)(Stanislovas & Kermorgant, 2022)(Boppart & Mahmassani, 2019). Additionally, Notch receptors are transmembrane proteins important in tissue formation and cell-cell communication. Cell survival and apoptosis are two biological processes that are regulated by notch signaling. Loss of cell-cell connections can cause notch signaling to be disrupted, which can lead to the triggering of apoptosis in detached cells(Rocchetti et al., 2023).

Cell development, survival, and migration are all influenced by the receptor tyrosine kinase known as PDGFR (platelet-derived growth factor receptor). PDGF (Platelet-derived growth factor), which is secreted by nearby cells, has the ability to activate it. PDGFR signaling activation can help cells survive and defend them from apoptosis brought on by a lack of cell anchorage(Deng et al., 2021)(Khan et al., 2022). Changes in these EMT-regulated receptors lead to irregular anoikis. Thus, there is a pivotal interconnection between EMT and anoikis. For instance, epithelial cancers primarily occur as an organ-restricted lesion, and afterward circulate via the bloodstream to distinct organs individually, generating tumor-associated lethality (Wong et al., 2014)(Hayashida et al., 2011). Withstanding detachment(from ECM) induced cell death can facilitate growth in cells without anchorage to ECM, and tumor cells can survive while propagating, therefore such growth is evolving as a trait of metastatic malignancies (Babaei et al., 2021) (Strauss et al., 2010)(Y. N. Kim et al., 2012)(Taddei et al., 2012). Interestingly, autophagy is also co-related to anoikis as well as apoptosis. Autophagy is one of the mechanisms that is related to cell-matrix dissociation to promote survival and avoid anoikis. It has been stated that autophagy and anoikis are associated to each other in numerous ways. For example, the transcription factors ATF4 (activating transcription factor 4) and DDIT3 (DNA damage inducible transcript 3) are regulated by EIF2AK3/PERK (eukaryotic translation initiation factor 2 alpha kinase 3), which stimulate the autophagy genes LC3 and ATG5 gene in response to hypoxia (Chaurasia et al., 2019). Anoikis resistance has been associated to enhanced cell migration and EMT; autophagy also promotes anoikis resistance, and it may inhibit or promote EMT; anoikis resistance, and apoptosis as prosurvival mechanism and all these factors work together to encourage tumor cell metastasis. The association between autophagy, apoptosis, and anoikis in tumor microenvironment is not fully known yet. Dissecting the pathways and chemicals that connect anoikis, apoptosis, and autophagy is critical for wide tumor targeting, tumor therapy design, and other medical and scientific goals.

It has been found out in numerous studies that breakdown in implementing anoikis, could result in the proliferation of adherent cells at ectopic sites and cells growing without anchorage (Guadamillas et al., 2011). As defined in numerous types of cells, it may be possible that cells of diverse origin initiate various pathways that result in anoikis (Adeshakin et al., 2021). Commencement and end of anoikis takes place through pathways that terminally unite and result in the stimulation of caspase enzymes activation and DNA fragmentation (Paoli et al., 2013a). Anoikis can take either the intrinsic or extrinsic route for caspase activation. The intrinsic process of anoikis is activated when certain cellular circumstances cause mitochondrial dysfunction, whereas the extrinsic pathway is triggered by cell surface death receptors (Malagobadan & Nagoor, 2018) (Paoli et al., 2013b). The proteins playing major role in both types of pathways are proteins from the Bcl-2 (B-cell lymphoma 2) family, which fundamentally controls cell fatality (Warren et al., 2019). Proteins such as Mcl1 (myeloid cell leukemia sequence 1), Bcl-XL, and Bcl-2, are anti-apoptotic and key players in the whole molecular mechanism of anoikis (K. Tan et al., 2013). Other protein molecules like pro-apoptotic proteins, such as multi-Bcl-2 homology 3 domain proteins, Noxa, Bax, Bid, Bik, Bmf, Bim, Bad, Noxa along with Bcl-2 homology-only proteins are also involved in the anoikis molecular mechanism (Shamas-Din et al., 2013). The overexpression and suppression of these proteins has been noted in numerous types of cancer cells, which suggests that targeting these proteins to regulate their expression can suggest an anti-tumor therapy that can be clinically beneficial to control tumor metastasis (Shahar & Larisch, 2020). These proteins are involved in a variety of cancers like multiple myeloma, acute myeloid leukemia (AML), and some solid tumors such as breast, lung, and colorectal cancers (Wei et al., 2020). Overexpression of Bcl-2 and Bcl-xL can promote cancer cell survival, resistance to apoptosis and resistance to treatment and therapy (Kapoor et al., 2020). Pro-apoptotic proteins (Bax, Bid, Bik, Bmf, Bim, Bad, Noxa) are members of the multi-Bcl-2 homology 3 (BH3) domain family and play a role in promoting apoptosis (Knight et al., 2019). Different members of this protein family may be implicated in specific cancer types. For example, Bax is involved in various cancers, including colorectal, lung, and ovarian cancers, while Bid is associated with liver cancer and gastric cancer (Knight et al., 2019) (Lopez et al., 2022) (Sarosiek & Wood, 2023) (Carneiro & El-Deiry, 2020). Bim is often involved in hematological malignancies, such as leukemia and lymphoma (Zaman et al., 2014). The prime cause of lethality in cancer patients is metastasis (Taddei et al., 2012). Anoikis resistance provides a supportive environment for successful tumor metastasis by consenting cancer cell survival without attachment to their primary site and “seeding” in modest sites (Figure 1) (Simpson et al., 2008). For example, CDCP1 (CUB domain containing protein 1) phosphorylation by SFKs (Src family kinases) due to cell detachment boost metastasis in melanoma. It also directs a ‘feed-forward loop’ for sustaining amplified SFKs’ activity all through tumor progression (H. Liu et al., n.d.) (Uekita & Sakai, 2011). Another important factor FLIP (FLICE inhibitory protein) is overexpressed in the malignant cells that evade anoikis (Raeisi et al., 2022) (González-Llorente et al., 2020). Interestingly, suppressing the expression of FLIP has been found linked to sensitization of cells to anoikis in tumor growth in distal organs in mouse models corresponding to metastasis in prostate cancer (Oh & Sun, 2021). In numerous malignancies Twist protein is upregulated and helps in epithelial-to-mesenchymal transition, and Snail along with Zeb is also frequently overexpressed in tumors under metastasis (Baulida, 2017). Additionally, cadherin switch induced anoikis resistance and EMT is connected to metastasis of tumor. Cadherin switch is induced by Twist, Snail, and Zeb1 transcriptional factors, and these are activated by TGF- β and FGF (Fibroblast growth factor) EMT-inducing factors (Loh et al., 2019) (Y. N. Kim et al., 2012). Amongst N and E cadherin, the former is considered more crucial concerning malignancy of tumor and has been found encouraging cell survival and growth by inhibiting signals for apoptosis. It has been depicted in several clinical studies that N-cadherin is prompted in situ by tumors that are

aggressive(Maurizi et al., 2022)(F. van Roy, 2014). Alongside EMT and associated transcription factors irregular growth factors receptors and components of their signaling pathways correspondingly have significance in anoikis resistance related metastasis and tumorigenesis. Activation of prosurvival signaling pathways like Ras/MAPK, PI3/Akt, Rho-GTPase and NF- κ B results in anoikis resistance induced metastasis(J. Wang et al., 2022)(Adeshakin et al., 2021). From these examples it can be clinched that suppression of death signals and stimulation of pro-survival signals are linked to anoikis resistance which further helps in metastatic progression of tumor cells.

The genes Bcl-2, Bax(Bcl-2 regulated X, apoptosis regulator), CHUK(conserved helix-loop-helix ubiquitous kinase), BIRC4(Baculoviral IAP Repeat Containing 4) , BIRC3 (Baculoviral IAP Repeat Containing 3), IKK β (I κ B kinase β), Mcl-1, Bcl-xl etc have been identified as prevalent in all crucial cancer-related phenomena. The dysregulation of these genes help cancer to grow and gain resistance to treatments because of irregular EMT, anoikis (cell detachment-induced apoptosis), autophagy, and metastasis. These genes are differentially regulated in various tumor types(J.-L. Luo et al., 2005) (Suvana et al., 2019) (Moldoveanu & Czabotar, 2020) (D. Wang et al., 2016) (Paul et al., 2018). The active involvement of these common and shared genes in transcription, gene expression, and apoptosis-related pathways can either promote or impede tumor development. For example, several apoptotic pathway inhibitors like Bcl-2 anti-apoptotic proteins are overexpressed in malignancies, making it difficult to treat cancer using common methods of therapies like chemotherapy and radiation(Cavalcante et al., 2019) (Dadsena et al., 2021). The anti-apoptotic proteins are overexpressed in response to numerous disparities, including lack of growth hormones, hypoxia, ROS(reactive oxygen species) generation, and oxidative stress, and they effect the function of pro-apoptotic proteins as well(Jing et al., 2019).

Several medicinal treatments work through the ER(endoplasmic reticulum) stress pathway or the extrinsic mitochondrial pathway to encourage apoptosis in tumor cells(Sakamoto & Kyprianou, 2010a) (Paoli et al., 2013b) (Dipalo et al., 2018) (Was et al., 2019). Anoikis-resistant cells or cells proliferating on their own can undergo apoptosis via the modulation of apoptotic proteins involved in these pathways(Was et al., 2019) (Noel et al., 2020) (Zhai et al., 2021). The interplay of several cellular processes such as epithelial-to-mesenchymal transition (EMT), apoptosis, autophagy, and anoikis in the tumor microenvironment plays a critical role in determining the prognosis of cancer. EMT can decrease apoptosis and enhance the ability of tumor cells to migrate to distant organs, contributing to anoikis resistance. Autophagy, selective autophagy and mitophagy notably, can also inhibit apoptotic pathways and generate therapeutic resistance. Unfortunately, the dysregulated coordination of EMT, apoptosis, autophagy, and anoikis can result in resistance to cancer treatment. It is crucial to regulate the genes responsible for these pathways to combat the formation and spread of tumors. Deactivating anti-apoptotic proteins and increasing the expression of pro-apoptotic proteins can promote cell death in cancer cells and improve the efficacy of modern therapies. Overall, a better understanding of the molecular mechanisms that underlie these cellular processes is essential for developing effective cancer treatments. Huge variety of drugs have been designed for targeted inhibition of pro-survival proteins and genes. ABT-263(Navitoclax), for instance, is used as a Bcl-2 and Bcl-xl inhibitor because it interacts and binds to the BH3(Bcl-2 homology-3)-binding domain of Bcl-2 family proteins and causes apoptosis in a variety of tumors and tumor like conditions. Another example is ABT-199 (Venetoclax), which is a well-known Bcl-2 inhibitor (FDA Approved), that downregulates Bcl-2, and also helps avoiding the problem of thrombocytopenia, a side effect associated to navitoclax (Souers et al., 2013). Bcl-2 proteins are the ones among the groups of survival factors that stand out in drug designing. A phytochemical that may act as a strong inhibitor of anti-apoptotic protein by directly binding

to it has been found out in this study using *in silico* methods. In silico drug discovery is a computational strategy that uses computer-based approaches to identify and create prospective therapeutic candidates. It entails the use of various computational tools, algorithms, and databases to simulate and analyse molecular behaviour in order to anticipate medication efficacy and safety (Lyu et al., 2022) (S. Kumar & Das, 2023). In this study, common genes among cancer markers, and their function and association with each other was analyzed. The study begins by examining the gene data sets for EMT, metastasis, autophagy, apoptosis, and anoikis to identify common genes in these processes. The outcomes of every pathway connected to these genes are either caspase activation (apoptosis) or cell survival and proliferation (Herishanu et al., 2011) (Y. Wang et al., 2020) (Morgan et al., 2020). Thus, the study suggests that a drug that can block any one of these genes may be helpful in the treatment of cancer, as four of the selected genes are connected. Several analyses were conducted to investigate the expression of one of the short-listed genes in a number of commonly occurring cancers followed by screening of natural compounds having high binding affinity for the most commonly irregularly regulated anti-apoptotic protein. The compound with the most stable interaction was selected and analysed further by MD (molecular dynamics) simulation. This methodology allows to identify potent anti-apoptotic and pro-survival protein inhibitors in drug designing via analysing the gene expression profiles of common genes among EMT, apoptosis, autophagy and anoikis to that of genes treated with 102 bioactive natural compounds. This study highlights and provides a framework for identifying new oncogene inhibitors using a combination of target-driven and ligand-driven methods.

The dysregulation of gene expression in the tumor microenvironment has a significant impact on cancer prognosis. Dysregulated gene expression within the tumor microenvironment can suppress immune responses, allowing tumors to evade immune surveillance. This can lead to immune tolerance and immune escape, enabling tumor progression and reducing the effectiveness of immunotherapies (Dunn et al., 2002). The primary goal of treatments in cancer is to completely eliminate or destroy tumor cells while minimizing harm to normal cells. Over the years, bioactive natural compounds, natural compounds and their derivatives have shown significant promise in terms of chemotherapy and chemoprevention. The wide range of biological functions found in natural compounds offers numerous advantages over conventional chemotherapeutic agents and currently used drugs (K.-H. Lee, 2010) (H. Yuan et al., 2016) (Kingham, 2010). The utilization of natural chemicals and their combinations holds great promise in serving as powerful resources for synergistic cancer treatments (Dehelean et al., 2021) (Cragg & Pezzuto, 2016). This is due to their ability to interact with multiple biological targets associated with tumor growth, drug resistance, and metastasis (Z. Li et al., 2020). By exerting a multitargeting effect, natural compounds have the potential to enhance the efficacy of current cancer treatments and combat the development of treatment resistance (Nabizadeh et al., 2022). Most natural substances exhibit lower toxicity, cost less, and have fewer side effects compared to conventional therapies. Extensive research has meticulously examined their potential carcinogenic properties (Guilford & Pezzuto, 2008) (Seca & Pinto, 2018). With the observed drawbacks of conventional therapy, including adverse effects in chemotherapy and drug resistance, the significance of natural substances as potent anticancer agents has become more apparent (Seca & Pinto, 2018) (Nikolaou et al., 2018). Therefore, there is an urgent demand for therapies that can restore normal gene regulations without causing any side effects (K.-H. Lee, 2010) (H. Yuan et al., 2016) (Kingham, 2010). A comprehensive investigation was conducted on a collection of 102 natural compounds, utilizing diverse computational approaches, to explore their potential for treating cancer. One of the studies involved analysing differentially expressed genes in anoikis resistant prostate cancer to find out genes that can be regulated by the 102 bioactive natural compounds.

The primary approaches employed in cancer treatment involve surgery, radiation, and chemotherapy, either distinctly or in various combinations (L. Zhu et al., 2021). Unfortunately, these methods have been linked to a considerable decrease in quality of life and a high rate of illness (A. Huang et al., 2020) (Attwood et al., 2021). One of the most commonly occurring cancer is prostate cancer (PCa) affecting men globally, particularly in older age groups (De Silva & Alcorn, 2022). Prostate cancer stands as the primary reason for male cancer-related fatalities. In 2020, it was responsible for an estimated 375,000 deaths worldwide, making up around 3.8% of all cancer deaths (Y. Zhu et al., 2021). The treatment choices for prostate cancer are determined by multiple factors, including the stage of the cancer, the patient's general well-being, and individual preferences. Possible treatments encompass active surveillance (closely monitoring cancer without immediate action), surgical procedures (eg- radical prostatectomy), hormone therapy, radiation therapy, chemotherapy or immunotherapy (Kord et al., 2022) (Sekhoacha et al., 2022). The primary challenge posed by PCa is its inclination to spread to other parts of the body. This inclination is a result of specific molecular mechanisms and interactions that contribute to the invasion of nearby tissues, movement through blood vessels, and establishment of metastases at distant sites (S. S. Yadav et al., 2018). While there have been recent advancements in understanding the fundamental aspects of this complex process, several crucial elements remain inadequately comprehended. Once PCa has metastasized, it spreads to other organs such as the liver, lungs, lymph nodes, and occasionally to the brain (Vachon, 2011). It also gives rise to additional complications such as metastatic bone disease (MBD). This condition increases the risk of intractable bone pain, pathological skeletal fractures, spinal cord compression, and osteosclerotic lesions characterized by abnormal bone production (Oloruntobi Famuyiwa, 2017) (Logothetis & Lin, 2005). Moreover, metastatic PCa becomes resistant to therapy and drugs, ultimately leading to decreased survival and prostate cancer-related mortality (Leão et al., 2017) (P. Zhang et al., 2023) (Pungsrinont et al., 2021) (B. L. Sun, 2021).

The growth and survival of PCa cells rely on signals from the activated androgen receptor (AR). Therefore, a common treatment approach for PCa is androgen deprivation therapy (ADT), since these cancer cells often depend on male hormones, particularly testosterone, for their survival and growth (Pungsrinont et al., 2021). ADT works by reducing hormone levels or by blocking their effects on cancer cells. Medical ADT involves the use of medications like luteinizing hormone releasing hormone (LHRH) agonists or antagonists to suppress or block testosterone, while anti-androgens also hinder testosterone's impact. Surgical options like orchiectomy can also lower testosterone levels (Perlmutter & Lepor, 2007). Although ADT initially proves to be effective, metastatic PCa cells in due course grow into castration-resistant prostate cancer (CRPC) after an average of 18-24 months of ADT (Chandrasekar et al., 2015) (Sekhoacha et al., 2022) (Armstrong & Gao, 2015). CRPC, also known as hormone-refractory prostate cancer, refers to a stage where cancer cells adapt and become resistant to the effects of ADT, continuing to proliferate (Zong & Goldstein, 2013). Notably, the androgen axis remains crucial for the function and growth of CRPC. The development of castration resistance is marked by elevated levels of prostate-specific antigen (PSA), a tumor marker used to monitor PCa progression (Sekhoacha et al., 2022) (Ohlendorf et al., 2017). Key cellular features of CRPC include signaling through the androgen receptor and the activation of pathways associated with cell proliferation and survival (Formaggio et al., 2021).

A key factor contributing to the progression of metastatic prostate cancer is resistance to anoikis. Prostate cancer cells with metastatic potential exhibit a high concentration of intracellular reactive oxygen species (ROS) and can evade anoikis. This allows cancer cells to survive without adhesion and support like attachment to ECM, escape apoptosis, and travel through the circulatory and lymphatic systems, leading to metastasis at secondary sites

(Sakamoto & Kyprianou, 2010b) (Nepali & Kyprianou, 2023) (S. Kumari et al., 2018). In prostate cancer cells, ROS produced independently of ECM contact leads to the continuous activation of pro-survival pathways, mediated by Src kinase-dependent sustained EGFR activation. This activation leads to the degradation of pro-apoptotic proteins that play a crucial role in anoikis. Src and EGFR kinases actively contribute to resistance to anoikis (Doldi et al., 2016) (Giannoni et al., 2009). Proteins like anti-apoptotic Bcl-2, Bcl-xL, and IAPs, which are upregulated in cells resistant to anoikis, are also elevated in androgen-dependent tumors such as PCa (Nepali & Kyprianou, 2023) (Livas et al., 2020). This association directly relates to the ability of PCa to survive in an androgen-depleted environment. On the other hand, TGF- β (Tumor growth factor- β) acts as a tumor suppressor in the normal prostate and early stages of prostate cancer but acts as a promoter in advanced stages of PCa. Interestingly, Bcl-2 can suppress the apoptotic signaling of TGF- β and dihydrotestosterone (DHT), playing a crucial role in the emergence of CRPC (Castration-resistant prostate cancer) (Z. Cao & Kyprianou, 2015) (M.-L. Zhu et al., 2010) (Thompson-Elliott et al., 2021). To overcome the aggressiveness of PCa and CRPC, it is essential to manage the tumor cells in a way that makes them susceptible to agents inducing anoikis, regulatory proteins, and pathways involved in cancer cell survival, and prevents tumor initiation at secondary sites.

Currently, the popular treatment options available for metastatic PCa and CRPC involve ADT in combination with LHRH agonist-antagonist, along with taxanes and docetaxel. However, taxanes show poor outcomes, severe side effects, and resistance to treatment (Seruga et al., 2011) (Chandrasekar et al., 2015). Anoikis resistance actively contributes to CRPC and resistance to therapy. CRPC can become resistant to ADT and progress with an upsurge in serum levels of PSA despite using ADT (Sattari Fard et al., 2023a) (Nakazawa & Kyprianou, 2017). Consequently, there is a pressing demand for an improved treatment strategy to address CRPC in men. The dysregulation of gene expression in the tumor microenvironment has a significant impact on cancer prognosis. Dysregulated gene expression within the tumor microenvironment can suppress immune responses, allowing tumors to evade immune surveillance. This can lead to immune tolerance and immune escape, enabling tumor progression and reducing the effectiveness of immunotherapies (Dunn et al., 2002). The primary goal of treatments in cancer is to completely eliminate or destroy tumor cells while minimizing harm to normal cells. The utilization of natural chemicals and their combinations holds great promise in serving as powerful resources for synergistic cancer treatments (Dehelean et al., 2021) (Cragg & Pezzuto, 2016). This is due to their ability to interact with multiple biological targets associated with tumor growth, drug resistance, and metastasis (Z. Li et al., 2020). By exerting a multitargeting effect, natural compounds have the potential to enhance the efficacy of current cancer treatments and combat the development of treatment resistance (Nabizadeh et al., 2022). Most natural substances exhibit lower toxicity, cost less, and have fewer side effects compared to conventional therapies. Extensive research has meticulously examined their potential carcinogenic properties (Guilford & Pezzuto, 2008) (Seca & Pinto, 2018). With the observed drawbacks of conventional therapy, including adverse effects in chemotherapy and drug resistance, the significance of natural substances as potent anticancer agents has become more apparent (Seca & Pinto, 2018) (Nikolaou et al., 2018). Therefore, there is an urgent demand for therapies that can restore normal gene regulations without causing any side effects. In this study, a comprehensive investigation was conducted on a collection of 102 natural compounds, utilizing diverse computational approaches, to explore their potential for treating prostate cancer.

Natural compounds that we have used in this study are known for their potential anticancer properties. Among them, the polyphenolic compound gallic acid—a compound found in fruits, herbs, nuts, raisins, green tea, and vegetables—has been efficiently studied for its potential

anticancer and antioxidant effects. We have focused on the stage-specific regulation of cell death by gallic acid and its impact on expression of DEGs associated with anoikis in this study. We analysed the RNA sequencing data of the GSE158788 dataset to probe gene expression profiles of cells undergoing cell death at different time intervals upon treatment with gallic acid: 2 hours, 4 hours, 6 hours, and 9 hours. This particular part of our study aims to confirm the dynamic interaction between gallic acid and gene expression over time and to highlight its ability in surmounting the resistance mechanisms of cancer cells.

The fact that gallic acid can induce certain apoptotic events makes it a potential targeted therapeutic agent: caspase activation, free radical scavenging, downregulation of anti-apoptotic proteins, and mitochondrial release of cytochrome c. These molecular events contribute to gallic acid-induced cytotoxicity toward cancer cells but spare normal cells, hence showing great therapeutic promise with minimal side effects. This can be further articulated by providing information on the cell death stage-specific regulation of gallic acid to explore its potential as a therapeutic agent in cancer treatment. Its dynamic gene expression and molecular mechanisms underlying its anticancer effects would be understood, and further investigations on the use of gallic acid in combination therapies might open new avenues for improving treatment responses and helping to circumvent the mechanisms of resistance.

Natural compounds alone may not have enough potential for an effective and targeted drug delivery. For this purpose we conjugated gallic acid on gold nanoparticle. Nanotechnology is one of the most promising approaches to the resolution of these challenges and represents a new method of cancer diagnosis and treatment. Gold nanoparticles have gained great attention among many types of nanomaterials due to some unique physical and chemical properties, such as high surface area, biocompatibility, and ease of functionalization(Patil et al., 2023)(Georgeous et al., 2024)(Nejati et al., 2022). AuNPs can be functionalized to deliver chemotherapeutic drugs directly to tumor sites, enhancing drug accumulation in cancer cells while minimizing systemic toxicity. This targeted approach improves therapeutic efficacy and reduces adverse side effects(Siddique & Chow, 2020). AuNPs possess unique optical properties that enable their use in photothermal therapy (PTT). Upon exposure to specific wavelengths of light, AuNPs convert light energy into heat, selectively destroying cancer cells while sparing healthy tissue(Kesharwani et al., 2023). Additionally, AuNPs can enhance the effects of radiotherapy by acting as radiosensitizers, increasing the susceptibility of cancer cells to radiation-induced damage(Jain et al., 2012). AuNPs, owing to these advantages, represent an appropriate platform for the targeted delivery of several therapeutic agents, including natural compounds with anti-cancer potential.

Nanotechnology is one of the most promising approaches to the resolution of these challenges and represents a new method of cancer diagnosis and treatment. Gold nanoparticles have gained great attention among many types of nanomaterials due to some unique physical and chemical properties, such as high surface area, biocompatibility, and ease of functionalization. AuNPs, owing to these advantages, represent an appropriate platform for the targeted delivery of several therapeutic agents, including natural compounds with anti-cancer potential. Even though gallic acid exhibits very good therapeutic potential, its clinical application is greatly hampered by poor bioavailability and fast clearance from the body. Hence, a proper delivery system is a requirement in enhancing the stability, bioavailability, and targeted delivery of gallic acid.

In this work, we explored gold nanoparticles as a delivery vehicle of gallic acid to improve its in vitro therapeutic efficacy against colon cancer. The gold nanoparticles used for this study were synthesized by the citrate reduction method and characterized in size, shape, and surface properties. Gallic acid was loaded onto the synthesized AuNPs and optimized. A gallic acid

drug release profile from the AuNPs was assessed under different physiological conditions in vitro to ensure its controlled and sustained release. We performed cytotoxicity studies using the MTT assay on the human colon cancer cell line, Col-320, to evaluate the gallic acid-loaded gold nanoparticles for therapeutic potential. The MTT is a colorimetric assay that permits the measurement of metabolic activity, thus proportionately indicating cell viability and cytotoxicity. In this study, we found that gallic acid-loaded AuNPs have appreciable cytotoxicity against the Col-320 cells. This opens new dimensions in cancer therapy. This approach will not only help with enhanced bioavailability and controlled release but also reduce side effects, which eventually will help in developing more effective and safer treatments for cancer. The outcome of this study unravels the potential use of AuNPs as a delivery vehicle for natural compounds against cancer and lays the foundation for further studies in drug development.

CHAPTER 2 REVIEW OF LITERATURE

2.1 Anoikis Resistance in Cancer Progression

A conceptual model for this phenomenon is referred to as Anoikis, which functions as a specific form of programmed cell death wherein epithelial cells experience apoptosis upon detachment from the ECM(Zia et al., 2021). In instances of cancer, the inability to convey signals indicative of detachment from their designated microenvironment obstructs proper adherence to the surrounding ECM, thereby disrupting tissue homeostasis. Conversely, cancer cells acquire anoikis resistance, which enables tumor cells to endure and proliferate following their disassociation from the ECM, thereby facilitating their migratory capabilities during the metastatic process(Farahani et al., 2014)(Abdollahiyan et al., 2021). Subsequent to the development of resistance, these tumor cells can sporadically infiltrate the vascular system (a hallmark characteristic of cancer) or lymphatic vessels, enabling their translocation from the primary site to distant tissues, thereby promoting metastasis(Kepp et al., 2009). In solid tumors, exemplified by prostate cancer, the phenomenon of anoikis resistance has been recognized as a pivotal determinant facilitating the survival of tumor cells within the circulatory system and their subsequent extravasation into secondary sites(Kamarajugadda et al., 2012)(Nepali & Kyprianou, 2023). The perturbation of anoikis in malignant cells encompasses a multitude of oncogenic signalling pathways that enhance cellular viability in a suspended state, thereby carrying profound implications for the process of tumor metastasis(Grimm et al., 2022). The capacity of cancer cells to circumvent anoikis has been correlated with unfavourable prognostic outcomes and the emergence of therapeutic resistance across various malignancies, notably including breast, prostate, and pancreatic cancers.

2.2 Molecular Mechanisms of Anoikis Resistance

The phenomenon of anoikis resistance is orchestrated by an intricate assembly of molecular pathways, predominantly encompassing those implicated in apoptosis, integrin signalling, and cellular survival mechanisms such as PI3K/Akt and Ras/MAPK(Khan et al., 2022)(Adeshakin et al., 2021). Within the framework of prostate carcinoma, empirical investigations have elucidated that these signalling cascades are frequently subject to dysregulation, thereby enabling neoplastic cells to circumvent apoptosis and persist in non-anchored environments(Nepali & Kyprianou, 2023).

Central to the regulatory network governing the apoptotic apparatus, particularly the Bcl-2 protein family, are crucial determinants that mediate anoikis resistance(Y. Yu et al., 2022a). Anti-apoptotic proteins, exemplified by Bcl-2 and Bcl-xL, are often found to be overexpressed in malignant cells, thereby obstructing the activation of pro-apoptotic agents such as Bax and Bak(A. Yadav et al., 2015), (Lopez et al., 2022),(X. Zhong & Rescorla, 2012)(Paoli et al., 2013a)(Girnus & Davis, 2017). This overexpression culminates in the attenuation of mitochondrial outer membrane permeabilization (MOMP), an essential phase in the apoptotic cascade(H.-S. Choi & Baek, 2022). Investigative work by Westaby et al., 2021 underscored the pronounced overexpression of Bcl-2 in prostate cancer cell lines, revealing that its pharmacological inhibition resulted in augmented sensitivity to anoikis and a concomitant decrease in metastatic capacity.

Another significant contributor to the phenomenon of anoikis resistance is the activation of the PI3K/Akt signalling pathway, which is frequently upregulated in metastatic neoplastic cells(Toren & Zoubeydi, 2014). The phosphorylation of Akt facilitates cell survival by suppressing pro-apoptotic entities such as Bad while simultaneously activating downstream survival pathways. Research conducted by Pungsrinont et al., 2021 illustrated that the pharmacological blockade of PI3K/Akt signalling in prostate cancer experimental models was

correlated with a marked increase in anoikis sensitivity and a reduction in tumor cell dissemination.

The Ras/MAPK signalling cascade similarly plays a pivotal role in mediating resistance to anoikis, with ERK and JNK signalling promoting cellular survival and proliferation under detached conditions (Cox & Der, 2010). Aberrant activation of this signalling pathway has been associated with a multitude of malignancies, including prostate cancer, where it fosters the expression of anti-apoptotic proteins and aids in the evasion of anoikis (Fatemipour et al., 2021). This intricate interplay between apoptosis inhibitors and survival signalling pathways constitutes the molecular foundation for anoikis resistance in neoplastic cells.

2.3 Role of EMT in Anoikis Resistance

EMT represents a vital developmental program that is usurped by neoplastic cells to facilitate metastatic dissemination (Z. Cao et al., 2016). During the EMT process, epithelial cells undergo a loss of polarity and cell-cell adhesion characteristics, while simultaneously acquiring mesenchymal features, which encompass augmented migratory and invasive potentials (Thiery et al., 2009). This morphological transition is orchestrated by a complex network of transcription factors, including Snail, Slug, Twist, and Zeb1, which inhibit epithelial markers such as E-cadherin and promote mesenchymal markers like N-cadherin and vimentin (Nieszporek et al., 2019) (Usman et al., 2021). EMT has been prominently associated with anoikis resistance, as it equips cancer cells with the capacity to endure in a suspended state, which is essential for successful metastasis. Investigations conducted by S. Chen et al., 2021 elucidated that EMT transcription factors, particularly Twist, attenuate pro-apoptotic signals and enhance anoikis resistance by activating survival pathways, including NF- κ B and Akt. In the context of prostate cancer, the initiation of EMT correlates with augmented resistance to anoikis, thereby facilitating the metastatic colonization of distant organs such as bone and lymph nodes (Begemann et al., 2018).

Moreover, the EMT-induced inhibition of integrin signaling significantly contributes to anoikis resistance. Integrins serve as transmembrane receptors that mediate interactions between cells and the ECM, and their dysregulation during EMT permits cancer cells to disengage from the ECM without experiencing apoptotic cell death (Desgrosellier & Cheresh, 2010). Notably, the downregulation of integrin $\alpha 5 \beta 1$ and $\alpha v \beta 3$ has been linked to anoikis resistance in prostate cancer, thereby facilitating metastatic progression (W. Guo & Giancotti, 2004).

2.4 Interplay Between Autophagy, Apoptosis, and Anoikis

Autophagy constitutes a catabolic mechanism through which cells degrade and recycle cytoplasmic constituents, particularly in response to various stressors. Within the oncological milieu, autophagy is recognized to possess a bifunctional role, wherein it can either promote cellular survival or elicit cell death, contingent upon the specific cellular environment (G. Yu & Klionsky, 2022). Autophagy has been implicated in conferring anoikis resistance, particularly in metastatic cancer cells, where it serves to sustain energy homeostasis and alleviate oxidative stress during the process of detachment from the ECM. Empirical studies have demonstrated that the activation of autophagy-related proteins such as LC3-II and Beclin-1 enhances anoikis resistance across multiple cancer types (Long et al., 2019) (Y. Yu et al., 2022b). By employing pharmacological inhibitors of autophagy or utilizing genetic knockdown strategies targeting Beclin-1, researchers have observed a notable increase in anoikis sensitivity, thereby suggesting the protective role of autophagy in detached neoplastic cells (Dower et al., 2018a) (M. Delgado & Tesfagzi, 2014).

The relationship between autophagy and apoptosis is intricate, characterized by shared molecular regulators such as Bcl-2 that govern both processes. Bcl-2 functions to inhibit

autophagy through its binding to Beclin-1, while its overexpression fosters anoikis resistance by suppressing apoptotic pathways (Kang et al., 2011). Investigative work by Vitto et al., 2022 revealed that targeting the Bcl-2/Beclin-1 interaction reinstated autophagic flux and induced anoikis in prostate cancer cells and cancer of other organs, thereby underscoring the potential therapeutic implications of modulating autophagy in metastatic cancer. Table 2.1 summarizes the key players that coordinate control and regulate anoikis, autophagy and apoptosis.

Table 2.1 Examples of genes and signalling pathways that are common among autophagy, anoikis and apoptosis

S.No	Gene name/pathway	Role in autophagy	Role in apoptosis	Role in anoikis
1	Bcl-2	Interact with BH3 domain of Beclin-1 and downregulates autophagy mediated by Beclin-1, downregulation of Bcl-2 activates autophagy(H.-D. Xu et al., 2013), Bcl-2-Beclin-1 complex controls autophagy in ER(H.-D. Xu & Qin, 2019).	Bcl-2 prevents apoptosis by obstructing release of cytochrome c by preventing Bak/Bax oligomerization(Warren et al., 2019), preventing their permeabilization(Souers et al., 2013), and preventing the activators Bim and Bid from binding to Bak/Bax pro-apoptotic proteins(Edlich, 2018)	Helps in gaining anoikis resistance via Src-FAK pathways and E-selectin(Y. Yu et al., 2022a), increases cell survival without adhesion(Paoli et al., 2013b), counter act the function of pro-apoptotic and cell death proteins (A. Yadav et al., 2015)
2	Bax	Triggers autophagy initiation, autophagosome formation, has been shown to induce autophagy which contributes in apoptosis triggered by mitochondrial stress, has been discovered to interact with Beclin1, leading to the suppression of autophagy(Saleem, 2021)(Ojha et al., 2015).	Counteracts anti-apoptotic proteins(Lopez et al., 2022), participates in MOMP by oligomerizing with Bak which facilitates release of cytochrome c leading to activation of cascade of caspases(Wolf et al., 2022)(Edlich, 2018)(Kale et al., 2018)	Bax activation and subsequent mitochondrial outer membrane permeabilization contribute to the induction of cell death in cells that lack proper anchorage(Lopez et al., 2022), sensitizes cells to anoikis via regulating the intrinsic pathway of anoikis (X. Zhong & Rescorla, 2012)(Paoli et

				al., 2013a)(Y. N. Kim et al., 2012)
3	CHUK/IKK α	regulation of transcription factors involved in autophagy modulation, for example, transcription factor EB (TFEB) (Dong et al., 2012), inhibition of AMPK and autophagy suppression ²⁷⁸], regulation of p53 dependent autophagy, Atg16 and mTOR (X. Xu et al., 2020)(Q. Tan et al., 2020)	promotes the transcription of anti-apoptotic genes, such as Bcl-2, IAPs, FOXO3(J. Yuan et al., 2021), positively regulates apoptotic pathways of death receptors DR4/5 and DR3(Rahal et al., 2014), contributes to mitochondrial pathway of apoptosis and apoptotic DNA fragmentation (Y. Cao, 2011)	Activation of anti-apoptotic proteins such as Bcl-2 and IAPs which increases anoikis resistance by preventing detachment induced apoptosis, also downregulates pro-apoptotic proteins (H. Yu et al., 2020) (X. Li & Hu, 2021)(N. Chen & Debnath, 2013)
4	BIRC3/cIAP2	Plays crucial role in fusion of lysosomes with autophagosomes(Gradzka et al., 2018), downregulation of cIAP2 leads to blockade in both general autophagy and mitophagy (Z. Yin et al., 2016)	Inhibits caspases by binding to caspase-3 and -9 thus inhibiting apoptosis(Berthelet & Dubrez, 2013), regulates TRAF, NF- κ B and modulates NF- κ B signaling (Frazzi, 2021)(Safa, 2022)	Supports anoikis resistance by modulating NF- κ B pathway(Cetraro et al., 2022), Interacts with FAK and Src and modulates their activation (D. Wang et al., 2016)

5	BCL2L11/BIM(Bcl-2 interacting mediator of cell death)	Inhibits autophagy by interacting with Beclin-1 and mis localizing it(Dai & Grant, 2015), inhibition of BCLL11 leads to drug resistance in certain cancers due to enhanced autophagy, induces autophagy during starvation (Dower et al., 2018a)	Counteracts the function of anti-apoptotic proteins like BCL-2, Bcl-xL, Myc1 etc. (Dai & Grant, 2015), activates pro-apoptotic proteins and MOMP leading to caspase activation (Whelan et al., 2010)(N. Chen & Debnath, 2013)	Interacts with BECN-1 leading to induction of anoikis, Influences FAK signaling and integrin signaling(Dower et al., 2018a). Modulates Akt pathway and reduces Akt activity in cells leading to susceptibility to anoikis, effects PI3/Akt/mTOR pathway ,E-cadherin and β -catenin (Whelan et al., 2010)
6	BMF(Bcl2 modifying factor)	Balances the interaction among Bcl-2 and BECN1 leading to autophagy inhibition in adherent cells(Dower et al., 2018a), promotes autophagy in non-adherent cells, (M. Delgado & Tesfaigzi, 2014)	Activation of intrinsic apoptotic pathway, activates MOMP and subsequent release of cytochrome, coregulates Bcl-2 and pro-apoptotic proteins like Bim, Puma etc.(Zhi et al., 2022) (Fan et al., 2018)	Sensitizes cells to undergo anoikis by interacting with anti-apoptotic proteins Bcl-2, Bcl-xL etc. and neutralizing them(M. Delgado & Tesfaigzi, 2014), downregulation of BMF leads to anoikis resistance (Zhi et al., 2022)
7	CASP2 (Caspase-2)	Suppresses autophagy via mTOR and AMPK dependent pathway(Shalini et al., 2015), regulates the autophagy related protein AMBRA1 and ATG4 (M. Tiwari et al., 2014)(Pagliarini et al., 2012)(Betin & Lane, 2009)	Acts as an initiator caspase in intrinsic pathway of apoptosis, cleaves caspase-3, participates in MOMP and release of pro-apoptotic factors, acts as a tumor suppressor(Bouchier-Hayes & Green, 2012)	Is activated in response to apoptotic stimuli, loss of cell-matrix adhesion or disruption of adhesion signaling (Lossi, 2022)

8	CASP8 (Caspase-8)	Has dual role, cleaves Beclin-1 which leads to formation of Beclin-1-VPS34 complex thus enhancing autophagic flux(Mandal et al., 2020), inhibits ATG3 gene and LC3 leading to impairment of autophagosome formation, cleaves p62 leading to inhibition of autophagy and survival of tumor cells (M. Kumar et al., 2018)	Regulates the extrinsic pathway of apoptosis(Norman et al., 2010), activated in response to apoptotic stimuli which leads to formation of DISC and caspase-3,-9 activation , dysregulation of CASP8 can lead to promotion of tumor survival (Mandal et al., 2020)(M. Kumar et al., 2018)	Regulates the activation of anoikis(Mandal et al., 2020), impairment of CASP8 activation or downstream signaling can contribute to anoikis resistance and promote tumor cell survival under anchorage-independent conditions, downregulated CASP8 confers malignancy in cancer as well as resistance to treatment (M. Kumar et al., 2018)
9	MCL1 (Myeloid cell leukemia-1)	Interacts with Beclin-1 preventing the initiation of autophagy(Elgendy et al., 2011)	interacts with other Bcl-2 family members, such as Bcl-2 and Bcl-xL, forming heterodimers that enhance their anti-apoptotic functions, High levels of MCL1 confer resistance to apoptosis, while downregulation of MCL1 sensitizes cells to apoptotic signals(Orrenius et al., 2015), binds to pro-apoptotic proteins Bax-Bak preventing their oligomerization(Ghaemi et al., 2020)	Makes tumor cells resistant to anoikis via interactions with Cav-1,(Chunhacha et al., 2012)
10	PI3K-AKT-MTORC1	A major regulator of starvation induced autophagy and detachment induces	Negatively regulates apoptosis increasing cell survival(S. K. Roy et al., 2010)	Confers anoikis resistance via autophagy induced EMT(S.

		autophagy(Criollo et al., 2010)		K. Roy et al., 2010)(Zada et al., 2021)
11	MAPK/ERK pathway	Regulates ATG genes and controls autophagy(Braicu et al., 2022)(L.-Z. Xu et al., 2017)	Promotes apoptosis in cells lacking apoptosis, inhibits anti-apoptotic proteins(S. K. Roy et al., 2010)	Confers anoikis resistance to detached cells(P. Zhang et al., 2016)(S. K. Roy et al., 2010)
12	EIF2AK3-ROS-ATF4 pathway	Induces the expression of Becn-1 and Atg5 protein(Dower et al., 2018b)	Suppresses apoptosis (Gupta et al., 2021)	Enhances autophagy mediated anoikis-resistance(Dower et al., 2018a)
13	TNF receptor signaling pathway	Suppresses autophagy in cancer cells via NF- κ B stimulation(Z. J. He et al., 2017)(T. Niu et al., 2020)	Participates in extrinsic pathway of apoptosis and gives rise to DISC formation(Redza-Dutordoir & Averill-Bates, 2016)(Raeisi et al., 2022)	Regulates anoikis by activating the caspases and death signals(Raeisi et al., 2022)

2.5 Therapeutic Targeting of Anoikis Resistance

Given the significant role of anoikis resistance in the process of metastasis, the targeting of this phenomenon emerges as a promising therapeutic paradigm(Neuendorf et al., 2023). A variety of molecular targets have been delineated, encompassing anti-apoptotic proteins (e.g., Bcl-2, Bcl-xL), integrins, and survival pathways such as PI3K/Akt and Ras/MAPK(Z. Zhu et al., 2022)(Sattari Fard et al., 2023b). Small-molecule inhibitors directed against these targets have demonstrated potential efficacy in preclinical models, particularly in reinstating sensitivity to anoikis and mitigating the progression of metastasis(Nagaprashantha et al., 2012). Natural compounds have also attracted considerable scholarly attention due to their capacity to modulate the pathways implicated in anoikis resistance. Gallic acid, curcumin, and resveratrol have been evidenced to induce apoptosis and enhance the sensitivity of cancer cells to anoikis by targeting critical signalling molecules(Benvenuto et al., 2020)(Akhtar et al., 2020). (Jang et al., 2020)elucidated that gallic acid instigated the downregulation of Bcl-2 and activated caspase-dependent apoptotic pathways in prostate cancer, thereby reinstating the sensitivity to anoikis. Furthermore, computational studies employing molecular docking and dynamics simulations have recognized gallic acid as a formidable inhibitor of Bcl-2, thereby corroborating its therapeutic potential (Vijayalakshmi et al., 2023).

2.6 Computational Approaches in Cancer Research

Computational methodologies have become essential in the realm of cancer research, particularly in the identification of novel therapeutic targets and the screening of prospective inhibitors(McDonald et al., 2023). Molecular docking and molecular dynamics simulations have been extensively utilized to predict the binding affinity and stability of small-molecule inhibitors targeting proteins associated with anoikis resistance(Qiao et al., 2024). For instance, docking investigations have demonstrated that natural compounds such as gallic acid and

curcumin interact with the BH3 domain of Bcl-2, disrupting its interaction with pro-apoptotic proteins and reinstating anoikis sensitivity in cancer cells (Bahadar et al., 2024)(Sadik et al., 2022).

Moreover, bioinformatics methodologies, including RNA sequencing (RNA-seq) and differential gene expression analysis, have offered invaluable insights into the molecular mechanisms that underpin anoikis resistance(Sui et al., 2024). Pathway enrichment analyses have further illuminated the signalling networks involved in anoikis resistance, thereby presenting novel avenues for targeted therapeutic interventions(C. Guo et al., 2019). The investigation conducted by (Palorini et al., 2016)underscores the paramount significance of computational methodologies in enhancing the comprehension of anoikis. Utilizing bioinformatics and systems biology methodologies, scholars are equipped to scrutinize extensive datasets in order to elucidate pivotal molecular pathways implicated in anoikis resistance. By simulating these pathways, computational strategies can assist in forecasting innovative therapeutic targets and refining approaches for mitigating metastasis, thereby illustrating the efficacy of in silico techniques within the domain of oncological research.

CHAPTER 3 FINDING KEY TARGETS OF ANOIKIS RESISTANCE

3.1 Rationale of the study

Anoikis is a form of programmed cell death resulting from detachment of cells from the extracellular matrix. The mechanisms deter the detached cells from reattaching to and colonizing other tissues, hence acting as a barrier for cancer progression. However, cancer cells elude this barrier and metastasize. Once the cancer cells invade the lymphatic or circulatory system it does not receive signals from ECM because it doesn't interact further with the basal membrane. Now, these cells must attain the capacity to subsist without adhesion to ECM for their survival (Figure 2). This independent survival potential in cancer cells is termed anoikis resistance (Y. N. Kim et al., 2012). Cancer cells undergo various environmental and cellular stresses while they go through the stepwise progression of metastasis (Bergers & Fendt, 2021). In this case, when tumor cells attain anoikis resistance they can subsist even when detached from their primary location or when they pass through the lymphatic system, cardiovascular system, circulatory system, water vascular system, systema lymphaticum and portal system, up until colonization in distal organs (Khan et al., 2022) (Paoli et al., 2013a). The biology of cancer metastasis has been greatly unraveled, yet the molecular mechanisms of anoikis resistance in cancer remain poorly understood. The present research identified a number of genes and pathways involved in anoikis resistance, which is still lacking in overall understanding. There is need to further explore these mechanisms to develop targeted therapies so as to prevent metastasis in cancer patients. This study involves identifying genes and pathways involved in the process of anoikis resistance, hence exposing potential therapeutic targets and biomarkers for cancer. The knowledge of these mechanisms will certainly go a long way to provide ways of preventing metastasis and improve the survival as well as the quality of life of afflicted patients. Further, these findings can contribute to general mechanisms of metastasis and resistivity to cell death from the context of cancer studies. This work identifies the overlap and cross-talk between pathways genes in various cancer-related pathways that are common in apoptosis, autophagy, EMT, anoikis and metastasis. It further explores the common genes in the pathways involved in aforementioned processes and their connection to anoikis and metastasis. The interaction and binding of the anoikis-associated protein Bcl-2 has been studied using computational biology methods.

Besides, this study includes analysing differential gene expression in anoikis-resistant cancer cells using datasets from the Gene Expression Omnibus with the aim of reusing the same in order to identify genes promoting the state leading to resistance to anoikis and progression of tumor.

The presented research will strive to reach a deeper understanding of the genetic and molecular basis of anoikis resistance in prostate cancer, which may, in turn, possibly result in some novel possible therapeutic strategies to prevent metastasis and improve patient outcomes.

3.1 Methodology

3.1.1 ASSORTMENT AND ANALYSIS OF DATA

From online public sources, a list of all the genes implicated in apoptosis, anoikis, EMT, and metastasis was retrieved. From deathbase.org, the collection of apoptotic genes (128 genes) data was downloaded. The dataset of genes engaged in anoikis (134 genes) was obtained from Harmanizome and MaayanLab.cloud. The collection of genes for metastasis (2241 genes) was downloaded from hcmdb.i-sanger.com. A database called HADb (Human Autophagy Database) was utilized to compile data for autophagy genes (231 genes). 'Epithelial-mesenchymal transition gene' database was used for collecting the dataset for EMT-linked genes (1184 genes). Four genes that are shared by EMT, anoikis (apoptosis), metastasis, and autophagy were found by comparing the data sets for these processes (M. Zhao et al., 2015) (M. Zhao et al., 2019). Set of natural compounds that were screened was downloaded from Gene expression omnibus database (GEO).

3.1.2 Gene expression profiling

Using GEPIA2 (Gene expression profiling interactive analysis) (Tang et al., 2019), the expression levels of Bcl-2 were compared in normal and tumor samples and their paired normal samples. GEPIA2 is a tool used for analysis of RNA sequencing expression data in tumor samples and their matched normal samples. The data for these samples is collected from GTEx projects and TCGA.

3.1.3 Data collection for differential gene expression analysis

Three datasets GSE106363, GSE100629, GSE46602 were collected from GEO (Gene expression omnibus) (Barrett et al., 2012). GSE106363 (Yue et al., 2018) contains total 6 samples out of which 3 samples are PC3 cell line (Prostate cancer cell line) and 3 samples are circulating tumor cell (CTCs) derived from PC3 cell line. GSE100629 (P. Zhang et al., 2018) contains 6 samples out of which three are anoikis resistant cells derived from PC3 cell lines and three are non-anoikis resistance cells also derived from PC3 cell line. The third dataset, GSE46602 (Mortensen et al., 2015) consisted of 50 samples out of which 36 are prostate cancer sample and 14 are adjacent non-tumor samples of patients.

3.1.4 Identification of total differentially expressed genes

Differentially expressed genes were identified with the help of GEO2R Tool (Smyth, 2004). GEO2R is an online tool that analyses the microarray data and helps in comparing raw data files and gives DEGs. GEO2R typically entails the selection of a dataset sourced from the Gene Expression Omnibus (GEO) database or the upload of microarray or RNA-seq data. Subsequently, the establishment of experimental comparison conditions is undertaken, delineating factors like distinctions between diseased and healthy samples or disparities between treated and untreated samples. In this study, we study samples of anoikis resistance vs non-anoikis resistance samples. Data normalization, correction of errors, selection of suitable algorithms to find DEGs (via GEO2R) were achieved and indicated by various graphs. GEO2R provides gene tables with $-\log$ (Fold change value) and P-value which can be used for statistical analysis.

3.1.5 Identification of common differentially expressed genes

Differentially expressed genes from the three datasets were compared and identified in Microsoft (MS) Excel. MS Excel, created by Microsoft, is a highly potent spreadsheet software utilized extensively for a range of tasks. These tasks encompass data analysis, numerical and

text data organization, calculation, visualization, and more. This categorization was done by using conditional formatting followed by highlighting cell rule and finding out duplicate values. Common differentially expressed genes were identified from all three datasets using the described method.

3.1.6 Enrichment analysis

The genes which were downregulated and upregulated in all samples were further subjected to functional enrichment analysis in relevance to biological processes and pathways involved in prostate cancer. For this purpose, FunRich was used which is a popular bioinformatics program used for biological data network visualization and functional enrichment studies. It incorporates data from numerous open-access databases, including Gene Ontology (GO), KEGG, Reactome, and Disease Ontology. A list of genes or proteins names is entered on FunRich and it performs functional enrichment analysis to find molecular functions, cellular components and biological processes that are associated with the input list. FunRich employs statistical algorithms, and the results are presented in various forms like Venn diagram, bar plot, pie chart, doughnut plot etc.

3.2 Results and discussion

3.2.1 Intersection and cross talks between key genes in multiple cancer related pathway

Cancer cells must acquire migratory potential before passing through the basement membrane and entering circulatory or lymphatic system, a process known as intravasation. After entering these systems, the cancer cell cannot communicate with the basal membrane and it attains survival capacity without adhesion to ECM, which is called anoikis resistance. Anoikis resistance improves the potential of the cell to travel to distant organs, and now the cell can survive and proliferate in a new environment and develops into a metastatic tumor (secondary tumor). Anoikis resistance involves dysregulation of numerous process and pathways which are linked to each other. These pathways have common genes that are involved in their regulation and functioning. It has been evidenced that EMT, apoptosis, autophagy and anoikis which are linked to each other, are found highly dysregulated in tumor microenvironment. The connections and links between the pathways in which the shared genes- BIRC3, Bcl-2, Bax and CHUK are active and how they can affect each other's functions were examined and a model was proposed (Figure 2.2). Figure 2.2 illustrates the key signalling pathways that control apoptosis, inflammation and survival of cells, and it also depicts the role of activation of caspases. These pathways are predominantly in relevance to anoikis and anoikis resistance. In the extrinsic pathway of anoikis binding of FASL to death receptors like FAS leads to the recruitment of FADD adaptor protein and further activation of caspase 8 and caspase 10 initiation caspases. The intrinsic pathway is activated by BAX/BAK and cytochrome c release. This leads to binding of Apaf-1 to form the apoptosome, activating caspase-9 and executioner caspases leading to DNA fragmentation and apoptosis. The NF-KB signalling pathway plays a critical role in anoikis resistance. Activation of TLR, CD40 receptors and IL1-R stimulates a signalling cascade which involves proteins like NIK, IKK α /IKKB, TRAF2 and TRAF3 which phosphorylates IKB, releasing NF-KB. NF-KB is then translocated to the nucleus to promote transcription of the genes that facilitate inflammation, anti-apoptotic responses and cell survival. Upregulation of survival factors such as BCL2 and CIAPs enables cells to evade apoptosis by blocking caspase activation and maintaining mitochondrial integrity. Additionally, BCL2 inhibits pro-apoptotic proteins like BAX and BAK, while CIAPs suppress caspase activity, preventing anoikis. The figure also highlights the crosstalk between survival

and apoptotic pathways, particularly in FAS-FASL and TNF/TNFR1 signalling. While these pathways can activate apoptosis through Caspase 8, they also promote NF- κ B signalling, which drives cell survival and inflammation. Cancer cells exploit this crosstalk to suppress apoptotic signalling and enhance survival, thereby increasing their ability to resist anoikis. The functional outcomes of these pathways include either apoptosis—characterized by caspase activation and DNA fragmentation—or cell survival through NF- κ B activation and upregulation of anti-apoptotic proteins

After a thorough examination of these pathways and comparing lists of genes and proteins involved in these pathways, we found out that BIRC3, Bcl-2, Bax and CHUK are such genes that are common among genes involved pathways associated to EMT, apoptosis, autophagy and anoikis. Therefore, we theorized that if these genes are targeted and regulated, multiple pathways associated to anoikis and anoikis resistance can be regulated.

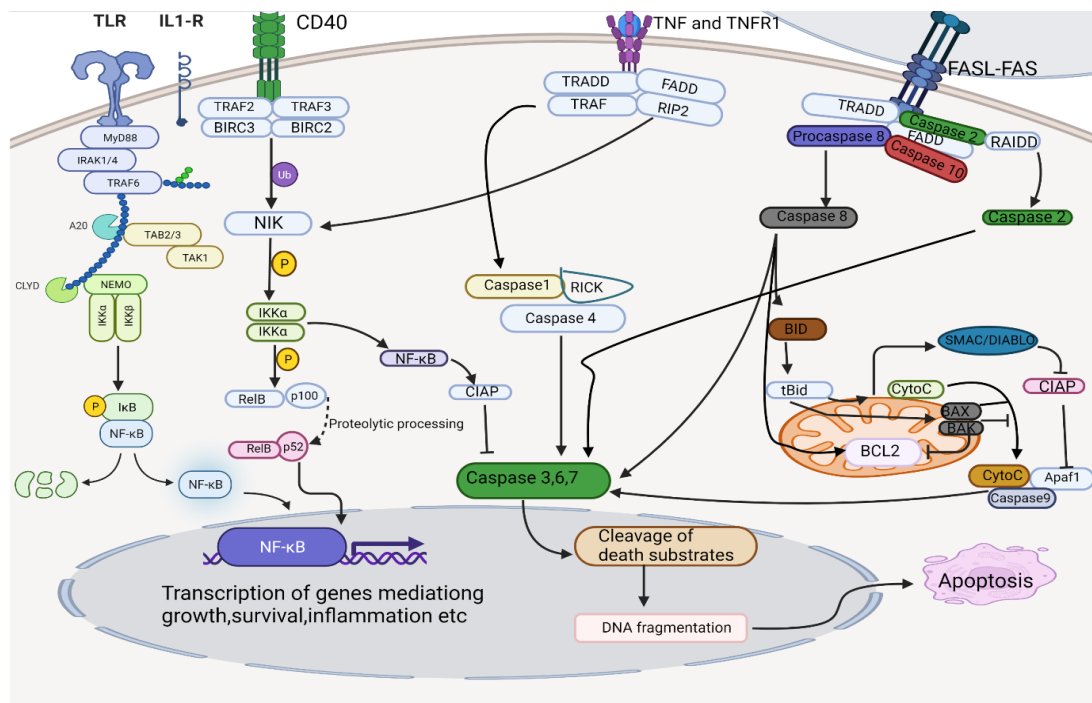


Figure 3.2 The common genes BAX, BCL2, IKK α (CHUK), and BIRC3 are involved in cross-talks between non-canonical and canonical pathway NF- κ B, TNF, and FAS receptor pathway. T bars indicate inhibition, whereas arrows indicate activation.

Analysis for connection among these genes using STRING showed that BIRC3 and CHUK are linked directly to each other while they are connected indirectly to Bax proapoptotic protein and Bcl-2 (Figure 3.3). Bax and Bcl-2, on other hand, are directly linked to each other. The nodes represent the genes and coloured lines depict the connection between genes. Bcl-2 showed highest degree of connection. The model created using STRING database (Szklarczyk et al., 2023), shown in Figure 3.3 depicts the connections between the common genes Bcl-2, BIRC3, Bax, and CHUK- which are central regulators of apoptosis and cell survival. In the context of anoikis and anoikis resistance, this network provides insights into how cancer cells evade detachment-induced cell death. BAX acts as a pro-apoptotic protein that promotes mitochondrial outer membrane permeabilization (MOMP), triggering apoptosis by releasing

cytochrome C. In contrast, BCL-2 is anti-apoptotic, stabilizing mitochondrial integrity and counteracting BAX activity. While anoikis-sensitive cells rely on BAX to initiate apoptosis upon loss of adhesion, anoikis-resistant cancer cells often overexpress BCL-2, which suppresses BAX and prevents apoptosis.

BIRC3, a member of the Inhibitor of Apoptosis Protein (IAP) family, plays a significant role in inhibiting caspases and apoptosis. It interacts with TRAF2 to activate the NF- κ B pathway, which drives the transcription of survival genes, including BCL-2 and BIRC3 itself. This pathway is critical for anoikis resistance, as it allows cancer cells to suppress apoptotic signaling and evade cell death upon detachment. CHUK (IKK α), a key regulator of NF- κ B activation, further amplifies this survival response by promoting the transcription of genes that inhibit apoptosis and enhance cell survival.

The TRAF2 protein acts as a bridge between survival signaling and NF- κ B activation. Its connection with CHUK and BIRC3 highlights its role in transmitting anti-apoptotic signals to ensure cellular survival in stressful conditions, such as loss of adhesion. Overall, this network illustrates the delicate balance between pro-apoptotic factors like BAX and anti-apoptotic factors such as BCL-2 and BIRC3, with NF- κ B signaling serving as a major mediator of survival. The precise role of certain genes in cancer progression remains incompletely understood. To address this, we employed a multitargeting strategy by identifying common genes across pathways associated with anoikis resistance. This approach aims to shed light on the complex interactions that contribute to cancer metastasis.

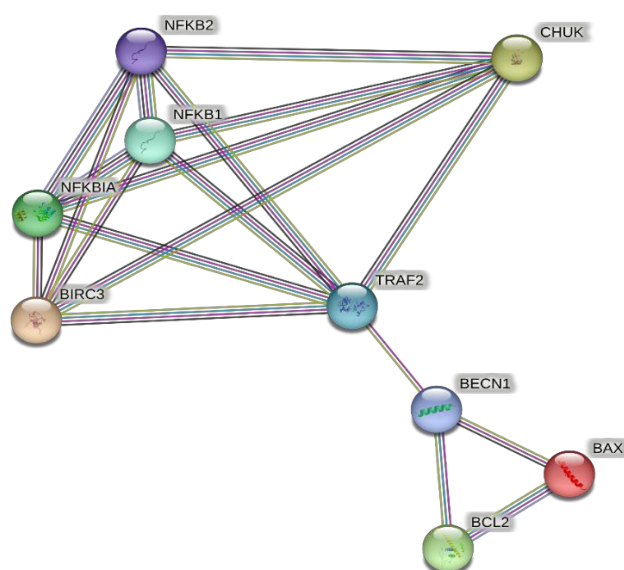


Figure 3.3 Network of connections between common genes Bcl-2, Bax, BIRC3 and CHUK

Bcl-2 inhibitors are clinically approved and demonstrate significant promise, whereas CHUK and BIRC3 inhibitors require further evaluation for safety and effectiveness. The expression of Bcl-2 was analysed in some commonly occurring cancers like breast cancer, acute myeloid leukaemia, thymoma and kidney renal clear cell carcinoma (figure 3.4). Results indicated that Bcl-2 is overexpressed in all these tumor samples as compared to their paired normal samples. Figure 3.4 demonstrates the comparison in the expression levels of Bcl-2 in some commonly

occurring tumors and their paired normal samples. Highest expression of Bcl-2 was noted in Acute myeloid leukaemia, followed by KIRC and BRCA.

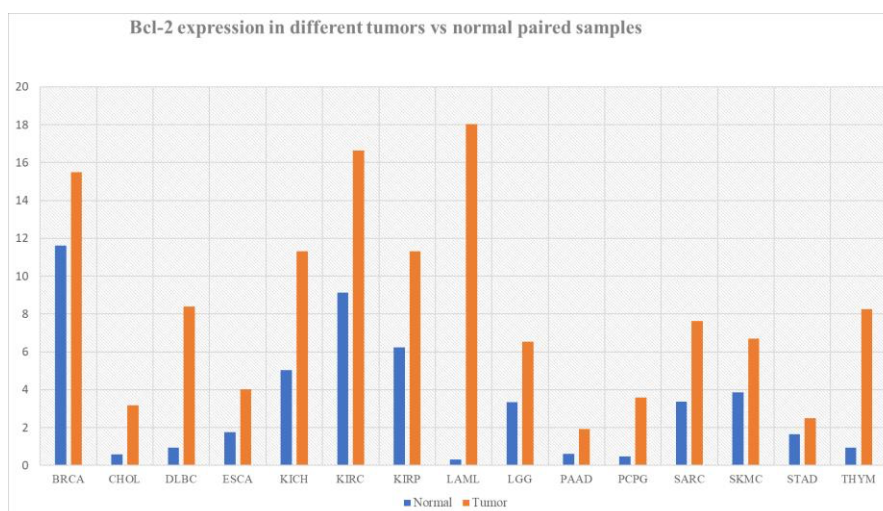


Figure 3.4 Expression levels of Bcl-2 in different tumors and their paired normal samples. BRCA-Breast invasive carcinoma, CHOL-Cholangio carcinoma, DLBC - Diffuse large B cell lymphoma, ESCA- Oesophageal carcinoma, KICH-Kidney Chromophobe, KIRC-Kidney renal papillary cell carcinoma, LAML-Acute Myeloid Leukemia, LGG-Brain Lower Grade Glioma PAAD-Pancreatic adenocarcinoma, PCPG-Pheochromocytoma and Paraganglioma, SARC-Sarcoma, SKCM-Skin Cutaneous Melanoma, STAD-Stomach adenocarcinoma, THYM-Thymoma.

3.2.2 Study of anoikis resistance related genes for understanding the role of genes for regulation of anoikis

The analysis of differential gene expression was carried out to identify genes that were upregulated and downregulated significantly in the control conditions compared to the test conditions to better understand how these genes regulate anoikis resistance. This comparison helps in identifying key genes involved in the processes that enable cells to evade anoikis, which is crucial for cancer progression and metastasis. <test conditions> GEO2R was used to normalize the data and analyse DEGs of the datasets-GSE106363, GSE100629 and GSE46602. A criterion was set for this purpose. Therefore, only genes having p-value less than 0.05 were measured as statistically significant, confirming that the chances of these findings coming up due to random variation is not more than 5%. Genes with logFC values less than -1 and greater than 1 were considered having statistically significant change in gene their expression. Figure 3.5-3.7 depicts the volcano plot and normalization plot for these data sets. In the data set GSE100629 which was for anoikis resistant PCa cells vs non-anoikis resistant PCa cells, there were 756 genes that were upregulated and 941 genes were such that were downregulated. In the dataset GSE106363 which consisted of CTCs and PC3 cells, 523 genes were up regulated and 1508 genes were downregulated. Third dataset which was for samples from prostate cancer patients having tumorous prostate cells and adjacent normal prostate cells (GSE46602) had 1648 upregulated genes and 1382 downregulated genes.

Anoikis resistant vs non-anoikis resistant PCa cells

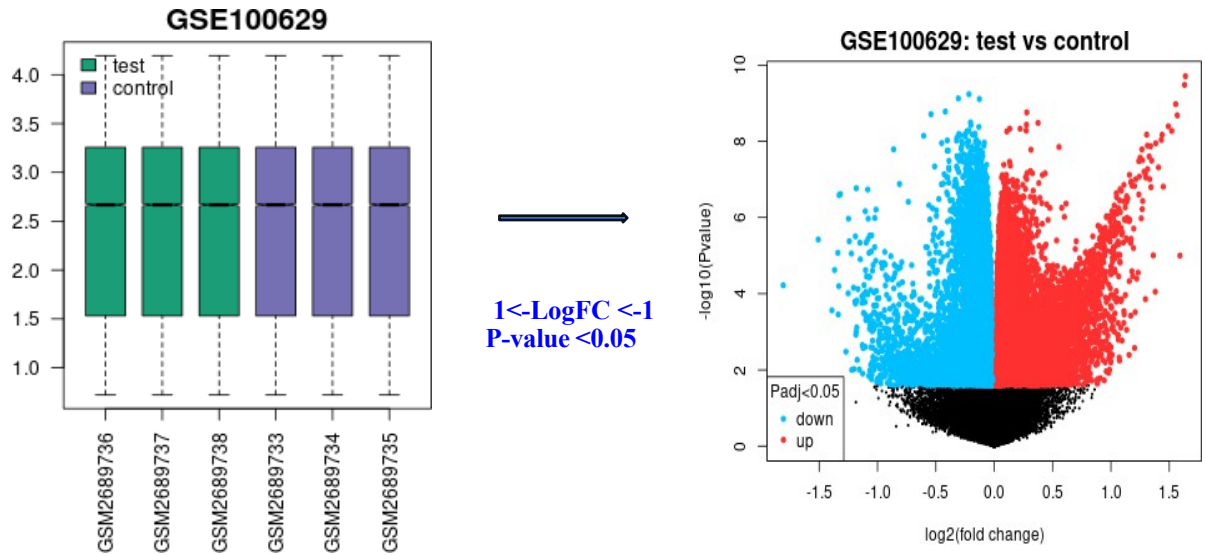


Figure 3.5 Dataset: GSE100629, Anoikis resistant vs non anoikis resistant PCa cells. Left graph is a box plot showing distribution of expression levels in test control samples while graph on right shows volcano plot illustrating differentially expressed genes between test and control samples in the dataset. Genes with $1 < -\text{LogFC} < -1$ and $\text{P-value} < 0.05$ were considered statistically significant

CTCs and PC3 cells

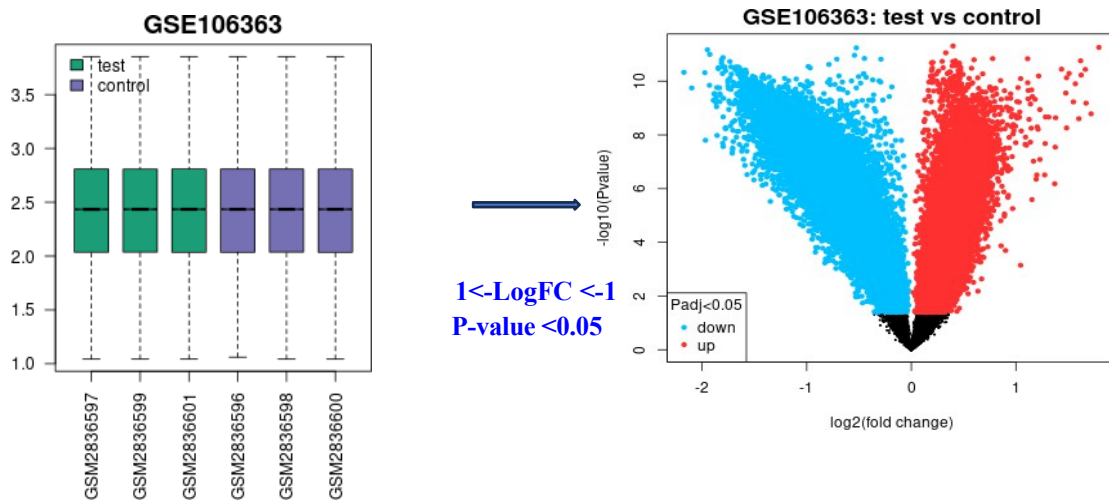


Figure 3.6: Differential Gene Expression Analysis of GSE106363 Dataset, Left panel: Boxplot. Represented are gene expression levels for test, green, and control, purple, samples for the six different GSM identifiers (GSM2836597 through GSM2836600). Boxes correspond to the interquartile range with a black line at the median and whiskers extending to 1.5 times the IQR values.

Prostate cancer cells and adjacent normal prostate cells

cells

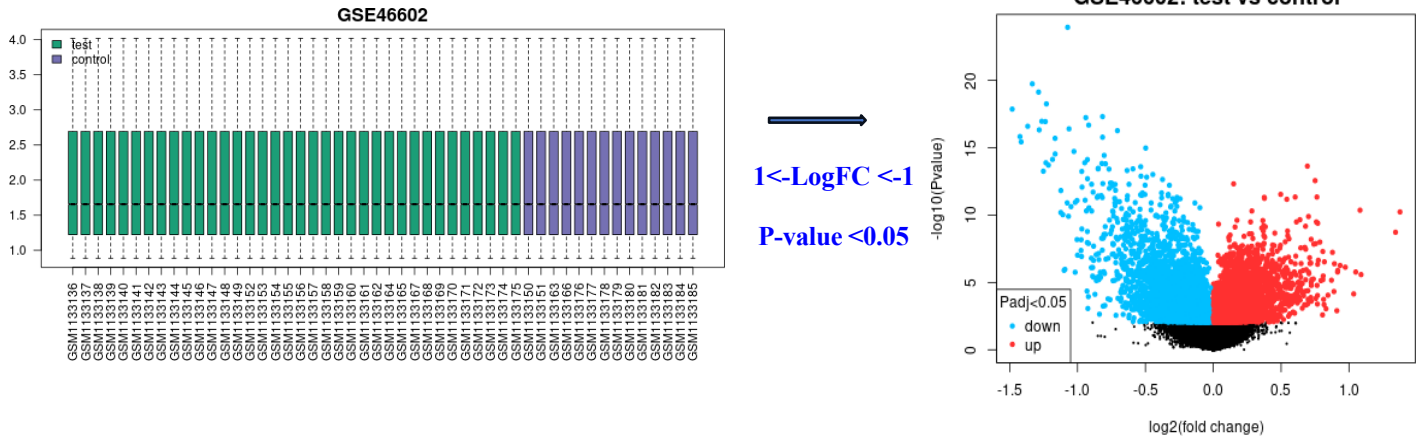


Figure 3.7 Dataset: GSE46602, Prostate cancer cells and adjacent normal prostate cell. The Box plot on left represents the distribution of gene expression levels in test and control samples for this dataset. Volcano plot on right shows DEGs between test and control samples in this dataset. Genes with $1 < -\text{LogFC} < -1$ and $P\text{ value} < 0.05$ are considered statistically significant.

Screening of common differentially expressed genes in anoikis resistance PCa.

DEGs from three distinct gene sets were compared for identifying their expression in biological pathways and process. It was found that there were 19 genes that were common in all the genes categorized as upregulated in all three paired samples. The flow chart for this step is demonstrated in the accompanying figure 3.8 and 3.9. Similarly, a comparison of the total number of downregulated genes across all three pairs of samples revealed that 34 genes were consistently downregulated in all three samples.

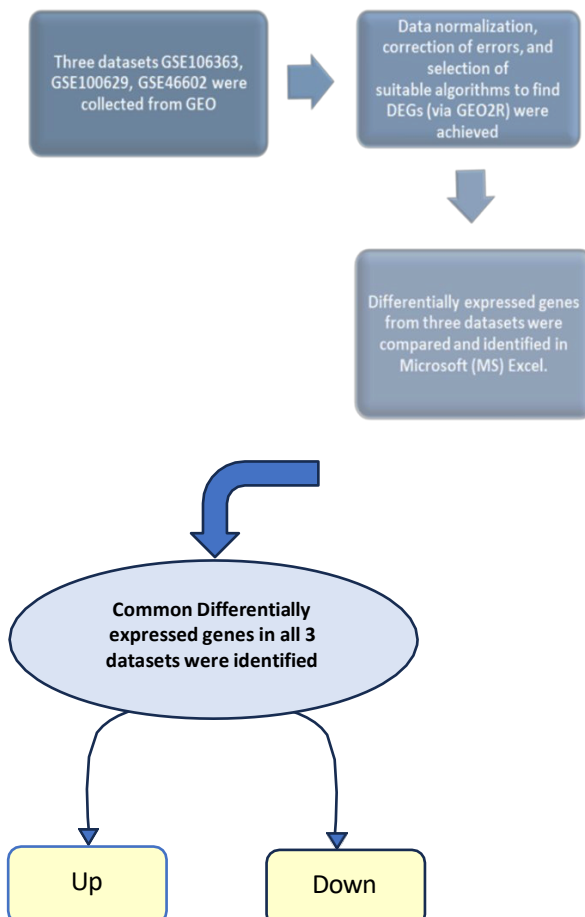


Figure 3.8 Work flow demonstrating brief methodology and steps involved in processing of data and classifying differentially expressed genes

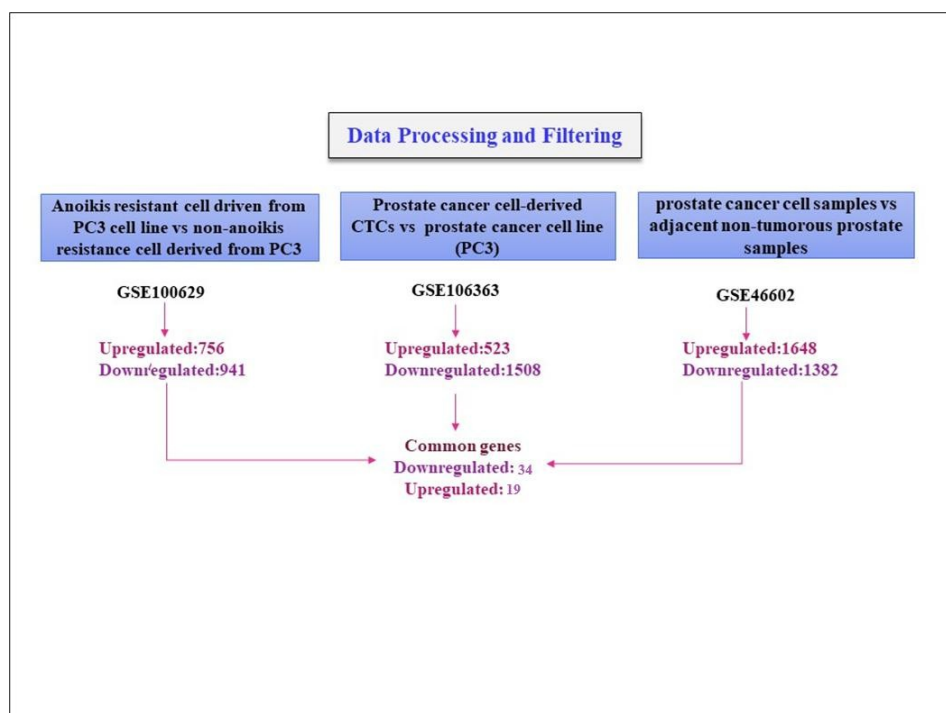


Figure 3.9 The data processing and filtering of all 3 datasets in PCa. The total number of upregulated genes was 2927 and the total number of downregulated genes that were obtained was 3831, from all three datasets together. While, the total number of genes that were common and downregulated were 34, total common upregulated genes were 19.

We used GEO2R for categorization and normalization of the datasets. During this process the adjusted P-value was ≤ 0.05 and logFC Value ≥ 1 for upregulated genes and for downregulated genes the logFC Value was ≤ -1 . In genome-wide studies, where huge number of genes are simultaneously tested, there are high chances of false positives to occur. To prevent this, adjusted p-values are frequently used. Secondly, Log fold change is measured as the magnitude of change in the expression of a gene between two different conditions. It is the logarithm base 2 of the ratio of levels of expression in the test to control condition. Genes having logFC value less than 1 (negative value) or greater than 1 (positive value) are considered having biologically significant change in expression. This parallels to a two-fold increase or decrease in gene expression levels. A total of 756 genes and 941 genes were upregulated and downregulated respectively, in the three datasets that procured for differential gene analysis from GEO. All these genes actively contribute to pathways like EMT, apoptosis, cell communication, caspase activation etc which are linked to anoikis and any disparities in these lead to anoikis resistance resulting in tumor progression. The dataset GSE100629 comprises prostate cancer cell lines (PC3), and consisted of various potential molecular targets that regulate metastasis and metabolism in anoikis-resistant prostate cancer cells, which are non-adherent and metastatic (P. Zhang et al., 2018). The second dataset, GSE106363 Circulating tumor cells (CTCs) are clusters of cells that have detached from the original cancer site and entered the bloodstream and lymphatic system (Lin et al., 2021). The matched normal samples in this sample are the

parental cells of PCa. In analogy to parental cancer cells, CTCs exhibit a higher capacity to invade and establish in distant organs (Micalizzi et al., 2017). During the process of CTC release, several factors come into play. These factors include morphological changes in tumor cells, regulation of expression of crucial genes (Miyamoto et al., 2015), changes in proteomic expression (Reza et al., 2021) etc. Together, these cellular and genetic changes contribute to the cells' capability to disseminate to distant organs within the body while escaping programmed cell death processes and surviving without attachment as happens when the cells become anoikis resistant (A. Yadav et al., 2015) (Deng et al., 2021). These genes were also found to be associated with disease recurrence following surgery, cell cycle and mitosis (Z. Sun et al., 2021). It is therefore essential to analyse these genes together with those associated with anoikis resistance genes and CTCs in PCa. GSE46602 (Mortensen et al., 2015) contains 50 samples out of which 36 are prostate cancer sample and 14 are adjacent non-tumor samples of patients. The third dataset (GSE46602) was derived from pairs of samples that had 50 samples, having 14 adjacent non-tumorous prostate samples and 36 prostate cancer cell samples. This dataset had 1648 upregulated genes and 1382 downregulated genes. When immune cells interact with the TME, they often undergo changes in their gene expression patterns resulting in changes in the properties of immune cells like immune response, response to treatment, tumor progression, metabolic adaptation, migratory properties and their ability to colonise (K. Tan & Naylor, 2022) (H. Zhao et al., 2021) (Mao et al., 2021). Therefore, it is necessary to analyse the expression data of such cells (for example, anoikis resistant cells) separately. By doing so, we can identify the genes that are differentially expressed in the anoikis-resistant cells. This analysis is valuable because it paves the way to target immune cells in TME, in addition to tumor markers, for effective tumor regression. DEGs identified in this study have the potential to modify the functionality of immune cells and facilitate the evasion of tumor cells from the immune system. These DEGs genes can be examined to identify immunological biomarkers specifically relevant to PCa as well as other tumor types involving these genes. Thus, computational screening process can aid in the discovery of valuable markers in an efficient and expedient manner which can be used for immunological assessments in cancer patients. After comparison of datasets in MS Excel we deduced 19 genes that were upregulated and were common in all three datasets and 34 genes that were downregulated and common in all three datasets. Anoikis resistant cells and CTCs have the ability to infiltrate the tumor microenvironment and evade cell death, thereby impacting tumor progression. TME has an impact on the expression of these genes. Due to the intricate interactions between cells within the TME, these genes may be responsible in altering gene regulation specifically within the subset of immune cells present around the tumor in cancer patients. Therefore, DEGs from these datasets were subjected to enrichment analysis which helped us study their contribution and role in various biological pathways and processes which are vital in cancer progression, cancer onset and fighting cancer.

3.2.3 Biological process affected by common DEGs and their influence on anoikis in cancer progression

Genes and proteins are vital in controlling the pathways and mechanisms linked to anoikis and apoptosis, which are crucial for preserving cellular balance. When these processes are disrupted, it can lead to the development, advancement, and spread of cancer. When gene expression is improperly regulated, cancer cells can escape anoikis resulting in a heightened potential for metastasis (Y.-N. Kim et al., 2012). In our study, we focused on 53 genes recognized as dysregulated in tumors that are resistant to anoikis and metastatic. These genes were scrutinized for their viability as therapeutic targets in the treatment of cancer. To gain a

clearer understanding of their roles and involvement, we performed enrichment analyses to explore their connections with various biological pathways and processes.

The enrichment analysis enabled us to pinpoint significant pathways and molecular mechanisms influenced by these dysregulated genes. By associating these genes with established signaling networks, we revealed their participation in processes such as cell adhesion, survival signaling, epithelial-to-mesenchymal transition (EMT), and immune evasion—all of which are essential elements in cancer metastasis. This comprehensive approach establishes a framework for prioritizing candidate genes for further exploration as potential therapeutic targets. Focusing on these pathways may present new avenues for intervention, with the goal of reestablishing anoikis sensitivity and curbing metastatic progression in cancer. The enrichment analysis of differentially expressed genes (34 downregulated and 19 up-regulated genes) involved in mainstream biological process, which are also associated to cancer onset and progression is depicted in graphical form in Figure 3.10.

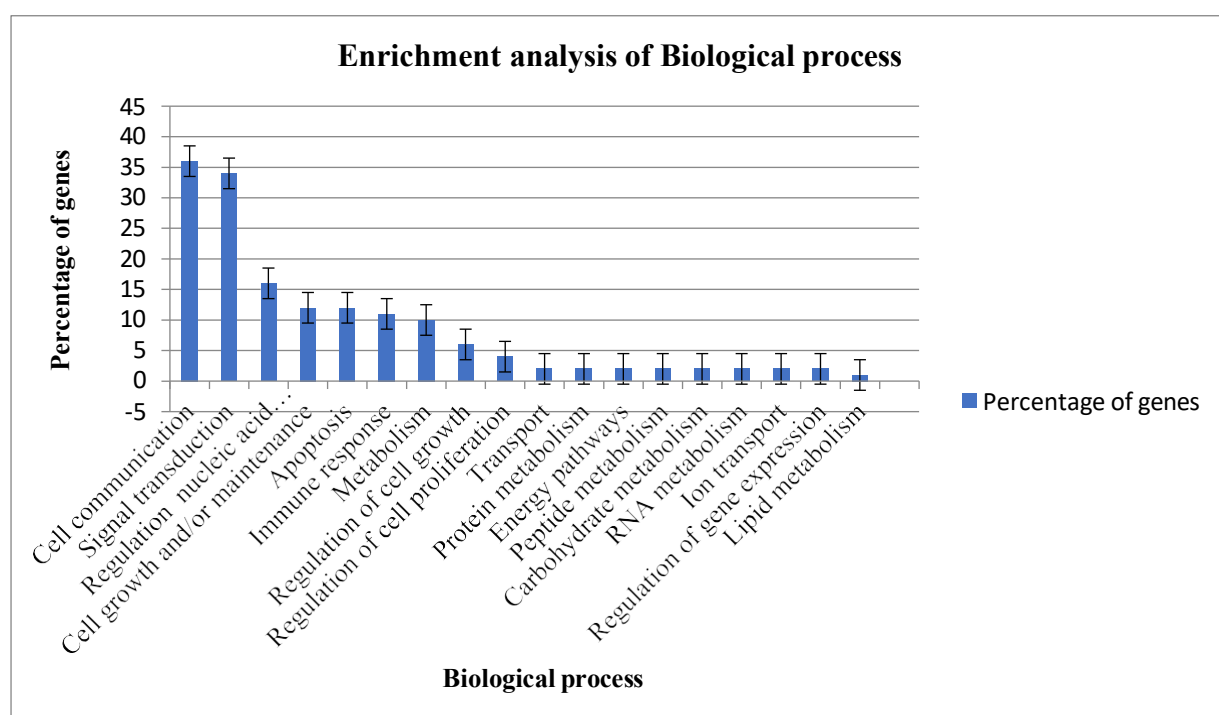


Figure 3.10 Following figure demonstrate the gene enrichment analysis of 19 common upregulated genes and 34 common downregulated genes relating to biological processes.

The percentage of genes involved in the biological processes are represented on Y-axis and the common differentially expressed genes are listed on x-axis (figure 3.10). Among the identified genes, SKIL, SSTR1, CAV2, ST7L, and ANXA2 are engaged in a range of biological processes and are crucial in the advancement and spread of cancer.

SSTR1 (Somatostatin Receptor 1) is a G-protein-coupled receptor (GPCR) that plays a critical role in somatostatin signaling. Upon binding to its ligand, somatostatin, it inhibits adenylyl cyclase activity, thereby reducing cyclic AMP (cAMP) levels and regulating downstream pathways. SSTR1 is particularly important in modulating hormone secretion, cell proliferation, and neuronal communication. It is linked to signaling pathways such as MAPK and PI3K/AKT, which are involved in apoptosis and cell cycle arrest, as well as neurotransmission through effects on calcium and potassium ion channels. CAV2 (Caveolin-2) is a scaffolding protein localized in caveolae, specialized membrane domains. It regulates signal transduction by

interacting with multiple signaling molecules such as G-proteins, EGFR, and Src-family kinases. CAV2 is involved in pathways like MAPK/ERK and nitric oxide signaling, contributing to processes such as endothelial cell signaling and vascular homeostasis. This protein plays a significant role in lipid metabolism and endocytosis, further enhancing its importance in cell communication. ANXA2 (Annexin A2) is a calcium-dependent phospholipid-binding protein that mediates diverse cellular processes, including membrane trafficking, cytoskeletal dynamics, and exocytosis. It forms a complex with S100A10, which is essential for extracellular signal regulation and fibrinolysis. ANXA2 is also associated with cancer cell migration and invasion. It interacts with Rho-GTPases and participates in pathways such as TGF-beta signaling, inflammation regulation, and plasminogen activation, highlighting its role in both normal and pathological cellular functions. SKIL (Ski-Like Proto-Oncogene) acts as a transcriptional corepressor and plays a key role in TGF-beta signaling by inhibiting SMAD proteins. This modulation affects cell proliferation, differentiation, and tumorigenesis by altering transcriptional responses to TGF-beta ligands. SKIL's involvement in the TGF-beta/SMAD pathway makes it critical for cellular processes like epithelial-mesenchymal transition (EMT), immune responses, and fibrosis, emphasizing its relevance in both development and disease. ST7L (Suppression of Tumorigenicity 7-Like) remains less well-characterized, but evidence suggests it may function in cell signaling and tumor suppression. It is potentially involved in modulating the Wnt/beta-catenin pathway or other oncogenic signaling pathways. ST7L has been associated with transcriptional regulation or chromatin remodeling, which may indirectly influence signal transduction. Though its precise biological roles are unclear, it has been identified in cancer studies as a potential tumor suppressor, further underscoring its importance in cellular communication and regulation. These genes collectively demonstrate significant involvement in critical signaling processes such as cell communication, signal transduction, and pathway regulation, with roles spanning normal cellular functions to contributions in diseases like cancer. Many of the up-regulated genes were involved in process such as cell communication (~35%), immune response and apoptosis (10%), signal transduction (~30%), regulation of nucleic acid metabolic process (~15%), cell proliferation etc. Cell communication, which is a key factor in maintenance of anoikis and anoikis resistance included around 35% of the common genes involved in anoikis. Signal transduction, a process crucial for transmitting signals within and between cells involved around 30% of the common genes associated to it, therefore proven to be second most significant process in relevance to anoikis and cancer. For example the gene ILK is a critical player in integrin-mediated signal transduction leading to anoikis protection. Following cell- ECM adhesion, ILK binds to integrin receptors and phosphorylates its target PKB/Akt on Ser473, hence activating it. Activated forms of either FAK or ILK were shown to inhibit anoikis in suspended cells, underscoring that these kinases positively contribute to anoikis protection. Notably, ILK transmits integrin-mediated survival signals even in the absence of FAK, as shown by the fact that dominant-negative FAK was unable to reverse the ILK- mediated protection from anoikis, this finding indicating that the two kinases are working through different and parallel pathways to affect anoikis. Regulation of the processes associated to nucleic acid metabolism and cell growth and maintenance involved about 15% of the genes. Nucleic acid metabolism is crucial in anoikis and anoikis resistance. Nucleotides that are essential for RNA and DNA synthesis, as well as NADPH (electron donor) support nucleic acid and other biosynthetic pathways which are important for cancer cell survival. For instance, Pentose phosphate pathway (PPP) produces pentoses and NADPH, helping cancer cells to proliferate and becoming anoikis resistant. Another metabolic pathway, Glycolysis, breaks down glucose to pyruvate generating energy and supporting cancer cell survival in detachment from neighbouring cells and ECM. Mitochondrial pathways like TCA cycle, fatty acid oxidation produce energy by NADH and ATP thus enabling cancer cells to withstand anoikis.

To conclude, these metabolic pathways play crucial roles in encouraging anoikis resistance, therefore facilitating tumor metastasis. In the graph (Figure 3.10) process such as protein metabolism, transport and energy pathways, peptide metabolism, carbohydrate metabolism, RNA metabolism, ion transport, regulation of gene expression, and lipid metabolism are represented to a lesser extent. Although processes like protein metabolism, transport, and lipid metabolism involve fewer genes, they play critical roles in cellular functions such as maintaining energy balance, regulating gene expression, and supporting tumor cell survival. This cumulative effect promotes tumor metastasis and underscores the importance of targeting these pathways for therapy. Therapies aimed at disrupting cell communication, signal transduction, or metabolic pathways may sensitize tumor cells to anoikis, reduce metastasis, and improve treatment outcomes. By focusing on these processes, targeted therapies can weaken the tumor's survival mechanisms, offering promising avenues for cancer treatment.

3.2.4 Biological pathways associated with anoikis and anoikis resistance and their roles.

The common differentially expressed genes were analysed for their contribution in key specific pathways, affecting biological processes. Among the 53 mapped genes, SSTR1, SKIL, and CAV2 are notably active in biological pathways associated with cancer progression. SSTR1 (Somatostatin Receptor 1) is a G-protein-coupled receptor that, upon activation by somatostatin, inhibits adenylyl cyclase activity, leading to reduced cyclic AMP (cAMP) levels. This inhibition can suppress cell proliferation and hormone secretion, mechanisms particularly relevant in neuroendocrine tumors where somatostatin analogs are employed therapeutically to control tumor growth and hormonal activity. SKIL (Ski-Like Proto-Oncogene) functions as a transcriptional co-repressor, modulating pathways such as mTOR, PI3K, and EGF receptor signaling. By interacting with SMAD proteins, SKIL can inhibit TGF- β signaling, leading to increased cell proliferation and reduced apoptosis. Overexpression of SKIL has been associated with oncogenic transformation and tumor progression in various cancers, including prostate cancer. CAV2 (Caveolin-2) is involved in the regulation of syndecan-mediated signaling and EGFR1 pathways. In cancer, CAV2 has been shown to promote tumor progression by modulating the Wnt/ β -catenin signaling pathway. For instance, in pancreatic cancer, CAV2 regulates the miR-4723/Wnt7A signaling axis, enhancing cell proliferation, invasion, and metastasis. Additionally, CAV2's interaction with EGFR signaling can influence cell growth and survival, further contributing to cancer progression. Collectively, the dysregulation of SSTR1, SKIL, and CAV2 in these signaling pathways can lead to enhanced cell proliferation, survival, and metastasis, underscoring their roles in cancer progression. In figure 3.11, percentage of genes in various biological pathways has been demonstrated. EMT is associated with around 20% of the common genes, while CDC42 signalling events regulation activity was associated with ~30% of the 53 common genes. Around 30% of genes were involved in $\alpha 9\beta 1$ integrin signalling pathways. It was identified that CDC42 signalling events and the regulation of CDC42 activity pathways include the highest percentage of genes, encompassing about 30% of the genes. It has been studied that these pathways are crucial for regulation of cell cycle, cellular dynamics and cell morphology. The activity of CDC42 is vital for maintenance of cytoskeletal dynamics. When cells are in no contact with the cellular matrix, CDC42 aids regulation of the cellular response to detachment. Appropriate functioning of CDC42 guarantees the activation of apoptotic pathways that lead to anoikis, thus preventing the cells that have detached from surviving and colonizing. Tumor cells can exploit CDC42 signalling in order to activate the pathways that offset pro-apoptotic signalling like PI3K/AKT, MAPK/ERK etc generated upon cellular detachment. In addition to this, CDC42 can also promote reorganization of actin cytoskeleton and cell motility, helping in the dissemination of

tumor cells to distant organs and sites. The $\alpha 9 \beta 1$ integrin signalling pathway featured around 30% of the genes involved in its regulation and functioning. This pathway is vital in cell adhesion, cell migration and signal transduction. In relation to anoikis, $\alpha 9 \beta 1$ integrin signalling pathway plays an important role in maintenance of cell survival and cellular attachment to ECM. Dysregulation of $\alpha 9 \beta 1$ integrin signalling pathway can lead to anoikis resistance by supporting cell adhesion and migration in the absence of cellular matrix thus giving rise to cancer metastasis. In the enrichment analysis, the EMT pathway involved approximately 20% genes. EMT pathway is fundamental in metastasis and tumor progression. EMT aids the epithelial cells to gain mesenchymal features, enhancing their invasive and migratory potential that can help them evade anoikis and escape cell death during detachment. Other pathways RAC1 and RhoA signalling pathways included about 20% of the common genes. These two pathways are substantial for organisation of cytoskeleton and cellular movement. RAC1 and RhoA signalling can promote cellular motility thus letting cancer cells to resist detachment induced cell death or anoikis. mTOR signalling pathway, which is a key to cell proliferation, cellular growth and cell survival, involved about 15% of the common genes. Last but not the least, PI3K/Akt pathway and TRAIL signalling pathway involved around 15% of the genes. Tumor cells often exploit these signalling pathways to fight apoptosis and promote their survival, enabling themselves to become anoikis resistant cells. Other major pathways involved are the EGFR1, Trk signalling, TGF- β receptor signalling, and c-Src/Cx43 signalling, each requiring about 5 to 10% of the genes. Though involving a smaller percentage of genes, these pathways have key functions in cellular activities and the signalling mechanisms. For example, EGFR1 and Trk signalling pathways are known to activate downstream survival pathways, while TGF- β receptor signalling may be involved in EMT or anoikis resistance. Moreover, modulation of cell-cell communication and adhesion via the c-Src/Cx43 pathway may further support the survival of detached cells. The engagement of such signalling pathways in gene expression attributed to anoikis and anoikis resistance indicates their contribution to events involving cancer cell survival, movement, and metastasis. Under such a notion, the understanding of these pathways may point the way to possible therapeutic targets which may abrogate anoikis resistance and halt or slow cancer growth.

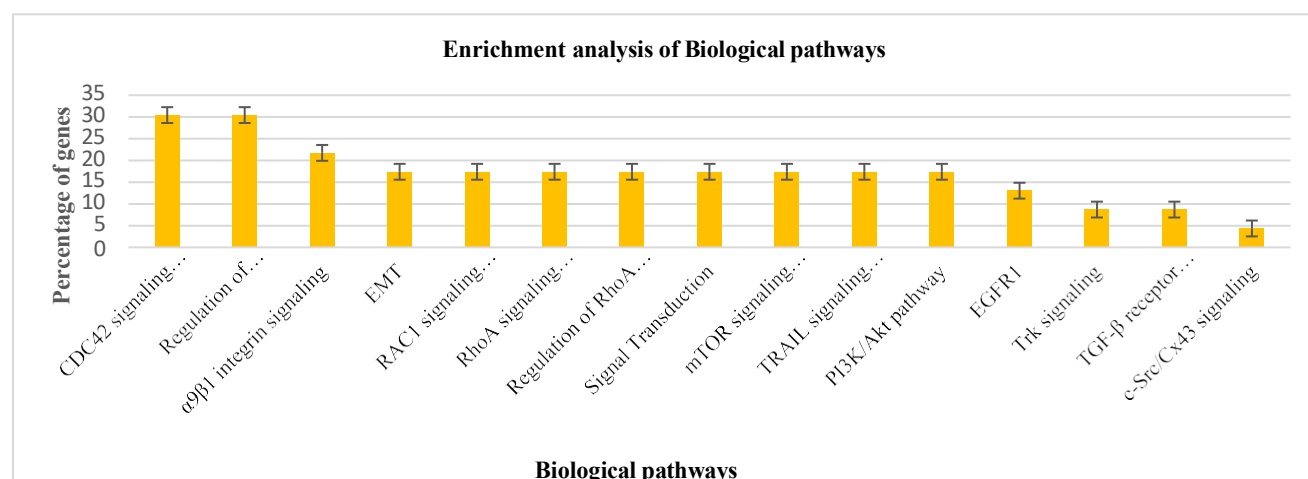


Figure 3.11 Involvement of the DEGs in pathways like EMT-associated pathways, Trk-signalling (Neurotrophic factor-mediated Trk receptor signalling), TGF- β signalling, mTOR (Mammalian target of rapamycin) signalling, PI3K/Akt (Class I PI3K signalling).

CHAPTER 4 IDENTIFICATION OF NATURAL COMPOUNDS FOR MODULATION OF ANOIKIS-RESISTANCE MEDIATORS.

4.1 Rationale of the study

Introduction:

Cancer is one of the major causes of death in the world. One of the main reasons why cancers become aggressive is tumor resistance to apoptosis which hampers efficient cancer therapy. Members of the B-cell lymphoma 2 protein family are key regulators of apoptosis and are commonly overexpressed in tumors, promoting survival and thereby contributing to drug resistance. Accordingly, the targeting of Bcl-2 apoptotic induction in cancer therapy may present a highly effective and prospectively potential method in this field.

Gap in the Current Research:

Currently, some treatments target Bcl-2 with the help of a Bcl-2 inhibitor, ABT-737, which has been rather effective in inducing apoptosis in tumor cells. However, such synthetic inhibitors usually have considerable side effects and limitations associated with their application, including development of resistance. Moreover, natural compounds are attracting much attention for their multi-targeting capabilities and minimum side effects. However, due to a lack of comprehensive studies, very few potential pro-apoptotic and drug resistance associated gene inhibitors from this natural compound group have been recognized and validated. There is a need of finding compounds having multi-targeting properties so that more than one of the pathways can be regulated in tumor conditions. Therefore, compounds having multitargeting as well as synergistic effect carry great potential in drug discovery and therapeutics.

Study objectives:

1. Investigate the potential of natural compounds as bcl-2 inhibitors:

The primary objective is to explore the potential of natural compounds, specifically Dioscin, as inhibitors of the Bcl-2 protein. By comparing the binding affinity and stability of Dioscin with the known Bcl-2 inhibitor ABT-737 via molecular docking and MD simulations, this study aims to identify more effective and less toxic therapeutic options for cancer treatment.

2. Investigate the combined effects of natural compounds on anoikis resistance related genes:

Another objective is to examine the antitumor and immunomodulatory effects of set of 102 natural compounds on 53 common DEGs which are associated to anoikis resistance, CTCs and metastatic prostate cancer. These compounds have shown potential in influencing various cellular processes and pathways relevant to cancer biology.

3. Assess the temporal regulation of gene expression:

Another objective is to study the DEGs at different stages of cell death induced by gallic acid treatment. By analyzing the RNA sequencing data at specific time intervals (2, 4, 6, and 9 hours), the study aims to elucidate the dynamic interaction between gallic acid and gene expression, particularly focusing on genes related to anoikis resistance.

4. Identify potential therapeutic targets:

Through comprehensive analysis, the study seeks to identify key genes, proteins, and pathways regulated by the natural compounds, which could serve as potential therapeutic targets for overcoming anoikis resistance and metastasis.

Importance of the Study:

1. Screening of Natural Inhibitors of Bcl-2: Dioscin, a plant-derived natural compound originating from traditional Chinese herbs, showed potential anti-cancer properties with reduced risks of side effects. The current research focuses on the efficacy of Bcl-2 inhibition by these compounds, which serve as a safer alternative to synthetic drugs.
2. Mechanistic Insights: According to this work, the assessment of Dioscin and Bcl-2 interactions through molecular dynamics simulations gave detailed insight into the binding mechanisms and stability of these complexes. This would be important in designing an effective Bcl-2 inhibitor.
3. Gene Expression Analysis: The research examines the effect in differential expression of anoikis, anoikis resistance and metastasis associated genes in cancer cells. Such analysis will further elucidate the impacts of bioactive natural compounds on the level of cellular pathways involved in the promotion and resistance of cancer.
4. Combination Therapy Potential: This study also investigates the synergistic effects of bioactive natural compounds, adopting the multi-targeted strategy for the prevention of drug resistance and enhancement of therapeutic efficacy.

Contribution to the field:

This work contributes to the area of cancer research by providing supporting evidence for the probable application of natural compounds as effective Bcl-2 inhibitors and regulator of other significant genes involved in anoikis and anoikis resistance. It is going to bridge the gap between traditional medicine and modern cancer therapy and thus open up new avenues for the development of safer and more efficient treatments. These findings could be pointers to additional research into natural compounds and their role in targeted cancer therapy, leading to the development of novel therapeutic strategies and better outcomes for patients.

4.2 Methodology

4.2.1 SCREENING PHYTOCHEMICALS AGAINST THE TUMOR-ASSOCIATED MOLECULE-LIGAND AND PROTEIN PREPARATION

Out of all the common genes, docking of Bcl-2 was performed with the structures of phytochemicals in order to study interactions between the protein and phytochemicals. The crystal structure of Bcl-2 solved by NMR (1ysw) and its inhibitor complex was retrieved from RCSB Protein Databank (PDB). The choice to use the Bcl-2 protein's NMR (Nuclear Magnetic Resonance) structures for molecular docking and MD (Molecular Dynamics) simulations was motivated by the clear benefits that NMR structures provide. NMR captures the dynamic nature of proteins in solution, revealing their capacity to adopt various conformations and undergo dynamic changes, in contrast to high-resolution crystal structures(Koehler Leman et al., 2018)(Krishnan & Rupp, 2012)(Harris, 2022). Bcl-2, which is known to exist in a variety of conformations in cellular contexts, benefits from NMR's ability to produce an ensemble of structures, giving molecular docking and MD simulations a full understanding(Oltersdorf et al., 2005)(Anantram et al., 2019). Understanding ligand binding and complex stability in actual cellular settings requires insights into protein-solvent interactions and solvation effects, which are made possible by NMR's solution-based methodology(Kovermann et al., 2016)(Koehler Leman et al., 2018). X-ray crystallography (XRD), provides high precision in revealing atomic details. NMR, on the other hand, is exceptional at revealing dynamics in solution

states(Krishnan & Rupp, 2012).Lastly, NMR structures ensure alignment with experimental data thus acting as a reliable reference for MD simulations(Tutumlu et al., 2020)(Bohg et al., 2021).

Using BIOVIA Discovery Studio v21.1.0.20298, unnecessary atoms and chains were removed and the protein was made ready for docking. From Pubchem, the 3-D structures of the ligands (phytochemicals) were retrieved. Known Bcl-2 inhibitor 3-nitro-n-{4-[2-(2-phenylethyl)-1,3-benzothiazol-5-yl]benzoyl}-4-{[2-(phenylsulfanyl)ethyl]amino}benzenesulfonamide (Pubchem CID-4369510,43B) also known as ABT-737 was used as control. ABT-737 is a senolytic, used in chemotherapy. Ligands were prepared using Lig prep and PDBQT file of the protein and phytochemicals was prepared using OpenBable(O'Boyle et al., 2011). Total Kollhman charges added on protein were -24.81 and total Gasteiger charge added on ligand was -0.9998. The dimensions of the grid box were 60×60×60 with 0.375Å spacing centred on protein. The X,Y and Z grid points were X=50, Y=66, Z=64 and the x y z center coordinates were 13.743, -1.631, -2.36 respectively(N. Kumari et al., 2022). Amino acid residues considered for grid generation were TYR199, TYR97, ASP100, ALA146, PHE101, VAL199 and PHE109(N. Kumari et al., 2022). Co-crystallized ligands were used to categorise the binding sites on Bcl-2, and residues in these areas as well as water molecules were taken into account while building grid lattice box for defining binding site during molecular docking.

4.2.2 MOLECULAR DOCKING AND MD SIMULATION, MM/PBSA AND MM/GBSA CALCULATION

Easydock vina 2.2 (*No Title*, n.d.-a) was used for docking of the protein and set of natural compounds which were used as ligands. The docking results were analyzed and validated using BIOVIA discovery studio, PyMOL by Schrödinger (<http://www.pymol.org/pymol>) and PLIP (Protein-ligand interaction profiler)(Adasme et al., 2021).

Using GROMACS (*No Title*, n.d.-b), MD simulation of Bcl-2 and bound ligands were run. MD simulation was performed on the model displaying the desired interactions and best docking score. In the cubic boxes with explicit water models the protein-ligand complex was set. Concentration of salt solution (NaCl) was 0.15 M and the temperature was set 300 K by Noose hover thermostat. All systems were neutralised by adding counter ions. The necessary conditions for the coulombic and van der waal interactions were established. Default values were set for equilibration and minimization of energy, and finally 100-ns simulation was run. In our study, we used rectangular water box that had dimensions 10 Å between box edges. We used the general protein-drug complex force field CHARMM and the CHARMM36 all-atomic force field to create topologies and co-ordinates for our experiment(J. Huang & MacKerell, 2013). We added counter-Na and Cl ions to keep the system neutral(Dalal et al., 2021). In order to reduce steric repulsions, we first performed a steepest descent energy minimization procedure that involved 50,000 steps. The system temperature was then stabilized by means of a 500 picosecond (ps) long NVT equilibration. In order to stabilize system pressure and enable system relaxation while maintaining protein restraint, we also implemented a short NPT equilibration lasting 500 ps(Chatterjee et al., 2022). Each system underwent 100 ns simulation without any imposed constraints. Using the GROMACS tools, we examined the trajectory files. We specifically used the "gmxrmsd" and "gmxrmsf" utilities to determine the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of the systems, respectively. We further performed Radius of gyration (Rg) and Hydrogen bond (h-bond) analysis in GROMACS, of entire 100 ns simulation frames to study the compactness and of the ligand and protein complexes and H-bond formation between amino acid residues of the protein and ligand(R. Kumari et al., 2022).

4.2.3 DIFFERENTIAL GENE EXPRESSION OF DIOSGIN

The expression of common variably expressed genes was matched with the expression profile of dioscin. GEO Dataset GSE79465 was analyzed for dioscin on MCF-7 cell line using GEO2R tool. Two replicates for each compound were considered as test set and compared with the vehicle control set in which only DMSO was present, one by one (S. Kumar & Das, 2023). The study's (GSE79465) experimental design involved the following steps: Human breast adenocarcinoma cells MCF-7 (showed high expression of Bcl-2) were cultured in phenol red-free DMEM-F12 medium supplemented with dextran charcoal-treated fetal bovine serum, penicillin-streptomycin, and l-glutamine. The cells were allowed to attach in culture flasks with a volume of 6 mL for 24 hours before treatment with dioscin (2.30 μ M) for three days. After removing the media, the cells were trypsinized, resuspended in the medium, and washed with PBS. RNA extraction was performed using Trizol reagent, and chloroform was added for phase separation. The concentration of RNA was determined using NanoDrop 2000c, and the samples were stored at -80 °C until sent to Phalanx Biotech Group for microarray analysis (Aumsuwan et al., 2016).

4.2.4 STUDYING THE IMPACT OF BIOACTIVE NATURAL COMPOUNDS ON DIFFERENTIALLY EXPRESSED GENES

GSE85871 dataset was used for studying the effect of bioactive natural compounds on the differentially expressed genes. This data set consisted of 102 bioactive natural compounds which are already known for their anti-cancer properties tested on the cancer cell lines, and cancer cells treated with Dimethyl sulfoxide (DMSO). It contains two replicates of each compound treatment. The GEO2R tool was used to analyse the effect of treatment of different natural compounds on the expression of different genes. DMSO treated samples were taken as control and compared with different natural compound treated samples as test set and differential expressed genes were identified. The expression profile of common genes that were differentially regulated was compared to that of natural compounds, and compounds that affected expression of highest number of genes were chosen using MS Excel. The same results were obtained for common downregulated genes. Number of genes were identified whose expression was altered by individual natural compound treatment from the selected genes.

4.2.5 SCREENING OF BIOACTIVE NATURAL COMPOUNDS

Bioactive natural compounds were evaluated based on the level to which they regulated the expression of common DEGs. Natural compounds that modulated the maximum number of genes from the group of common differentially expressed genes was selected first. Another compound from set of bioactive natural compounds was then chosen on the basis of which of the remaining genes (which were unaffected by the first compound) are affected by most of them. The remaining compounds were screened in a similar manner.

4.2.6 ANALYSIS OF COMBINATORIAL NATURAL COMPOUND FORMULATION

The analysis of effect of the chosen combination of natural compounds on anoikis resistance genes involved utilizing a Venn diagram to identify the genes regulated by these bioactive compounds. Additionally, FunRich tool (Version 3.1.13) (Fonseka et al., 2021) was used for performing enrichment analysis of the common genes(shared). To further enhance the understanding of the effect of these natural compounds on tumor, various biological pathways and processes influenced by these natural compounds were explored through an extensive review of literature from sources such as, Google Scholar, PubMed, SCOPUS, and others.

4.3 Results and discussion

4.3.1 TARGETING BCL-2 AS A KEY PIVOT FOR APOPTOSIS AND ANOIKIS

A set of 102 phytochemicals known for their anti-tumor properties were docked with anti-apoptotic, pro-survival Bcl-2 protein. The docked phytochemicals bind to chain A of the Bcl-2 protein structure. The greatest docking score (10.6 kcal/mol) was achieved by the Dioscin (CID119245) at the Bcl-2 cavity, which was less than the control. ABT-737(Navitoclax) used as control in this study, have been shown holding strong binding affinity towards Bcl-2 proteins. ABT-737 is a strong inhibitor of Bcl-2 and Bcl-XL anti-apoptotic protein. It binds to Bcl-2 chain A and downregulates it, giving away anti-tumor effects and proving beneficial in cancer therapy. However, there are certain side effects like thrombocytopenia, neutropenia, fatigue and gastrointestinal toxicity associated with ABT-737(Wilson et al., 2010)(Mohamad Anuar et al., 2020). A number of phytochemicals showed competent ligand efficiency scores, suggesting they may serve as the basis for more research. Dioscin, Phillyrin, Caryophyllin, Bacopside, 2-(2-methylphenyl)-5-phenyl-1,3,4-oxadiazole, Gamabufotalin, Silibinin, Notoginsenoside R1, Sennoside A and Tenuifolin were top ranked 10 bioactive natural compounds which showed most pertinent interactions with Bcl-2. Only the complex with the best interactions and docking score, that was Dioscin, was further studied via MD simulation and binding energy calculations. The top 10 ranked docked bioactive compounds and their binding energies are listed in Table 4.1. Among the 102 natural compounds docked, Dioscin exhibited interactions with Bcl-2 that were most similar to those of the control molecule, unlike the other 9 natural compounds in the top 10 list.

Table 4.1 Bioactive compounds with top docking score

S. No	Compound	PubChem ID	Binding affinity	Source	Anti-cancer property	Reference
1.	Dioscin	CID 119245	-10.6 kcal/mol	Dioscorea nipponica (wild yam)	Pro-apoptotic, suppresses MAPK signalling pathway	(S. He et al., 2020)
2.	Phillyrin	CID 101712	-9.9 kcal/mol	Forysythia suspensa	Pro-apoptotic, cell cycle arrest, inhibits metastasis	(Q. Luo et al., 2024)
3.	Caryophyllin	CID 10494	-9.8 kcal/mol	Caryophyllaceae family plants (e.g. Clove plant)	Cytotoxic against certain cancer cell lines	(C. Delgado et al., 2021)
4.	Bacopside	CID 21599442	-9.7 kcal/mol	Bacopa monnieri(Brahmi)	Induces apoptosis, inhibits proliferation in cancer cells	(Ghosh et al., 2021)
5.	2-(2-methylphenyl)-5-phenyl-1,3,4-oxadiazole	CID 259999	-8.9 kcal/mol	Certain alkaloids and secondary metabolites	Interacts with DNA and inhibits topoisomerase enzyme	(D. Tiwari et al., 2022)
6.	Gamabufotalin	CID 259803	-8.6 kcal/mol	Bufo gargarizans (Chinese toad)	Induces apoptosis, inhibits metastasis by targeting cellular pathways	(L. Liu et al., 2023)
7.	Silibinin	CID 31553	-8.5 kcal/mol	Silybum marianum (milk thistle)	Apoptosis, induction, inhibition of angiogenesis and metastasis, cell cycle arrest	(Binienda et al., 2020)

8.	Notoginsenoside R1	CID 441934	-8.5 kcal/mol	Panax notoginseng (Tienchi ginseng)	Regulates immune responses against neoplastic cell growth and tumor cells, cell cycle arrest, induction of apoptosis	(P. Wang et al., 2023)
9.	Sennoside A	CID 73111	-8.3 kcal/mol	Senna Alexandrina (Tienchi ginseng)	Inhibits cancer cell growth and metastasis, inhibits migration and invasion in cancer cell lines	(Le et al., 2021)
10.	Tenuifolin	CID 21588226	-8.2 kcal/mol	Polygola tenuifolia	Reduces NFκB activity, therapeutic effect in mice brain cells	(Garg et al., 2024)

Interactions between Bcl-2 and ABT-737 (Control molecule) as analysed by Bioviadiscovery studio and PLIP has been listed in table 4.2(a)-4.2(c) and the docked structure is depicted in figure 4.1.

The Bcl-2 homology (BH) domains have been preserved in all Bcl-2 family proteins and these proteins interact with other molecules through their BH3 pocket. There are four BH interaction sites present on Bcl-2 protein. It is anticipated that a BH3 mimetic will imitate the BH3 interacting domain of pro-apoptotic Bcl-2 protein and deactivate the anti-apoptotic proteins by saturating their BH3 binding sites. Hence Bcl-2 is a crucial target that can be directly downregulated in anti-tumor therapies(Emi et al., 2005)(Kaloni et al., 2023). The docked phytochemicals were observed to bind with chain A of the Bcl-2 protein via different interactions. Among these, Dioscin (CID119245) recorded the highest level of docking score at the Bcl-2 binding pocket, scoring -10.6 kcal/mol of binding energy, which is a good score as compared with the control compound ABT-737. The protein-ligand interaction between Bcl- 2 and Dioscin has been shown in figure 4.2 and figure 4.3

Table-4.2(a) Hydrophobic interactions between Bcl-2 and ABT-737(Control)

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	101A	PHE	3.65	1715	610
2	101A	PHE	3.40	1675	614
3	101A	PHE	3.58	1695	616
4	109A	PHE	3.71	1695	724
5	134A	LEU	3.62	1692	965
6	145A	VAL	3.59	1720	1082
7	145A	VAL	3.71	1721	1083
8	146A	ALA	3.77	1693	1089
9	199A	TYR	3.74	1720	1614

Table 4.2(b) Hydrogen bonds between Bcl-2 and ABT-737 (Control)

Index	Residue	AA	Distance H-A	Distance D-A	Donor angle	Side chain	Donor atom	Acceptor atom
1	97A	ALA	3.23	3.63	103.74	Absent	1673[Npl]	580[O2]
2	100A	ASP	3.26	4.03	133.11	Present	1672[Npl]	604[O.co2]
3	199A	TYR	2.11	2.90	137.89	Present	1616[O3]	1724[O2]

Table 4.2 (c) π - stacking interactions between Bcl-2 and ABT-737 (Control)

Index	Residue	AA	Distance	Angle	Offset	Stacking type	Ligand atoms
1	199A	TYR	5.45	65.21	1.22	T	1702,1703,1704,1705,1706,1707

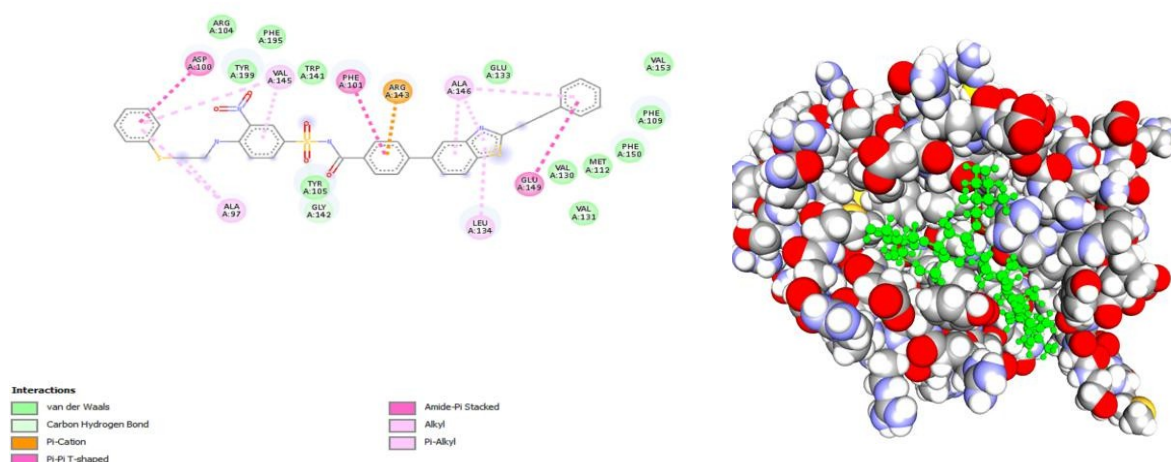


Figure 4.1 Docked structure of BCL-2 in complex with ABT-737, illustrating key interactions 2D (Left) and 3D (right) identified using BIOVIA Discovery studio

The molecular structure demonstrates the binding interactions between Dioscin and Bcl-2, highlighting key residues involved. The hydrophobic interactions were largely observed with the amino acid residues phe101a, phe109a, tyr199a and val145a. The distances between the atoms of the ligand Dioscin and protein atoms of the protein bcl-2, with the residues specifically interacting are listed in table 4.3(a)-4.3(c).

Table 4.3 (a) Hydrophobic interactions between Bcl-2 and Dioscin

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	101A	PHE	3.65	1715	610
2	101A	PHE	3.40	1675	614
3	101A	PHE	3.58	1695	616
4	109A	PHE	3.71	1695	724
5	134A	LEU	3.62	1692	965
6	145A	VAL	3.59	1720	1082
7	145A	VAL	3.71	1721	1083
8	146A	ALA	3.77	1693	1089
9	199A	TYR	3.74	1720	1614

Hydrogen bond formation occurred with the amino acid residues ALA97A, TYR199A, and ASP100A. The distance and bond angles between the ligand and protein atom bonds are detailed in table 3.5(a)-3.5(b). π - stacking interactions with TYR199A were observed which contributed to the stability of the docked molecules.

Table 4.3(b) Hydrogen bonds between Bcl-2 and Dioscin

Index	Residue	AA	Distance H-A	Distance D-A	Donor angle	Side chain	Donor atom	Acceptor atom
1	97A	ALA	3.23	3.63	103.74	Absent	1673[Npl]	580[O2]
2	100A	ASP	3.26	4.03	133.11	Present	1672[Npl]	604[O.co2]
3	199A	TYR	2.11	2.90	137.89	Present	1616[O3]	1724[O2]

Table 4.3(c) π - stacking interactions between Bcl-2 and Dioscin

Index	Residue	AA	Distance	Angle	Offset	Stacking type	Ligand atoms
1	199A	TYR	5.45	65.21	1.22	T	1702,1703,1704,1705,1706,1707

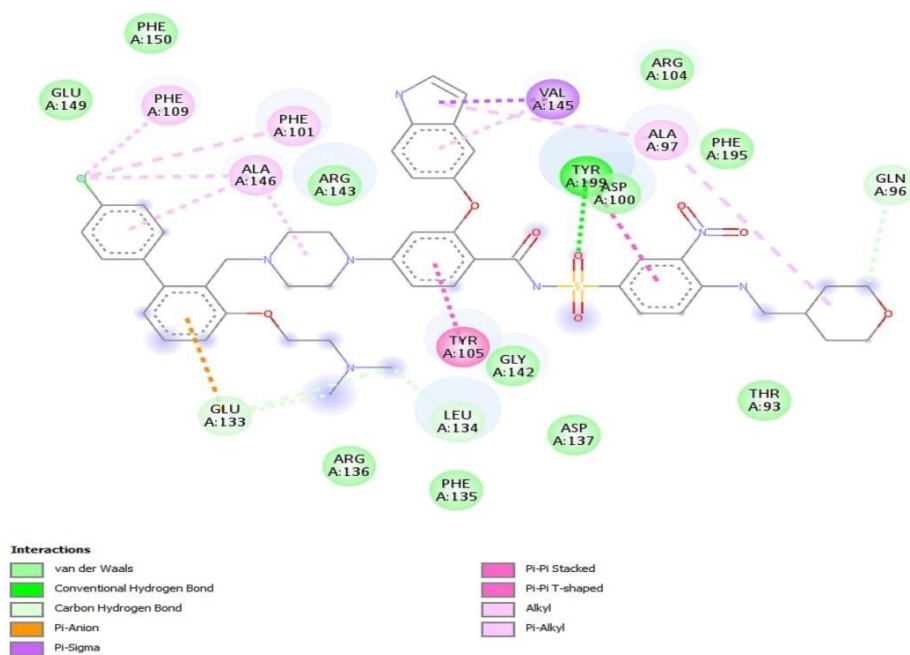


Figure 4.2 Dioscin and Bcl-2 complex after molecular docking, pertinent interactions by amino acid residues with ligand atoms.

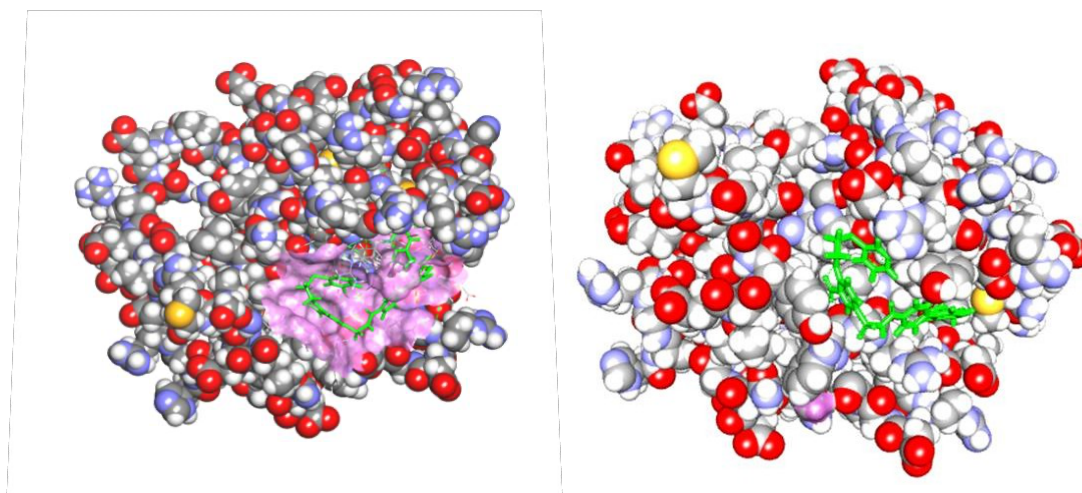


Figure 4.3 3D structure showing Bcl-2 and Dioscin complex bound to each other

Natural compounds have long been recognized for their targeted binding, minimal side effects, and diverse therapeutic properties, including anti-tumor, antioxidant, anti-apoptotic, and anti-inflammatory activities. Dioscin, which was the test molecule belongs to a Chinese herb and has been studied for its anti-cancerous properties. Bcl-2 on the other hand being the most frequently overexpressed protein in the tumor, is an appropriate target in order to induce anoikis (apoptosis upon detachment) in anoikis resistant conditions.

4.3.2 MOLECULAR DYNAMICS SIMULATION FOR STRUCTURAL AND BINDING DYNAMICS ANALYSIS

We extensively studied the interactions between Dioscin and Bcl-2 and ABT-737 and Bcl-2 in simulated conditions technically similar to the biological environment. To further explore how the two molecules interact with each other and whether they will be stable or not upon binding, we subjected these molecules to molecular dynamic simulations which helped us study their interactions at a deeper level. Therefore, we ran a 100 ns long molecular dynamic simulation using GROMACS software and examined the interactions between ligands and protein that were sustained throughout the simulation. The trajectory acquired from the simulation was examined to look at the stability of structure of receptor protein and ligand before looking at ligand-protein interactions. Root-mean square deviation (RMSD) and root mean square fluctuations (RMSF) were employed for quantifying the displacements of atoms and average distance between the atoms compared to the original structure for each frame and for observing major changes along protein and ligand structures. RMSF is the measure of flexibility at the residue level and is directly proportional to the deviation in the position of each residue over time. Figure 4.4 and 4.5 represents the bound molecular complexes of Bcl-2 and Dioscin and Bcl-2 and ABT-737 under simulation, respectively. In the structures obtained during simulations, the protein is shown in blue colour and the ligands in yellow. Dioscin interacted appreciably with a number of amino acid residue: PHE101, PHE109, TYR109, ALA97, VAL145, and ALA146. These interactions contributed to the complex's stability, mediated by hydrogen bonding, van der Waals, and hydrophobic interactions. Equally, ABT-737 showed interactions with residues like TYR109, GLY142, ASP100, and VAL145. ABT-737 is compared against this profile of interaction to determine how efficacies of Dioscin are for Bcl-2 binding.

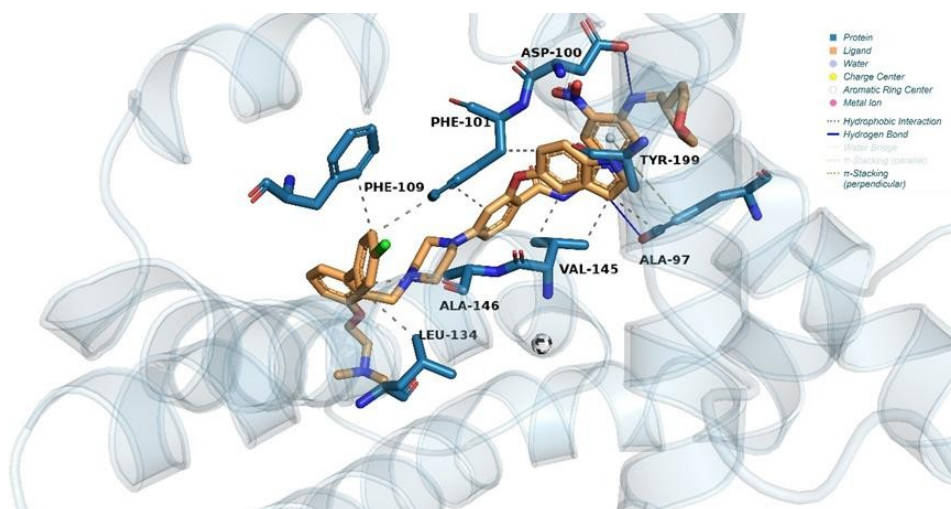


Figure 4.4 Residues interactions of natural compound Dioscin and antiapoptotic protein Bcl-2 during MDS. Protein is represented by blue colour and ligand is represented in yellow colour.

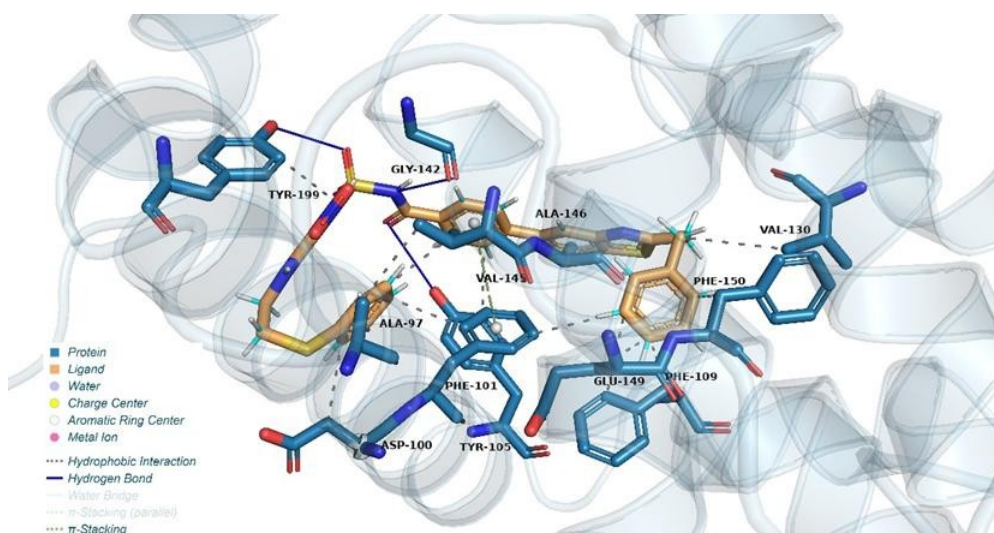


Figure 4.5 Residues interactions of the reference molecule (control) ABT-737 and antiapoptotic protein Bcl-2 during MDS. Protein is represented by blue colour and ligand is represented in yellow colour.

Chain A of Bcl-2 interacts with a variety of significant residues, including Asp100, Arg104, Tyr199, Tyr105, Phe101, Asp108, Phe109, Asn140, Gly142, Ala146, and Arg143, evident by the research conducted so far. An anti-apoptotic drug and popular down-regulator of Bcl-2 protein “Venetoclax” is able to fill four surface pockets, P1 through P4(Q. Niu et al., 2021) (Birkinshaw et al., 2019). Dioscin and ABT-737 had a variety of interactions similar to venetoclax, showing how they interact with Bcl-2. GLY142 and ARG143 emerged as key interacting residues in both control (ABT-737) as well as Dioscin complex with Bcl-2. This signifies that these residues may hold a pivotal role in the binding affinity and stability of the natural compound to the Bcl-2 protein. The conservation of these interacting residues across different compounds displayed their significance in designing a potent inhibitor of Bcl-2,

providing perceptions into the structural requirements for operative targeting, binding and inhibition of the anti-apoptotic function of this protein.

4.3.3 MM/GBSA AND MM/PBSA FOR ENERGY CALCULATIONS FOR THE PROTEIN-LIGAND COMPLEX

To further look into the binding affinity and stability of Dioscin with Bcl-2, binding free energy calculations, namely MM/GBSA and MM/PBSA, were carried out. To compare the outcomes, the values of same analysis obtained from the control molecule, ABT-737, were compared against Bcl-2. The binding free energy ($\Delta G_{\text{binding}}$) of the Bcl-2-dioscin and Bcl-2-ABT-737 complexes were calculated using the MM/GBSA and MM/PBSA methods, which fester the total binding energy into van der Waals, electrostatic (ΔG_{GAS}), and solvation (ΔG_{SOLV}) components. The MM/GBSA and MM/PBS results are detailed in the table 4.4(a) and 4.4(b).

Table 4.4(a) Binding energy calculations of Bcl-2 and Dioscin complex

Energy component (average)	MM/GBSA-Dioscin (Delta complex-receptor- ligand)	MM/PBSA-Dioscin (Delta complex-receptor- ligand)
$\Delta \text{Van der Waal}$	-69.74 kcal/mol	-69.74 kcal/mol
ΔG_{GAS}	-84.67 kcal/mol	-84.67 kcal/mol
ΔG_{SOLV}	-32.47 kcal/mol	-75.53 kcal/mol
$\Delta G_{\text{binding energy}}$	-53.21 kcal/mol	-9.18 kcal/mol

Table 4.4(b) Binding energy calculations of Bcl-2 and ABT-737 complex

DioscinEnergy component (average)	MM/GBSA-ABT-737 (Delta complex-receptor- ligand)	MM/PBSA-ABT-737 (Delta complex-receptor- ligand)
$\Delta \text{Van der waal}$	-55.37 kcal/mol	-55.420 kcal/mol
ΔG_{GAS}	-75.24 kcal/mol	-75.24 kcal/mol
ΔG_{SOLV}	45.85 kcal/mol	84.42 kcal/mol
$\Delta G_{\text{binding energy}}$	-29.39 kcal/mol	-9.14 kcal/mol

The analysis of binding free energy suggested that Dioscin possess a higher binding affinity than ABT-737 which is it's known inhibitor and is used as the control molecule. Favourable energy components like electrostatic energy, solvation energies and Van Der Waals interactions further support the strong binding energy and stability of the Bcl-2 and Dioscin complex.

Energy components of Dioscin and ABT-737 and their values obtained after MM/GBSA and MM/PBSA are mentioned in table 4.4(a) and 4.4(b), respectively. Binding free energy of

Dioscin calculated by MM/GBSA was -53.21 kcal/mol, and the same calculated by MM/PBSA was -9.18 kcal/mol.

The binding affinity of Dioscin is significant, suggesting its potential as a Bcl-2 inhibitor that directly targets its interaction sites, potentially suppressing Bcl-2 expression in tumor cells and inducing apoptosis. ABT-737, a well-established BH3 mimetic and Bcl-2 inhibitor, serves as a reference. Dioscin demonstrates strong interactions and direct binding with Bcl-2, comparable to the mechanism of ABT-737.

4.3.4 ROOT-MEAN SQUARE DEVIATION (RMSD) AND RMSF ANALYSIS FOR STUDYING CONFORMATIONAL CHANGES OF THE PROTEIN-LIGAND COMPLEXES

A comparative RMSD and RMSF graph of the examined ligands and protein receptor based on alpha carbons have been displayed in figure 4.6 and 4.7 respectively. The average distance between the atoms of the superimposed proteins depicts and narrates time variations in conformational stability of the protein-ligand complex. It can be observed from the RMSD graph (Figure 4.6) that both Dioscin and ABT-737 complexes with Bcl-2 stabilize after an initial fluctuation. However, the Dioscin complex shows a slightly higher RMSD value in comparison to ABT-737, thus showing a slightly higher degree of conformational flexibility. Nonetheless, Dioscin became stable in the interacting domain of the protein structure after around 50 ns and did not have much mobility. Dioscin has flexible sections and large structure in size, similar to the control molecule. As a result, it spreads from its first binding pocket to the another one (for example, hydrophobic grooves P1 to P4), which may explain why there were minor fluctuations. As shown in Figure 4.6, the RMSD values did not change much after around 50 ns and remained between 0.2-0.35 Å. Notably, the ligand was very stable in the first 10 ns and the RMSD values remained between 0.1-0.15Å before a fluctuation seen at around 20 ns. The protein structure fluctuated a little in the beginning of the simulation, but after 50 ns, RMSD stayed between 0.2-0.3 Å. ABT-737 interacted with Phe101 and Asp100 during most of the simulation period. Cation and H-bond interactions with Phe109 and Arg143 were also noted, besides Phe195 and Val 145 also had stacking interactions with Dioscin. Moreover, Dioscin interacted with the residues Arg143 & Phe101 and formed a sustained H-bond with Asp100.

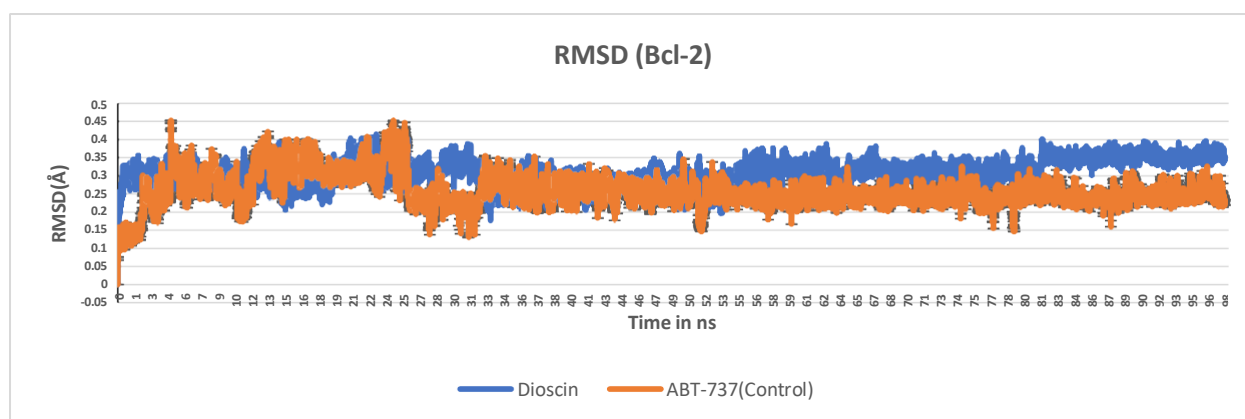


Figure 4.6 RMSD graph representing the comparative analysis of ABT-737 and Dioscin with respect to their binding to the Bcl-2 protein during MD simulations. The MD simulation's entire running time is depicted on the x-axis in nanoseconds. The RMSD values are displayed on the y-axis in Angstroms (Å).

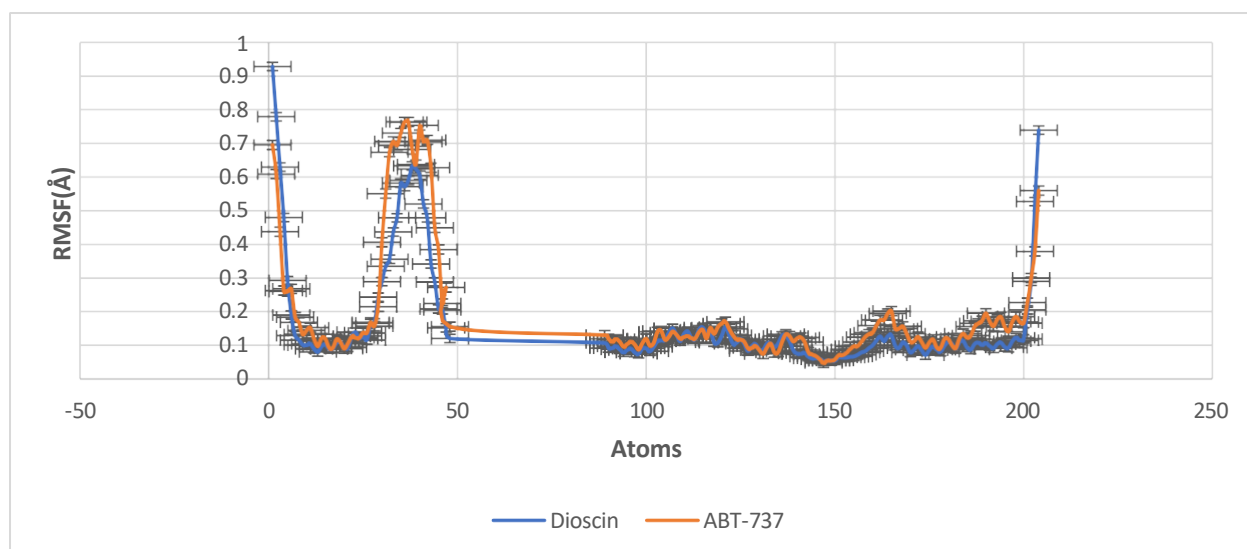


Figure 4.7 RMSF graph representing the comparative analysis of Dioscin and ABT-737 concerning their interactions with the Bcl-2 protein during molecular dynamics (MD) simulations. The RMSF values for both compounds are calculated, providing valuable insights into their dynamic behaviour and flexibility at the atomic level within the binding site.

The RMSF graph (Figure 4.7) depicts the flexibility of residues in the Bcl-2 protein bound with Dioscin (blue line) and ABT-737 (orange line). In general, the pattern of fluctuation is almost conserved in the two ligands, and only a few residues show a gain in flexibility in the Dioscin complex, indicating that the binding with Dioscin could significantly promote local flexibility to a greater extent.

4.3.5 RADIUS OF GYRATION

we did Radius of gyration (Rg) analysis of all the frames (10000) generated during 100 ns simulation, following MM/PBSA and MM/GBSA, RMSD and RMSF analysis. The Rg values for the Dioscin and Bcl-2 complex showed fluctuation at 10 ns point, falling between 1.34 and 1.45 nm (Figure 4.8). Following that, a decline was seen, and the values stayed between the range 1.36 nm-1.42 nm for the last 15 ns of the simulation.

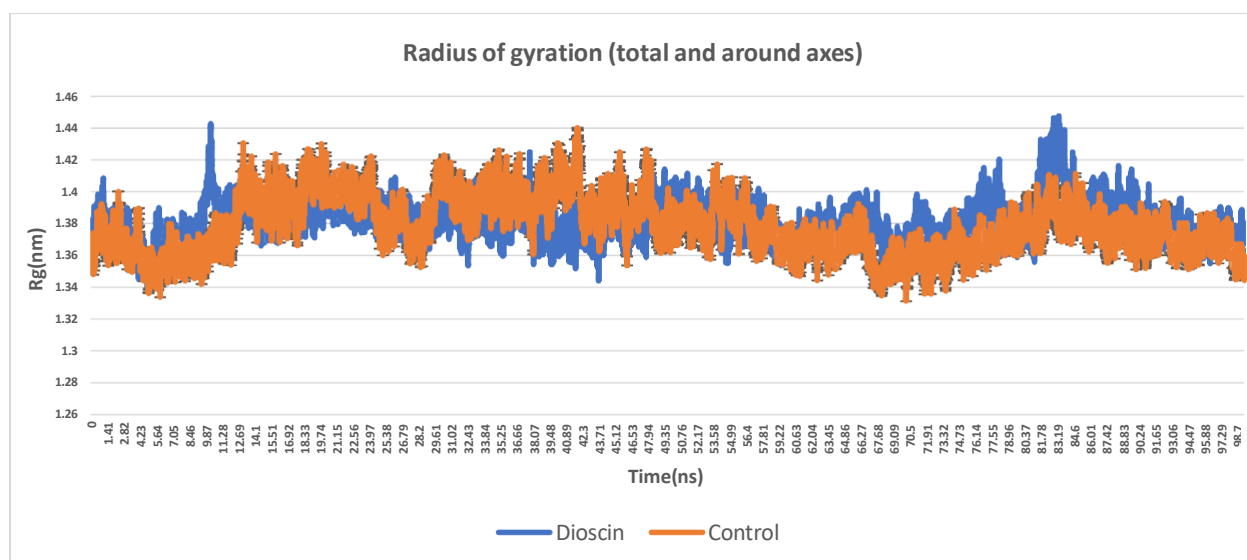


Figure 4.8 The results of the radius of gyration (Rg) analysis for the screened Bcl-2 systems during the 100 nanosecond molecular dynamics (MD) simulation are shown in this figure. The y-axis shows the Rg values in nanometres (nm), while the x-axis shows the simulation time in nanoseconds (ns).

During simulations, Rg, a structural parameter of the distance, spatial distribution and compactness of molecule, offers important insights into the compactness, stability, and folding behaviour of a receptor. Low and steady Rg values point to a tightly packed and well-folded structure, whereas high and erratic fluctuations point to folding instability (Ghahremanian et al., 2022). The Bcl-2-Dioscin complex is well-folded and stable, which is reflected in its Rg values. There were fluctuations for ABT-737(control) throughout the simulation, it is acceptable that they stayed within a reasonable range of 1.34 nm-1.44 nm (Figure 4.8). The normal folding pattern and ligand binding were not significantly affected by these fluctuations. It has been previously reported that the Rg value of complex Bcl-2-venetoclax (known inhibitor of Bcl-2 and FDA approved drug against several cancers) was 1.51 nm which displayed a highly stable structure(Birkinshaw et al., 2019)(Krishna et al., 2021). This analysis shows that the receptor attained a compact state during the simulation and that there were no sharp fluctuations, highlighting the protein's stable folded state upon binding to the target compounds.

4.3.6 HYDROGEN BOND FORMATIONS BETWEEN BCL-2 COMPLEXES.

Our main focus in this analysis was to study the amino acid residue involved in H-bond formation and the percentage of that particular bond. In the MD simulation, the "H-bond percentage" shows how hydrogen bonds are distributed among various amino acid residues. It details the relative importance of different residues in relation to the overall number of hydrogen bonds formed(Almansour et al., 2023)(Krishna et al., 2021). This knowledge sheds light on the role of various residues in maintaining the system's molecular interactions and structure(Dalal et al., 2021).

We analysed the MD trajectories to determine H-bond formation throughout the entire 100 ns simulation, to determine the number and percentage of H-bonds formed by Dioscin and ABT-737 in their interactions with Bcl-2 as shown in Figure 4.9 (a and b). ABT-737 formed noteworthy number of H-bonds, up to a maximum of 6 bonds at different times with the receptor protein. This finding suggests a robust affinity between ABT-737 and the target protein. Additionally, the complex consistently maintained the formation of two H-bonds that

involved the amino acid residues ASN40 (Asparagine) and GLY142 (Glycine) during simulation (Figure 4.9 a), demonstrating the stability of its structural composition.

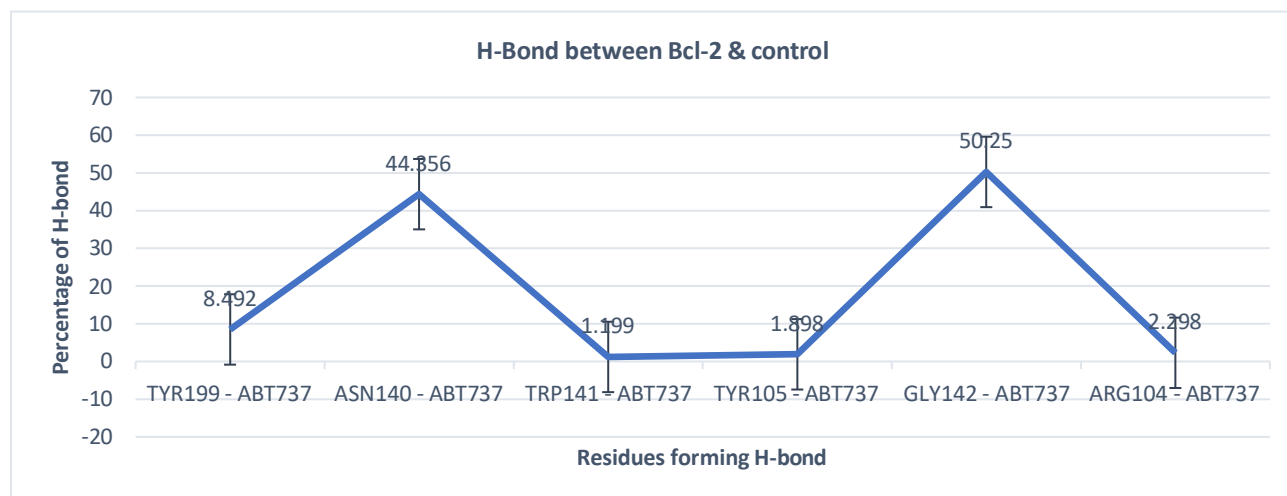


Figure 4.9 a) H-bonds formed between ABT-737(control) and Bcl-2 protein formed during entire MD simulation. Percentage of H-bond is represented on the Y-axis while amino acid residue involved in the H-bond formation is represented on the X-axis. Asparagine (ASN140) and glycine (GLY142) displayed highest percentage(44.5% and 50.25% respectively) of H-bond formation during MD simulation.

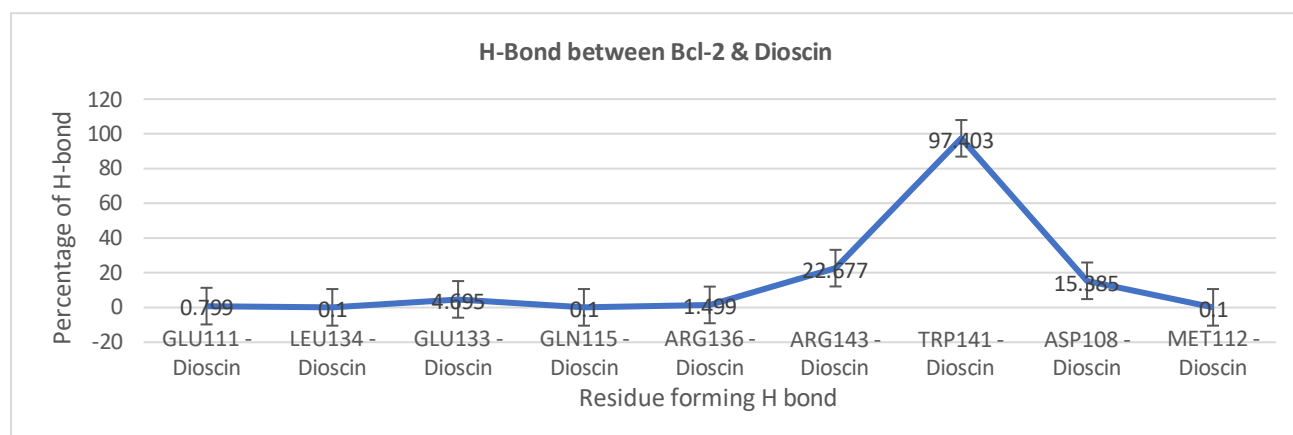


Figure 4.9 b) H-bonds formed between Dioscin and Bcl-2 protein formed during entire MD simulation. % of H-bond is represented on the Y-axis while amino acid residue involved in the H-bond formation is represented on the X-axis. Aromatic amino acid tryptophan represents highest percentage (97.4%) of H-bonds formed during MD simulation, while Arginine (ARG143) being the second highest (22.6%) of it.

Notably, Dioscin formed nearly 9 H-bonds in the BCL2-dioscin complex over the course of the most of the simulation. The interaction with the tryptophan (TRP 141) residue resulted in the formation of a H-bond which was nearly 97.4% of the total hydrogen bonds in the system involved during entire 100 ns MD simulation.

Hydrogen bonds play a significant role in the stability of protein–ligand complexes. Thus, the hydrogen bonds analysis gives us the number and percentage of hydrogen bond formation during the simulation run. The hydrogen bonding analysis of Bcl-2 shows a graphical

representation of the percentage of hydrogen bonds Bcl-2 makes with the ligands Dioscin and ABT-737 during the runtime of the simulation. Among those percentages, the residues ASN140 and GLY142 showed the highest percentage of hydrogen bonds when analysed along with ABT-737; 44.35% for ASN140 and 50.25% for GLY142 illustrated that these had more interactions with the ligand and this type of interaction was rather consistent. The remaining percentage of hydrogen bonding was with Dioscin, which was comparatively less in number but maintained high stability in the complex.

The number, percentage and amino acid residues of Bcl-2 involved in H-bond formation with ABT-737 and Dioscin, respectively, has been displayed by graphs in figure 4.9(a) and 4.9(b). During the majority of 100 ns simulation, Dioscin consistently formed a number of H-bonds with the Bcl-2 amino acid residues. The interaction with the amino acid tryptophan (TRP14) was notable, accounting for approximately 97.4% of all the H-bonds formed throughout the simulation (Figure 4.9b). Notably, tryptophan which is an aromatic amino acid bearing the indole-ring structure contributes effectively to the stability and strength of this interaction (Maksimov et al., 2020)(Ainsley et al., 2018)(de Jesus & Allen, 2013). The sturdiness and frequency of this particular interaction signifies that Dioscin binds to Bcl-2 structure in a durable and remarkably stable manner. As compared to ABT-737, this molecule also showed strong binding affinity by maintaining constant, multiple H-bonds with the protein. Nevertheless, noteworthy number of H-bonds formed by Dioscin, particularly the tryptophan-Dioscin H-bond, highlights its compelling and stable binding characteristics. This proposes that Dioscin could possibly be a more effective molecule for targeting and direct binding to Bcl-2 due to its capability to form such a stable interaction through H-bonding.

4.3.7 STATISTICAL ANALYSIS OF GENE EXPRESSION MICROARRAY DATA TO ASSESS THE EFFECT OF DIOSCIN ON COMMON GENES AMONG THE DIFFERENTIALLY EXPRESSED GENES ACROSS VARIOUS TUMOR SAMPLES.

The effect of Dioscin on gene expression of cancer cell line MDA-MB-231 was assessed using the gene expression analysis tool GEO2R. Data normalization and categorization of genes was first done using GEO2R (Figure 4.10). P-value threshold less than 0.05 was considered as statistically significant. LogFC value below 1 and above 1 was taken into consideration for downregulated and upregulated gene expression identification respectively. The results revealed that Dioscin has a regulatory impact on the four genes Bax, Bcl-2, BIRC3 and CHUK which are associated with apoptosis, anoikis, EMT, autophagy and metastasis. Bax which is a pro-apoptotic gene had LogFC value 0.0534 indicating that it can be affectively upregulated by Dioscin, while Bcl-2, BIRC3 and CHUK had a negative logFC value indicating that these 3 anti-apoptotic and pro-survival genes can be significantly downregulated by Dioscin.

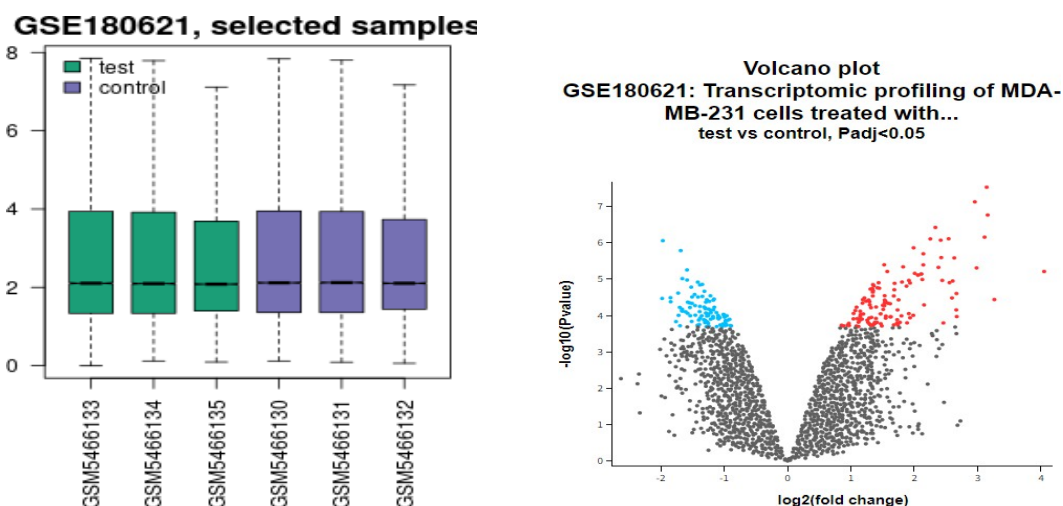


Figure 4.10 Dot plot (left) and box plot (right) illustrating the normalization and categorization of data points, representing differentially expressed genes.

Table 4.5(a): Gene expression regulation by Dioscin

Gene symbol	logFC	Value
BCL2	-6.79E-01	0.01292
BAX	5.34E-02	0.010049
BIRC3	-2.54E-01	0.026049
CHUK	-1.08E-02	0.00766

The negative logFC value for anti-apoptotic and pro-survival Bcl-2 expression level after Dioscin treatment indicates that Dioscin significantly downregulated the gene expression of Bcl-2 in MDA-MB-231 cells, where the p-value was 0.01292 and logFC value was -6.79, indicating a statistically significant downregulation of this gene (Table 4.5(a)). Low expression of Bcl-2 would result in less chances of inhibition of pro-apoptotic pathways, thus regulating apoptotic cell death, which in this context is a desired outcome of cancer treatment, since that can be effective in managing anoikis resistance. The logFC value of Bax expression after Dioscin treatment was 0.0534, hence implying that Dioscin upregulates the expression of Bax in MDA-MB-231 cells. The p-value that resulted from this upregulation is 0.010049, meaning that the result is significant. The induction of an increase in Bax levels after treatment with Dioscin indicates that Dioscin augments the apoptotic pathway such that it can cause the induction of programmed cell death in cancer cells by regulating the apoptotic protein and genes expression. Elevations in Bax levels arm the pro-apoptotic machinery of the cell and further push the cells into apoptosis. The BIRC3 gene which codes for the inhibitor of apoptosis proteins (IAPs) that maintain cell survival by inhibiting caspase activation had logFC value -0.254 indicating that Dioscin may cause the downregulation of this gene in MDA-MB-231 cells, by suppressing its expression. CHUK which is also termed as IKK α , is highly associated with the NF- κ B pathway regulating the activation of genes responsible for cell survival and cell proliferation. The negative logFC value (-0.0108) of CHUK after gene expression analysis upon Dioscin treatment indicates that Dioscin might affect the growth and survival of MDA-MB-231 cells through downregulation of the CHUK gene. Downregulation of CHUK points toward an inhibitory role of Dioscin on the activity of the NF- κ B pathway, which then correlates to reduced ability of the cells to proliferate and survive. This may challenge the cell to make it less likely to progress through the cell cycle. Therefore, it will contribute to apoptosis and anoikis activation. Dioscin has been extensively investigated in both cellular and animal

models, demonstrating its efficacy in combating cancer cells through diverse mechanisms. These mechanisms include the reduction in cancer cell population, inhibition of proliferation and survival, induction of cell cycle arrest, and promotion of cell death. According to the findings of our study, Dioscin downregulates anti-apoptotic proteins like Bcl-2, CHUK (IKK α) and BIRC3 while it upregulates pro-apoptotic proteins like Bax.

4.3.8 EFFECT OF NATURAL COMPOUNDS ON GENE EXPRESSION REGULATION OF THE DIFFERENTIALLY EXPRESSED GENES IN ANOIKIS RESISTANCE

The primary objective of this analysis was to recognize the genes whose expression can be significantly altered by specific bioactive natural compounds. To achieve this, a compiled list of DEGs encompassing all 102 bioactive compounds was tested and their corresponding logFC (log fold change) values were compared. From the huge dataset of all the natural compounds and thousands of genes, we screened those natural compounds that had maximum potential to downregulate the maximum number of genes among the identified 19 commonly upregulated genes while upregulating the maximum number of genes among the identified 34 commonly downregulated genes. This dual criterion ensured that the compounds selected would have a maximum impact on reverting the dysregulated gene expression profile associated with the cellular condition under study. Consequently, a comprehensive list of differentially expressed genes was compiled, encompassing all 102 bioactive compounds and their corresponding logFC values. From this list, natural compounds that demonstrated the highest potential to downregulate the highest number of genes among the 19 common upregulated genes, while simultaneously upregulating the maximum number of genes among the 34 common downregulated genes were identified. These DEGs that we obtained from the 3 GEO datasets (GSE100629, GSE106363, GSE46602) were compared to the DEGs affected by the 102 bioactive natural compounds. The bioactive compounds were evaluated and ranked according to their capacity to reverse the expression of the highest number of genes among common DEGs. The work flow and findings for this methodology is demonstrated in figure 4.11. Bioactive natural compounds exhibit multitargeting properties, enabling them to target multiple pathways involved in tumor suppression (Dehelean et al., 2021)(Grisoni et al., 2019). This characteristic is particularly advantageous as tumor cells exhibit rapid changes in gene morphology when exposed to drugs having a single-target in an attempt to escape their effects. Interestingly, by utilizing combinations of natural compounds, the issue of drug resistance can be minimized (Z.-F. Zhong et al., 2017)(Q. Guo et al., 2017). These combinations act on multiple pathways, effectively reversing tumor progression (Radziejewska et al., 2021).

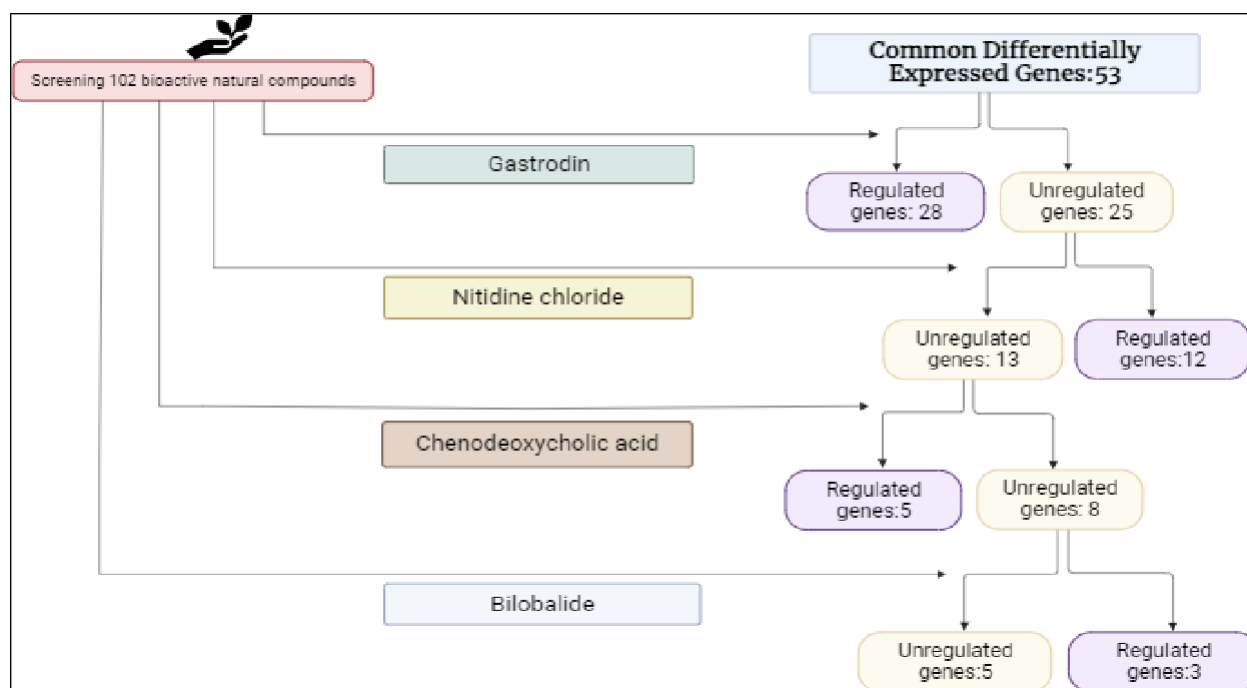


Figure 4.11 Effect of Gastrodin, nitidine chloride, CDCA and Bilobalide on common differentially expressed genes. Gastrodin alone regulated the highest number of genes while bilobalide alone regulated minimum number of genes. There were 5 genes which were not regulated by any compound alone.

The results, shown in a bar plot (Figure 4.12) and detailed in Tables 4.6 (a) and 4.6 (b), highlight the changes in expression levels of differentially expressed genes (DEGs) following treatment with four selected bioactive compounds: Gastrodin, Nitidine chloride, Chenodeoxycholic acid, and Bilobalide.

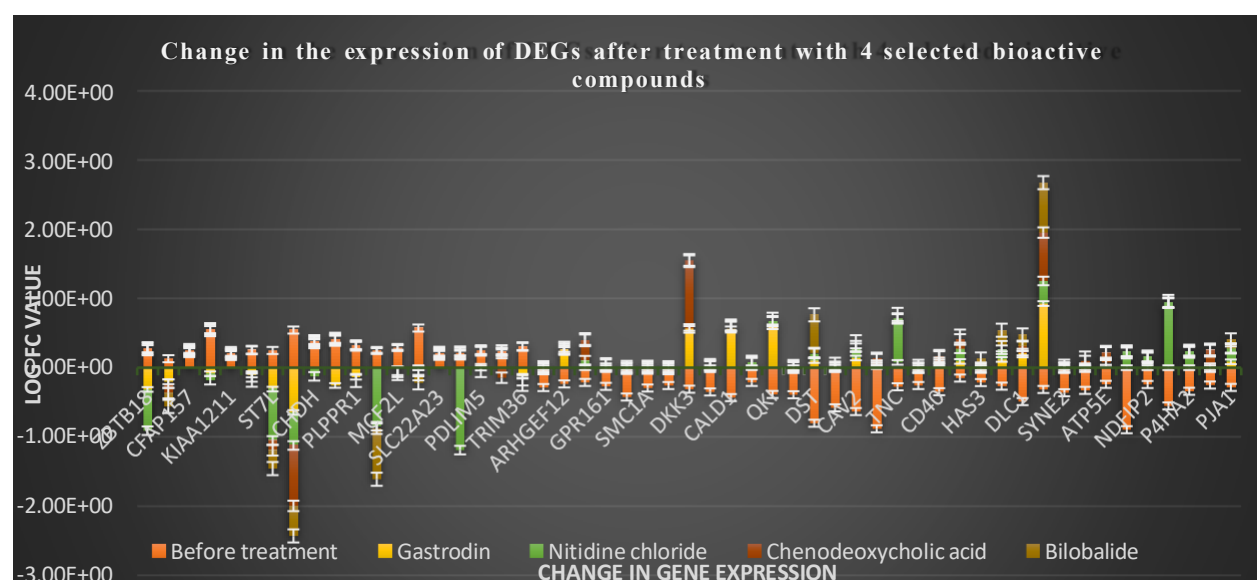


Figure 4.12 Each bar in the graph represents the fold change in gene expression levels upon treatment with the respective natural compound. Positive values indicate upregulation, while negative values signify downregulation. The differential expression patterns highlighted in this graph underscore the potential of these bioactive natural compounds in modulating anoikis resistance, thereby a promising avenue for therapeutic intervention in cancer treatment.

Various coloured bars used in the graph correspond to logFC for each gene with every compound tested. All natural compounds exhibited change in gene expression of these genes, but the above mentioned four bioactive compounds are those which affected the gene expression of maximum number of genes.

Table 4.6(a) Change in gene expression of the downregulated genes by natural compounds

S.No	Gene Symbol	Anoikis resistance cell	Gastrodin	Nitidine chloride	Chenodeoxycholic acid	Bilobalide
1.	ZNF827	-2.87E-01	NaN	NaN	NaN	NaN
2.	ARHGEF12	-2.35E-01	0.263231	NaN	0.013814	0.003265
3.	GJA1	-2.13E-01	NaN	0.108454	0.296321	NaN
4.	GPR161	-2.70E-01	NaN	NaN	0.043053	NaN
5.	STAT3	-3.24E-01	NaN	NaN	NaN	0.004055
6.	SMC1A	-2.94E-01	NaN	NaN	0.00668	NaN
7.	NFKBIZ	-2.58E-01	NaN	NaN	NaN	NaN
8.	DKK3	-3.11E-01	1.567387	NaN	1.980837	NaN
9.	CREM	-3.51E-01	NaN	0.031554	NaN	NaN
10	CALD1	-4.32E-01	0.562215	0.040697	NaN	NaN
11	IDH3A	-2.13E-01	NaN	0.079249	NaN	NaN
12	QKI	-3.87E-01	0.591117	0.079735	NaN	0.029429
13	LPAR1	-3.91E-01	NaN	0.017725	NaN	NaN
14	DST	-8.03E-01	0.102628	0.110166	NaN	0.551408
15	ADGRG6	-5.80E-01	0.006054	NaN	0.007432	0.036223
16	CAV2	-6.34E-01	0.139675	0.148186	0.024126	0.062891
17	NR5A2	-8.83E-01	0.044311	NaN	0.079335	NaN
18	TNC	-2.76E-01	0.076381	0.635391	NaN	0.059044
19	ZNF638	-2.58E-01	NaN	NaN	NaN	0.019456
20	CD40	-3.47E-01	0.083574	NaN	0.078604	NaN
21	SKIL	-1.50E-01	0.089878	0.182966	0.136163	0.047372

22	HAS3	-2.15E-01	NaN	NaN	NaN	0.125065
23	ANXA2	-2.67E-01	0.11882	0.140217	0.052083	0.230658
24	DLC1	-4.90E-01	0.211333	NaN	0.104308	0.15705
25	KIF5C	-3.15E-01	0.926634	0.327094	1.298901	1.121718
26	SYNE2	-3.62E-01	0.003317	NaN	NaN	0.017796
27	EPHA7	-3.28E-01	NaN	NaN	0.096632	0.042363
28	ATP5E	-2.41E-01	0.075144	NaN	0.146133	NaN
29	ZNF711	-8.96E-01	NaN	0.22867	NaN	NaN
30	NDFIP2	-2.43E-01	NaN	0.154308	NaN	NaN
31	PCDH7	-5.53E-01	NaN	0.940553	NaN	0.017829
32	P4HA2	-3.41E-01	NaN	0.23767	NaN	NaN
33	SCD5	-2.51E-01	NaN	NaN	0.254308	NaN
34	PJA1	-2.85E-01	0.0848	0.19389	NaN	0.123144

Table 4.6(b) Change in gene expression of up-regulated genes by natural compounds

S.No	Gene Symbol	Anoikis resistance cell	Gastrodin	Nitidine chlorid e	Chenodeoxycho lic acid	Bilobalid e
1.	ZBTB18	2.74E-01	-0.31432	-0.59629	NaN	NaN
2.	MYLIP	1.30E-01	-0.20438	-0.02506	-0.13868	-0.19241
3.	CFAP157	2.48E-01	NaN	NaN	NaN	NaN
4.	MICAL2	5.50E-01	-0.08765	-0.09322	NaN	NaN
5.	KIAA1211	2.05E-01	NaN	NaN	NaN	NaN
6.	TRIM3	2.55E-01	-0.10615	NaN	-0.02089	-0.05351

7.	ST7L	2.49E-01	-0.30959	- 0.7413 7	-0.14338	-0.26358
8.	SSTR1	5.44E-01	-0.64675	- 1.1772 1	-1.17335	-0.43359
9.	CHDH	3.76E-01	NaN	- 0.1251 5	NaN	NaN
10	PARD3 B	4.11E-01	-0.25721	NaN	NaN	NaN
11	PLPPR1	3.21E-01	-0.13811	NaN	NaN	-0.04636
12	MLXIP	2.49E-01	NaN	- 0.8450 2	-0.03702	-0.72997
13	MCF2L	2.92E-01	NaN	- 0.0454 2	-0.01546	-0.02348
14	ZSCAN 12	5.73E-01	-0.00016	- 0.0413 1	-0.066	-0.11182
15	SLC22A 23	2.08E-01	NaN	NaN	NaN	NaN
16	LILRB4	2.12E-01	NaN	- 1.1910 3	NaN	NaN
17	PDLIM5	2.49E-01	-0.00616	NaN	NaN	-0.03501
18	AZIN2	2.30E-01	NaN	NaN	-0.14542	NaN
19	TRIM36	3.06E-01	-0.12387	NaN	-0.05824	-0.05889

Total number of genes that were regulated by the natural compounds were 53. There were 3 genes-ZNF827, SLC22A23, KIAA1211 which were not affected by any of the natural compounds.

4.3.9 COMBINATORIAL EFFECT OF MULTIPLE NATURAL COMPOUNDS ON ANOIKIS REGULATION

In our study, we analyzed 53 DEGs to identify bioactive natural compounds capable of modulating their expression. Among the 102 compounds screened, gastrodin emerged as the most effective, significantly altering the expression of 28 genes. Gastrodin, a primary bioactive compound found in *Gastrodia elata*, has demonstrated anticancer properties in various studies. For instance, it has been shown to inhibit proliferation in human glioma cells by modulating gene expression related to apoptosis and cell cycle regulation(W. Cao et al., 2023).

Subsequently, we focused on the remaining 25 DEGs to assess whether their expression could be influenced by other potent bioactive natural compounds. Natural compounds have been recognized for their ability to modulate gene expression through epigenetic mechanisms,

thereby influencing cancer progression and treatment outcomes. For example, certain natural products can regulate microRNA expression, impacting cancer cell proliferation and apoptosis (Aly et al., 2024). The combination of four compounds—Gastrodin, Nitidine chloride, Chenodeoxycholic acid (CDCA), and Bilobalide—successfully restored the expression levels of 8 genes among the 53 common DEGs. To assess their impact, the expression of these 53 differentially expressed genes was compared with the expression induced by each compound individually. Gastrodin, nitidine chloride, chenodeoxycholic acid, and bilobalide individually regulated 28, 12, 5 and 3 genes, respectively. The selected combinations of bioactive natural compounds were further subjected to a comprehensive analysis. This analysis involved examining how the combinations of various bioactive natural compounds affected the anoikis resistance genes and process and pathways related to them. The combined effect of the bioactive natural compounds on gene regulation, cellular mechanisms, and signalling pathways was assessed by analysing the interactions and synergistic effects of these compounds. Additionally, the potential therapeutic benefits and implications of the combinations of these bioactive compounds in the context of prostate cancer were explored. This thorough analysis gave rise to a deeper understanding of effectiveness and potential uses that these bioactive natural compound combinations possibly have. The observations suggested potential synergistic effects among the compounds. Furthermore, the alteration of gene expression by multiple compounds proves beneficial in preventing drug resistance and mitigating toxic side effects, as redundant pathways can be engaged through alternative mechanisms (Mann, 2002) (Chamberlin et al., 2019). Figure 4.12 and figure 4.13 reveal that 8 genes—MYLIP (Myosin regulatory light chain interacting protein), ST7L (Suppression of tumorigenicity), SSTR1 (Somatostatin receptor 1), ZSCAN12 (Zinc finger and SCAN domain containing 12), SKIL (SKI-like proto-oncogene), ANXA2 (Annexin A2), KIF5C (Kinesin family member 5C), and CAV2 (Caveolin 2)—were regulated by all four compounds. The analysis revealed that these genes were associated with critical processes such as, regulation of nucleic acid metabolism, apoptosis, signal transduction, immune response and autophagy which was also made significant by the enrichment analysis study. The genes SSTR1, ST7L, SKIL, ANXA2, and CAV2 among all the genes which have undergone enrichment analysis, revealing their active involvement in various signaling pathways and biological processes. Notably, these genes are regulated by all four natural combinations. Collectively, these genes participate in critical signaling pathways that, when dysregulated, contribute to cancer progression through mechanisms affecting cell proliferation, survival, and metastasis. These factors are closely linked to immune system functionality along with cancer progression (C.-Y. Chen et al., 2018). Consequently, the combined targeting of these genes holds significant promise in effectively combating cancer. Furthermore, Figure 4.13 presenting a Venn Diagram represents the overlapping regulatory effects of the four bioactive compounds on the gene expression of the common up regulated and downregulated genes. The number of genes in each section of the Venn diagram represent the number of genes regulated by that respective compound. To start with, there are 8 genes MYLIP, ST7L, SSTR1, ZSCAN12, SKIL, ANXA2, KIF5C, and CAV2 that are altogether regulated by all four compounds, stating a noteworthy overlap in their mechanism of action. These 8 genes Nitidine chloride alone regulated 16.7% of the total DEGs. CDCA in green alone regulated 4 genes contributing to regulate 8.3% of the total DEGs. Gastrodin in blue area alone regulated only 1 gene (2.1% of the total DEGs). Lastly, Bilobalide independently regulated 3 genes representing 6.3% of the total DEGs regulated by all compounds.

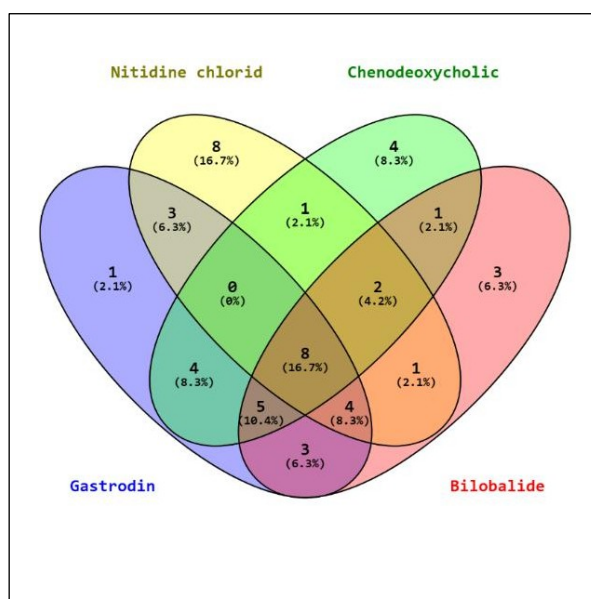


Figure 4.13 The Venn diagram illustrates how various bioactive natural compounds regulate the gene expression of 53 common DEGs. Among all the genes, eight genes were regulated by all 4 compounds.

In combinations of nitidine chloride and CDCA together they regulated 8 genes, that is 16.7% of the total genes. CDCA along with Bilobalide together regulated 3 genes that is 6.3% of the total number of genes. Gastrodin and Bilobalide in combination also affected 3 genes that is again 6.3% of the total genes. Given that natural compounds are known for their multitargeting nature, it is crucial to consider the potential side effects when targeting specific genes that strongly impact cancer regression (Ho et al., 2018) (Taylor et al., 2019) (Muniraj et al., 2019). Therefore, in order to evaluate the synergistic potential of the selected bioactive natural compounds, their individual mechanisms of action were thoroughly examined by retrieving information from the existing literature.

One of the screened bioactive natural compounds gastrodin is known for its anti-inflammatory properties. It lowers the activity of inflammatory enzymes and prevents the generation of pro-inflammatory cytokines, hence reducing inflammatory processes in the body. Gastrodin, derived from *Gastrodia elata* Bl., serves as a prominent monomer component and finds extensive clinical applications (Muniraj et al., 2019). Its therapeutic potential in neurological diseases is well-established, as it exerts neuroprotective effects through multiple pathways. These include combating oxidative stress, suppressing neuroinflammatory responses, modulating neurotransmitter levels, regulating neural remodelling, inhibiting apoptosis, and attenuating autophagy (Yao et al., 2019) (Y. Liu et al., 2018). Additionally, the combined administration of gastrodin and an alkloid Rhy (Isorhynchophylline) has demonstrated efficacy in mitigating brain damage induced by ischemia (H.-S. Zhang et al., 2019). Gastrodin has also been studied for its anti-cancer properties and it has been stated that it can act as an immunomodulator and anti-tumor agent in hepatocellular carcinoma. As gastrodin, has the ability to inhibit tumor cell growth in vivo with minimal toxicity, it holds promise as an adjunctive agent in hepatocellular carcinoma (HCC) therapy (Shu et al., 2013). Gastrodin has been found to downregulate the levels of interleukin-4 (IL-4), a representative Th2 cytokine known to impair the cytotoxic activities of CD8⁺ T cells and natural killer (NK) cells. Consequently, gastrodin administration leads to an increase in the cytotoxic activities of CD8⁺ T cells and NK cells, suggesting that gastrodin mitigates the systemic immune suppressive

Previous scientific studies have extensively investigated the diverse effects of CDCA, a primary bile acid, on various carcinomas. Natural bile acids like CDCA are produced in the liver and are stored within the gallbladder. Its primary role revolves around the metabolism of cholesterol and the absorption and digestion of fat. CDCA helps the body emulsify fats, create micelles for better absorption of substances that are fat-soluble, and excrete extra cholesterol through bile. Additionally, it supports healthy liver function and encourages gallbladder contraction during digestion. Certain animal-based sources contain CDCA, which is essential for lipid metabolism and a healthy digestive system. Additionally, it is employed in the treatment of particular liver and gallbladder ailments. In the scenario of prostate cancer cells, CDCA has shown the capability to control the cell cycle and prompt apoptosis. This is achieved by Cdk2 and therefore cyclin E-dependent kinase are inhibited but their expression levels are left unaffected (Y. H. Choi et al., 2003). Similar to this, CDCA has been reported to induce oxidative stress-induced apoptosis in cases of colon adenocarcinoma. This stimulation includes an increase in ROS production, which in turn causes the plasma membrane enzymes to get stimulated (Shen, n.d.). Additionally, it has been demonstrated that CDCA increases cell apoptosis in lung adenocarcinoma (LUAD) via upregulating the production of p53 through the integrin $\alpha 5 \beta 1$ signalling pathway. It is well-known that integrin $\alpha 5 \beta 1$ significantly contributes to the development of numerous cancer types, and CDCA has proven to be an efficient integrin $\alpha 5 \beta 1$ inhibitor (Shen et al., 2022). Previous studies looked into the possibility of a synergistic interaction between CDCA and sorafenib, a targeted therapy drug for cancer. The results suggest that by blocking the EGFR/Stat3 signalling pathway, the combination of CDCA and sorafenib may increase the anti-tumor effects of sorafenib (Y. Zhang et al., 2022).

Ginkgo biloba leaves contain the phytochemical called bilobalide that has a number of pharmacological properties, such as neuroprotection, anti-inflammatory, and anticonvulsant actions (Shi et al., 2010) (Jiang et al., 2014). Bilobalide has been found to inhibit the growth of FaDu human pharyngeal squamous cell carcinoma cells by causing nuclear condensation and DNA fragmentation in the context of cancer cells. This process is accompanied by the activation of caspase-3, caspase-7, caspase-8, and caspase-9, which are proteolytic enzymes involved in cell apoptosis pathways (Jeong et al., 2020). Bilobalide inhibits cell proliferation and induces apoptosis in FaDu through both the death receptor-mediated extrinsic apoptotic pathway and the mitochondrial-mediated intrinsic apoptotic pathway (Jeong et al., 2020). Taken together these findings indicate that, bilobalide influences the balance between antiapoptotic and proapoptotic proteins in cancer cell lines, as it downregulates the antiapoptotic protein Bcl-2 and upregulates the proapoptotic protein Bax. These actions result in a significant increase in the Bax/Bcl-2 ratio (Jeong et al., 2020). Figure 4.13 depicts the comprehensive impact of our drug combinations on multiple pathways, indicating their ability to target various processes and pathways simultaneously. By offering a multifaceted antitumor and immunomodulatory role, these drug combinations demonstrate high potency in promoting tumor regression. Additionally, the biological network illustrated in the figure 4.14 highlights the key genes regulated by these compounds, further emphasizing their therapeutic potential. Irregular expression of these factors within cancer leads to challenges like resistance to anoikis and the dissemination of CTCs. The four natural substances scrutinized in this study specifically address these elements, potentially positioning them as valuable tools in cancer treatment. Unlike contemporary approaches such as androgen receptor therapy in prostate cancer, these natural compounds target multiple entities, thereby mitigating the possibility of treatment inefficacy.

CHAPTER 5 IN-VITRO ANALYSIS OF NATURAL COMPOUND MEDIATED IMPACT ON ANOIKIS-RESISTANCE CELL LINE

5.1 Rationale of the study

Gallic acid is one of the natural compounds that have been widely recognized for their therapeutic properties, including anti-cancer, anti-inflammatory, and antioxidant effects. However, there is still little research done on the exact effect of these compounds on genes related to resistance to anoikis. It mainly investigates the effect of gallic acid on genes that express anoikis resistance in cancer cells at different time intervals. This may be completed by looking at differential gene expression profiles at various programs of cell death for the identification of critical genes and modulated pathways by gallic acid, which could contribute to its potential as a therapeutic agent.

The rationale of the present study is three-fold: Therapeutic Potential: Naturally abundant in many dietary sources, gallic acid is one of the bioactive compounds that have been comprehended rather promisingly against cancer with very minimal side effects. Therefore, understanding its influence on the anoikis-resistant phenotype, this research aims at building gallic acid as one of the potent candidates in the prevention of cancer metastasis for all-natural and low-toxicity options over traditional chemotherapy.

Temporal Regulation of Gene Expression: The study realizes the power of time-course analysis in explaining how gene expression changes at different stages of cell death. Because it is a temporal analysis of gene expression in response to gallic acid, the timeframe provides insight into when and how molecular events become timed and sequenced to bring about cell death—a very important aspect to be taken into consideration in the optimization of therapeutic interventions.

Gallic Acid-Loaded Nanoparticles for Enhanced drug efficacy: The gold nanoparticles are proposed as the carrier system for delivering the bioactive compound, gallic acid, in efforts to improve its bioavailability and therapeutic efficacy. These are some of the objectives of this study is to evaluate the time-dependent efficacy of gallic acid-loaded nanoparticles compared with free gallic acid and bare AuNPs, and to probe the enhancing mechanisms underlying in vitro efficacy, which may emanate from an improved cellular uptake and a sustained release. This study is expected to show that GA-AuNPs cause higher cytotoxicity against cancer cells than others, with lower IC₅₀ values at 12, 24, and 48 hours, thus indicating a synergistic effect that would improve treatment outcome.

Ultimately, this study serves to contribute to the rapidly increasing body of knowledge on natural compounds in cancer therapy and their potential role in the override of anoikis resistance, a major challenge in the treatment of metastatic cancer. The findings may hence offer new avenues for the establishment of novel treatment strategies against mechanisms of cell death resistance in cancer therapy, hence enhanced efficacy and reduced metastasis.

5.2 Methodology

5.2.1 STUDYING EFFECT OF NATURAL COMPOUNDS ON ANOIKIS RESISTANCE-ASSOCIATED GENES AT DIFFERENT TIME INTERVALS

We started by downloading dataset GSE158788 from GEO and categorizing this data in different time points categories. This data consisted of wet lab experiment results obtained by culturing cancer cell line that was treated with gallic acid at increasingly longer time points: 2, 4, 6, and 9 hours. The cells that remained untreated were used as controls so that their gene expression levels could serve as a comparison. After treatment, RNA was isolated from treated and nontreated cells for library preparation and RNA sequencing. From this sequencing, full expression profiles of genes at each time point were obtained which were further studied in detail for the time-dependent effects of gallic acid on cellular processes. Only high-quality data were advanced into bioinformatics analysis to ensure the reliability of results. Gene Ontology-based bioinformatics analysis was performed together with KEGG pathway analysis. This gave an insight into the biological pathways and processes modulated by gallic acid, showing its mechanistic effects on the cells. This current study focused on the time-dependent effect of gallic acid on gene expression. We compared these treated samples against their controls in order to find differentially expressed genes. By this filtration scheme, 53 genes related to anoikis resistance were found to be among DEGs. Notably, gallic acid significantly modulated the expression of 41 such genes at various time points, reflecting its high potential for the modulation of some critical cellular pathways.

We then took the fold changes in gene expression against the control at each time point for data visualization. Analysis of data was made possible through the aid of MS Excel, since it is a very powerful tool during the organization and interpretation of our findings.

5.2.2 GOLD NANOPARTICLE SYNTHESIS

The synthesis of gold nanoparticles was conducted using the citrate reduction method. Various nanoparticle solutions were prepared by taking 200 μ l, 300 μ l, 400 μ l, and 500 μ l from a 1% tetrachloroauric acid solution, which was then diluted to 50 ml to achieve concentrations of 10 mM, 15 mM, 20 mM, and 25 mM, respectively. These solutions were heated on a hot plate magnetic stirrer until reaching a temperature of 97° Celsius. Subsequently, 1.5% trisodium citrate was added quickly and continuously with stirring. Stirring was continued until the solution turned a brilliant red color. Finally, the solution was stored at 4 degrees Celsius for further use.

For the analysis of gold nanoparticle formation, the absorption spectrum of the gold nanoparticle colloid was measured at a wavelength of 550 nm using a spectrophotometer and further analysis was done using DLS, SEM and FTIR.

5.2.3 DRUG-NANOPARTICLE CONJUGATION

Gold nanoparticles (AuNPs) functionalized with gallic acid were synthesized using a modified Turkevich method. 1.5 mM stock solution of gallic acid was diluted to make 0.5 mM solution of gallic acid. Initially, 5 mL of gold nanoparticles solution was mixed with 0.5 mM gallic acid solution (2 mL). The mixture was stirred at room temperature for 24 hours, while being protected from surrounding light and sunlight to prevent any undesired photochemical reactions.

Following the reaction period, the solution was centrifuged at 7000 rpm for 15 minutes to remove any unreacted reagents and impurities. The supernatant was carefully decanted, leaving behind the pellet of functionalized gold nanoparticles.

The functionalized gold nanoparticles were then resuspended in a suitable solvent and stored at 4°C until further use. Characterization of the synthesized nanoparticles was performed using techniques such as UV-Vis spectroscopy, FTIR and dynamic light scattering (DLS). This procedure was repeated three times to ensure reproducibility, and the average values were reported. All glassware and equipment used were thoroughly cleaned and dried prior to use to prevent contamination.

5.2.4 DRUG-RELEASE OVER TIME

0.5 mM solution of gallic acid. Gallic acid (GA), like other polyphenols, undergoes significant degradation in the presence of factors such as light, oxygen, high temperatures, solvents, enzymes, proteins, and metallic ions. Preserving the stability of GA is critical, particularly during its release process, to ensure its activity and structural integrity. Ascorbic acid serves as an exceptional radical scavenger capable of retarding, inhibiting, and preventing the oxidation of susceptible materials like GA.

For experimentation, 10 mg of GA-loaded gold nanoparticles (NPs), suspended in deionized (DI) water, were introduced into 15 mL of phosphate-buffered saline (PBS) solution at pH 7.4, supplemented with 1% ascorbic acid, within 2-mL Eppendorf tubes. Subsequently, the samples underwent incubation at 150 rpm and 37°C for 48 hours. At intervals of 0.5, 2, 4, 8, 24 and 48 hrs the samples underwent UV vis analysis followed by centrifugation, followed by replacement of the buffers with fresh ones to sustain the release test. The wavelength at which gallic acid absorbs most strongly is 215 nm to 265 nm.

5.2.5 MTT ASSAY

Critical steps in the MTT assay protocol for the determination of cytotoxicity of nanoparticles loaded with gallic acid were as follows: Cells were seeded at a density of 3,500 cells per well in a 96-well plate. Culture media with various concentrations of gallic acid, GA-loaded gold nanoparticles, and gold nanoparticles were prepared. Control wells had only DMSO, the solvent used in the dissolution of the compounds. The cells were incubated for 48 hours with the treatments, and all experiments were repeated three times to improve the accuracy of the dose–response curves. Following incubation, the MTT reagent was added to the cells at 12, 24, and 48 hours to ascertain cell viability. The MTT reagent induced the formation of formazan crystals by mitochondrial dehydrogenase in living cells. This was followed by a further incubation period of 4 hours, which assisted in complete formazan crystal formation. Then, a solubilizer was added to the solution for the dissolving of formazan crystals; it was mixed well and incubated for an hour to allow complete dissolution. The formazan product, correlating to the number of viable cells, was quantified by measuring the absorbance at 570 nm using a microplate reader. Cell viability was calculated according to the formula:

$$\text{Percentage Viability} = (\text{Absorbance of treated sample} / \text{Absorbance of control}) \times 100\%$$

The above formula, comparing the treated samples against the control, gives a quantitative measure for cell viability and cytotoxicity. This MTT assay protocol puts forward the potential therapeutic efficacy of GA-loaded nanoparticles in the treatment of cancer.

5.3 Result and Discussion

5.3.1 NATURAL COMPOUND EFFECTING ANOIKIS RESISTANCE-ASSOCIATED GENES AT DIFFERENT TIME INTERVALS

In order to study the expression of 53 common DEGs associated to anoikis, at different time intervals of cell death we again performed the differential gene expression studies. For this purpose, we procured gene data set from GSE158788 from GEO. The data that we took was the RNA sequencing data revealing the gene expression profiles of cells undergoing cell death, after gallic acid treatment at- 2 hours, 4 hours, 6 hours and 9 hours. The work flow for this study is displayed in figure 5.1.

We matched the profile of these genes to anoikis resistance genes before and after treatment with gallic acid which was one the 102 natural compounds that we have investigated in this study. Figure 5.2 represents the effect of gallic acid on each gene after treatment with gallic acid after- 2-hour, 4-hour, 6 hour and 9 hours. Each bar in the graph represents the Log fold change in expression levels of the 53 common DEGs associated to anoikis resistance at each time point compared to the untreated ones. Positive LogFC value indicates upregulation while negative LogFC value indicates downregulation.

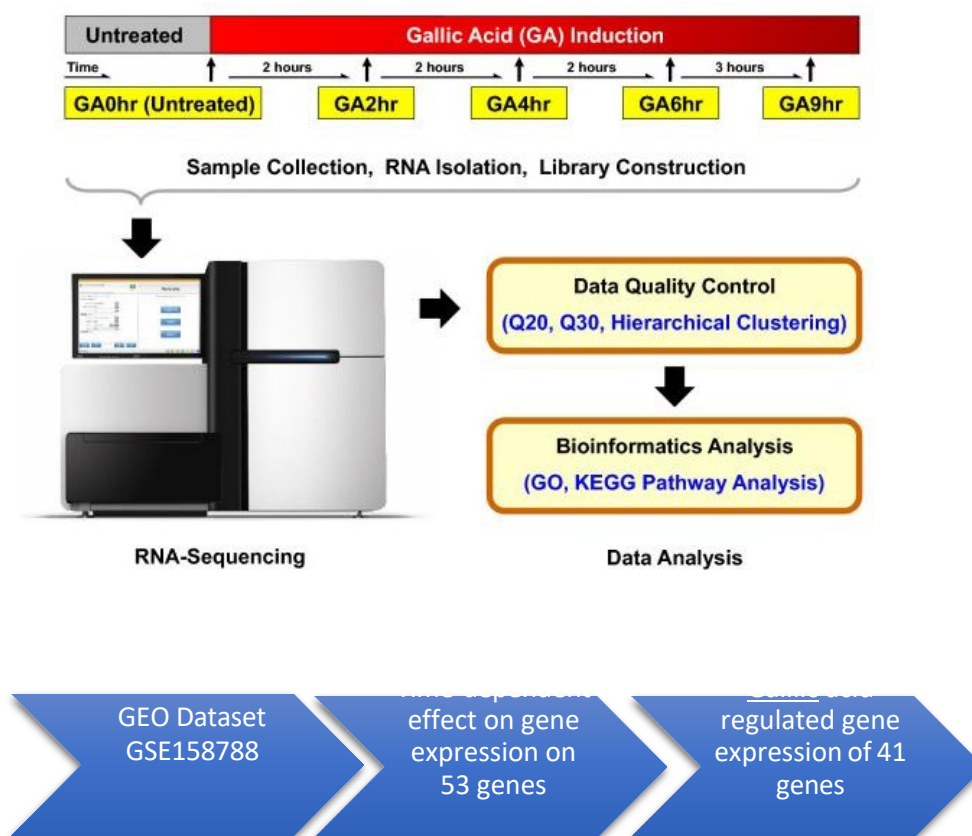


Figure 5.1 Demonstration of work flow for analysis of effect bioactive natural compound on 53 common DEGs.

The effect on expression level of each of the 53 DEG at the 4 time points has been detailed in Table 5.1. The change in logFC value of the 53 genes by gallic acid have been depicted in Table 5.1 at different time points. Every gene changed its LogFC value upon treatment with gallic acid at single and multiple time points.

Table 5.1 Effect on expression levels (logFC value) of 53 common genes by gallic acid at different time points

S.No	Gene	Before treatment	2 hours	4 hours	6 hours	9 hours
1.	PDLIM5	0.274	-0.2618658	-0.1318662	-0.078373	-0.290192
2.	ZSCAN12	0.13	-0.2820558	-0.1232039	0.0579458	0.2843895
3.	PARD3B	0.248	-0.3882722	-0.1033116	0.2337298	0.3885403
4.	TRIM36	0.55	-0.1092189	-0.1911248	0.5055024	0.3069014
5.	SLC22A23	0.205	-0.0782502	-0.223794	0.1535326	0.3085886
6.	MICAL2	0.255	-0.1405533	#N/A	#N/A	#N/A
7.	CHDH	0.249	#N/A	#N/A	#N/A	#N/A
8.	PLPPR1	0.544	#N/A	#N/A	#N/A	#N/A
9.	SSTR1	0.376	-0.1417052	-0.0013456	0.2661688	0.7698627
10.	LILRB4	0.411	#N/A	#N/A	#N/A	-0.470693
11.	ZBTB18	0.321	-0.0102103	-0.5900294	0.0594641	#N/A
12.	MYLIP	0.249	#N/A	#N/A	#N/A	#N/A
13.	ST7L	0.292	-0.0529292	-0.1303281	0.1076898	0.0983144
14.	AZIN2	0.573	-0.2351893	-0.266975	0.1945373	1.1528057
15.	MCF2L	0.208	-0.0668954	-0.8409403	0.7791845	1.9738996
16.	MLXIP	0.212	-0.179866	-0.2063712	0.1324206	-0.615793
17.	TRIM3	0.249	-0.2507022	-0.2007939	0.0176101	-0.180871
18.	CFAP157	0.23	-0.2486407	-0.1788378	0.0732241	0.3002527
19.	KIAA1211	0.306	#N/A	#N/A	#N/A	#N/A
20.	DST	-0.287	0.95627	1.3307412	0.699049	1.237887
21.	ZNF711	-0.235	0.9639563	1.4312434	0.391526	0.7885808
22.	ANXA2	-0.213	0.1415524	0.784282	0.2868731	0.2717415
23.	ZNF827	-0.27	0.6745027	0.5871634	0.6275331	0.6172335
24.	GJA1	-0.424	0.011893	0.1968262	0.0244708	0.5359024
25.	CAV2	-0.294	0.2237433	0.4648399	0.1719056	0.8009886
26.	DKK3	-0.258	0.0564504	0.3152302	0.0295847	0.1042643
27.	PCDH7	-0.311	0.0129447	0.0980003	#N/A	0.4424155
28.	SKIL	-0.351	0.0110926	0.8195715	0.0129448	0.5253413
29.	LPAR1	-0.432	0.742459	0.4556426	0.0348972	0.1729753
30.	SYNE2	-0.213	1.0222146	1.0815409	0.8563056	1.3359642

31.	CD40	-0.387	#N/A	#N/A	#N/A	2.374886
32.	CREM	-0.391	0.3539965	0.51923	0.5311116	0.2860555
33.	PJA1	-0.803	0.0344301	0.5527226	0.2754984	0.1719289
34.	SCD5	-0.58	#N/A	0.1826052	#N/A	#N/A
35.	GPR161	-0.634	#N/A	0.0504799	#N/A	#N/A
36.	CALD1	-0.883	0.2097592	0.9226615	0.1833622	0.7824892
37.	ADGRG6	-0.276	#N/A	#N/A	#N/A	#N/A
38.	TNC	-0.258	#N/A	#N/A	#N/A	#N/A
39.	HAS3	-0.347	0.3975648	0.4225112	0.2393033	0.2286521
40.	QKI	-0.15	#N/A	#N/A	#N/A	#N/A
41.	KIF5C	-0.215	0.2809256	1.5273884	0.1642923	0.8465477
42.	STAT3	-0.267	0.0933832	0.0987208	0.3818785	#N/A
43.	DLC1	-0.49	0.3156858	0.2180454	0.4991415	#N/A
44.	EPHA7	-0.315	0.298646	0.3647236	0.6363291	1.4838351
45.	ATP5E	-0.362	#N/A	#N/A	#N/A	#N/A
46.	SMC1A	-0.328	#N/A	#N/A	#N/A	#N/A
47.	IDH3A	-0.241	0.2313897	0.3198701	0.0055467	#N/A
48.	NFKBIZ	-0.896	1.2154933	0.7393304	1.4949205	1.0948101
49.	ZNF638	-0.243	0.3798816	0.713744	0.2486311	0.7199546
50.	ARHGEF12	-0.553	0.5952415	0.4672415	0.6303284	0.9855123
51.	NDFIP2	-0.341	0.1489297	0.42702	0.1897287	0.8734586
52.	P4HA2	-0.251	0.1575154	0.3472283	0.0132482	0.1112996
53.	NR5A2	-0.285	#N/A	#N/A	#N/A	1.6809859

Few of the genes were such that are not affected by the gallic acid at a particular time point but were affected at a different time point. In this manner, we studied the 53 common differentially expressed genes across various stages of cell death after gallic acid treatment which is of great significance in regulating cell death. For example, LILRB4 had no effect of Gallic acid at 2 hours, but it was downregulated at 9th hour having logFC value -0.470693. Gallic acid is a compound found in common dietary substances like fruits, various herbs, nuts and raisins, green tea and different vegetables. Gallic acid has been studied extensively for its anti-cancerous and anti-oxidant properties in colon cancer, lung adenocarcinoma, breast cancer, melanoma, blood cancer and hepatocellular carcinoma. Gallic acid has shown negligible side effects in research studies, displaying cytotoxicity to cancer cells only, upon treatment. Gallic acid regulates various factors in cell death resistant cells like caspase activation, free radical scavenging, downregulation of anti-apoptotic proteins etc which are otherwise highly irregular in tumor microenvironment.

5.3.2 GALLIC ACID AND STAGE SPECIFIC REGULATION OF CELL DEATH

Gallic acid has evidently shown various apoptotic events like cytochrome c-GFP release from the mitochondria at 4th hour of treatment, nuclear condensation starting at the 6th hour of treatment, caspase activation at 7th hour and plasma membrane rupturing at 9th hour of gallic acid treatment in HeLa cells, at dosage concentration 50 µg/ml.

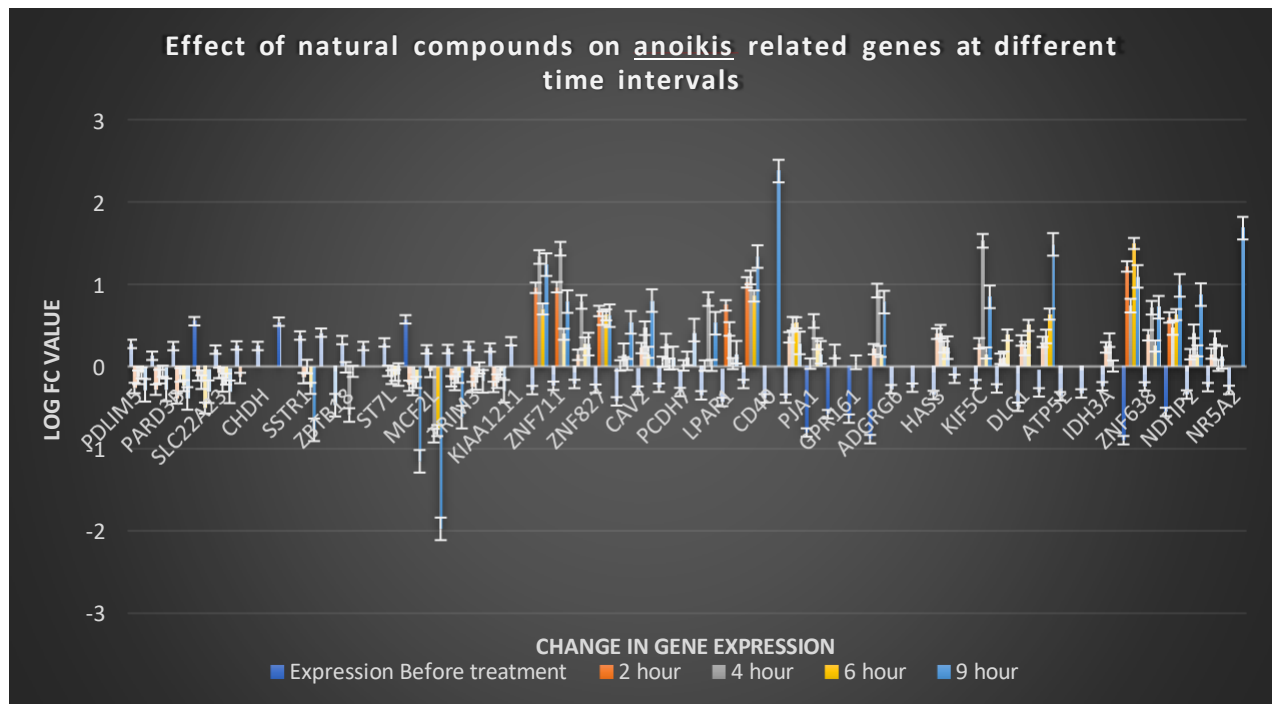


Figure 5.2 Each bar in the graph represents the fold change in gene expression levels at each time point compared to the untreated genes. Positive values indicate upregulation, while negative values signify downregulation. The differential expression patterns across these time points underscore the regulatory influence of gallic acid on anoikis resistance-associated genes providing valuable insights into its therapeutic potential in overcoming cancer cell resistance.

In the temporal gene expression profile study of the 53 common DEGs related to anoikis, we deduced that gallic effected a total of 39 out of 53 genes at 2nd hour, 4th hour and 6th hour, and 37 genes at 9th hour. Genes like PDLIM5, ZSCAN12, PARD38, TRIM36, SLC22A23 were affected by gallic acid at all 4 time points. PDLIM5(PDZ and LIM domain 5) for examples, is associated with regulation of cytoskeletal organization, cell proliferation, cell differentiation and survival. This gene is upregulated in tumor microenvironment aiding tumor cells to grow. According to results of this analysis, PDLIM5 can be downregulated by gallic acid at different time intervals reducing its logFC value to -0.0783 at 6th hour from 0.274 before treatment. Another tumor marker, ZSCAN12(Zinc finger and scan domain containing 12) transcription factor protein responsible of regulating transcription of various gene, cell growth and differentiation were upregulated prior to gallic acid treatment. This gene was downregulated by gallic acid at all 4 time point with the lowest logFC value -0.284 at 9th hour. PARD6B (Partitioning defective 6 homolog beta) had lowest log fc value at 9th hour which was -0.388. PARD6B is a constituent of the partition defective (PARD) protein complex which is required for cell polarity and is also involved in assymetric cell division, cell motility and formation of epithelial cell junctions. It is of great significance in cancer biology where cell migration and cell polarity can be a contributing factor in tumor metastasis and progression. TRIM36 (Tripartate motif containing 36) and SLC22A23 (Solute carrier family 22 member 23) are other genes which were regulated by gallic acid at all time intervals. The lowest logFC value recorded was -0.3085 at 9th hour for SLC22A23. SLC22A23 belongs to transporter protein family and they play significant role in drug absorption, metabolism, drug resistance and maintenance of cellular homeostasis.

Time -course

transcriptomic study is of great value and significance to unveil the mechanisms that control cell death, mainly apoptosis, ferroptosis and necrosis at different stages and time intervals.

The expression patterns of numerous genes across different time points evidence the regulatory effect of gallic acid toward the genes related to the mechanism of anoikis resistance. Such results further explain why gallic acid can be efficacious in terms of the therapeutic use in overcoming the resistance of cancerous cells. Temporal regulation indicates that the interaction of gallic acid and gene expression is very dynamic, highlighting the scope of this study in cancer therapy.

5.3.3 GOLD NANOPARTICLE FOR DRUG DELIVERY- FORMATION, SIZE AND STABILITY OF GOLD NANOPARTICLES AND GA-AUNP

The formation of gold nanoparticle was confirmed by UV-Vis spectrophotometry analysis. The UV-Vis spectrum obtained at 550 nm wavelength typically serves as a reference for assessing the presence and intensity of the surface plasmon resonance (SPR) absorption peak, which is indicative of AuNP formation.

In this case, the observed absorption peak between 400 nm to 500 nm wavelength suggests that the AuNP synthesized using the citrate reduction method exhibited SPR absorption in the visible range close to 550 nm. Characteristically, gold nanoparticles exhibit a SPR absorption peak around 550 nm which is a hallmark of their synthesis and size under 100 nm. The graph in figure 4.3(a) represents the absorption spectra of the synthesized AuNP using the citrate reduction method. Maximum absorption peak in the region of 400 nm to 500 nm indicates that the synthesized AuNPs were showing SPR absorption in the visible range, typical behaviour expected for gold nanoparticles but slightly shifted from their typical 550 nm peak.

The Gaussian curve of the figure 4.3(a) is given by the peak obtained for the UV-Vis analysis, indicating the distribution of absorbance values around the central wavelength. In this graph, a strong peak at around 440 nm, followed by an absorbance with a steep fall beyond 500 nm, dominates the absorption curve. This pattern therefore proves the successful synthesis of gold nanoparticles and also provides information about their optical properties.

The peak shift of the SPR from the usual 550 nm to around 440 nm may be a result of changes in size or shape or due to variations in the surrounding medium that affect AuNPs' plasmonic characteristics in some definite way. This analysis helps learn about the route of synthesis and the characteristics of the resultant nanoparticles, two very prime things while applying those nanoparticles for biomedical imaging, drug delivery, and photothermal therapy.

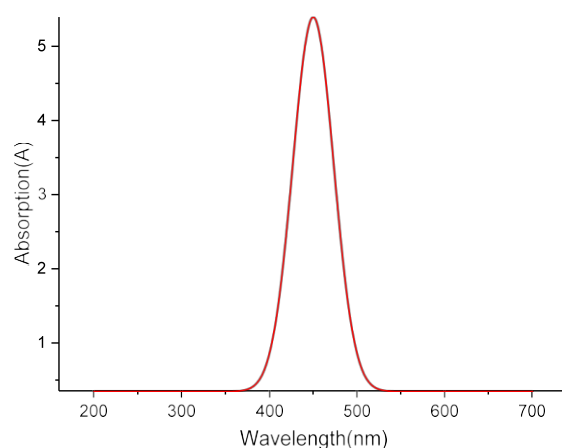


Figure 5.3(a) Absorbance spectra (gaussian curve) of gold nanoparticles, gold nanoparticle typically absorbs at 400-500 nm

5.3.4 SEM ANALYSIS

Figure 5.3(b) depicts SEM images taken in order to identify the size and surface morphology of gold nanoparticles obtained via the citrate reduction process. The instrument set with the following parameters: magnification 30.00 K X, electron high tension EHT 20.00 kV, working distance WD 5.0 mm, produced this image with Secondary Electron Imaging, SEI, to get details on the surface characteristics.

The left image contains two particles with dimensions, labelled P1 and P2. In particle P1, the size is 165.1 nm at an angle of 67.6°, while in particle P2, it is 85.0 nm at an angle of 81.9°. The image on the right gives a wider field of view of dispersion of nanoparticles without dimensions of any single particle and hence shows the general dispersion and morphology of the nanoparticles.

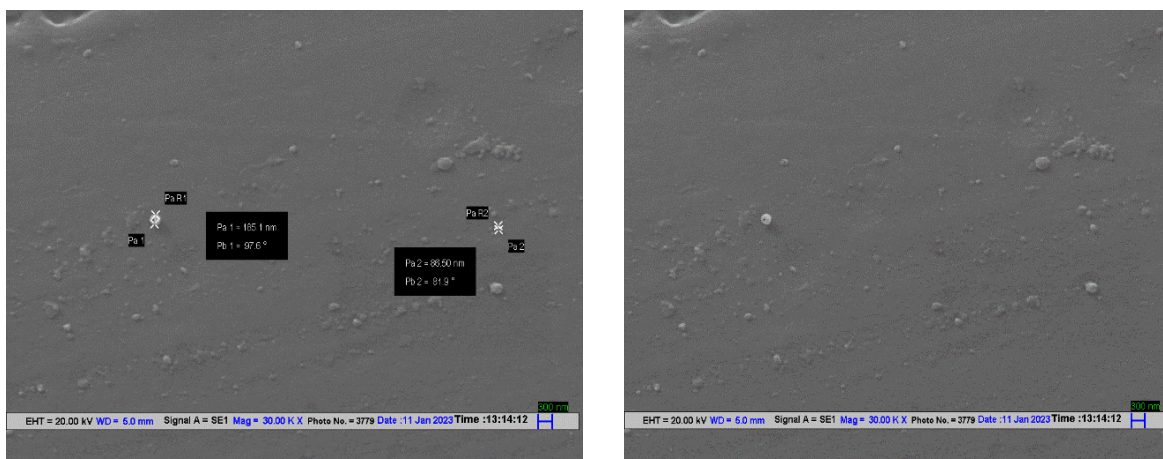


Figure 5.3(b) Scanning electron microscope (SEM) image revealing the surface morphology and size of gold nanoparticles synthesized through the citrate reduction method

These SEM images show that the surface morphology of these gold nanoparticles is predominantly spherical, with few cases of agglomeration. The surface is smooth, thereby exposing the uniformity in the formation of the particles, which typically occurs with the citrate reduction technique. This method of synthesis involves the reduction of chloroauric acid by trisodium citrate, which is not only a reducing agent at the same time but also acts as a capping agent to prevent the nanoparticles from agglomerating too much.

Such information on their morphology and size distribution is very critical to their application in medical imaging, drug delivery, and catalysis. It can be said that the control of nanoparticle size and shape can directly determine their optical, electric, and catalytic properties. The SEM images shown in Figure 5.3(b) nicely represent the surface morphology and size of gold nanoparticles synthesized by the citrate reduction method and provide information of great value related to the effectiveness of the synthesis process and quality of synthesized nanoparticles.

5.3.5 GALLIC ACID LOADING DYNAMICS:

The concentration of gallic acid loaded onto the AuNP was 0.5 mM over the experimental timeframe. Compared to the absorption spectrum of a solution of 0.5 mM gallic acid at 212 to 250 nm, this supernatant showed smaller absorption at 280 nm to 300 nm and another peak appeared between 320 nm to 370 nm (Figure 5.4). This result indicated that gallic acid molecules were successfully attached to the surface of AuNPs. Figure 5.4 shows that the absorption spectra of GA-AuNPs had an absorption maximum at 280 nm. The colour of the gold nanoparticles changed from dark purple to grey, indicating gallic acid attachment. The cumulative amount of gallic acid loaded increased steadily over time, reaching a maximum of 1.900 ml, indicating that the AuNP effectively adsorbed the drug molecules (Figure 5.5).

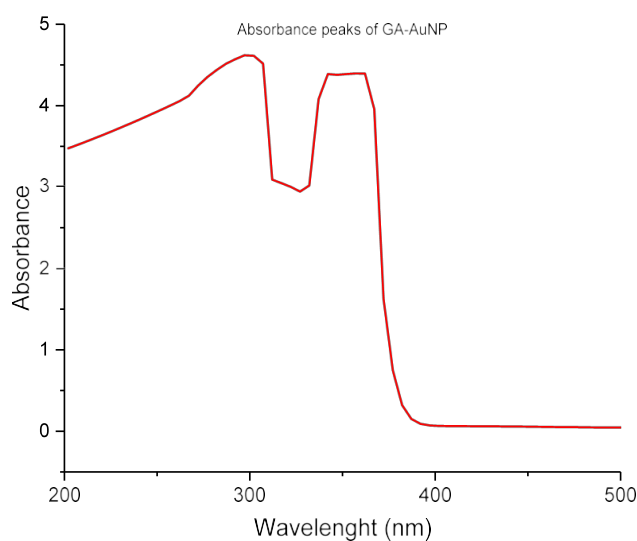


Figure 5.4 UV-VIS analysis of gallic acid-gold nanoparticle complex. Compared to 0.5 mM gallic acid solution (212-250 nm), the supernatant showed: Smaller absorption at 280-300 nm and another peak between 320-370 nm.

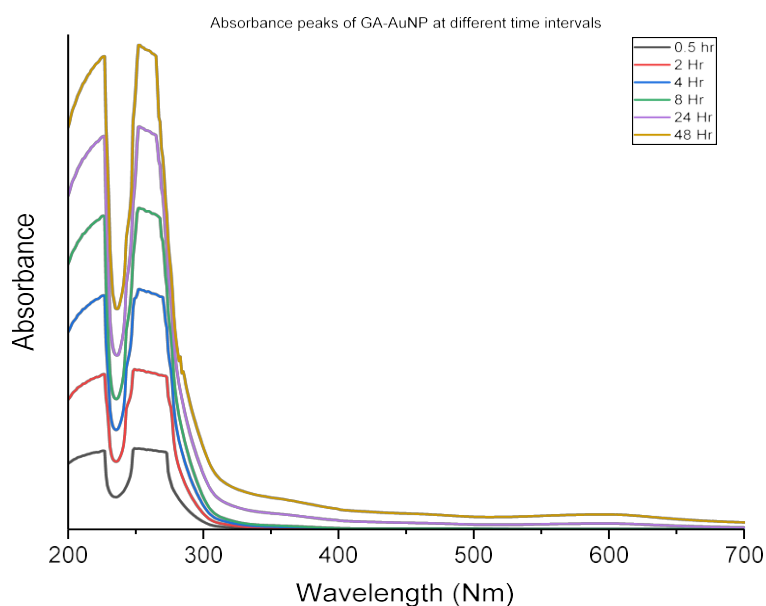


Figure 5.5 The time-dependent drug loading profile of gallic acid onto gold nanoparticles. The graph shown provides the time course invariances of gallic acid drug loading onto gold nanoparticles. Different coloured lines are obtained with the cumulative amount of gallic acid loaded at various times measured against the different time points in hours, measured every 2 hours. The initial loading could have been dependent on certain aspects such as availability of adsorption surface area and affinity of gallic acid towards AuNP surfaces. In all cases, the cumulative

gallic acid load increased with time to reach a maximum of 1.900 ml, an indication of effective AuNP adsorption ability for drug molecules.

The readings obtained from UV-Visible spectrophotometry analysis confirms the attachment of gallic acid molecules to the surface of gold nanoparticles successfully. The absorption spectra obtained by the Gallic and gold nanoparticle complex particles displayed distinct peaks and shifts as compared to the spectrum of unloaded gallic acid (free gallic acid) and gold nanoparticles indicating successful conjugation. The concentration of gallic acid that was loaded onto the gold nanoparticles was determined to be 0.5mM. The peaks obtained for this concentration were seen at two range of wavelengths, one at 280 nm to 300 nm and another one at 320 nm to 370 nm.

5.3.6 ANALYSIS OF SIZE DISTRIBUTION BY INTENSITY USING DYNAMIC LIGHT SCATTERING

Size distribution by intensity of gold nanoparticle and gallic acid loaded gold nanoparticle was studied by Dynamic light scattering method which have the hydrodynamic diameter giving the effective size of the nanoparticles in the solution. In figure 5.6(a), it can be seen that majority of gold nanoparticles fall in the range of 10 nm to 100 nm, while in figure 5.6(b) it is visible that the size of gallic acid loaded gold nanoparticle has exceeded and falls in the range 100 nm to 250 nm.

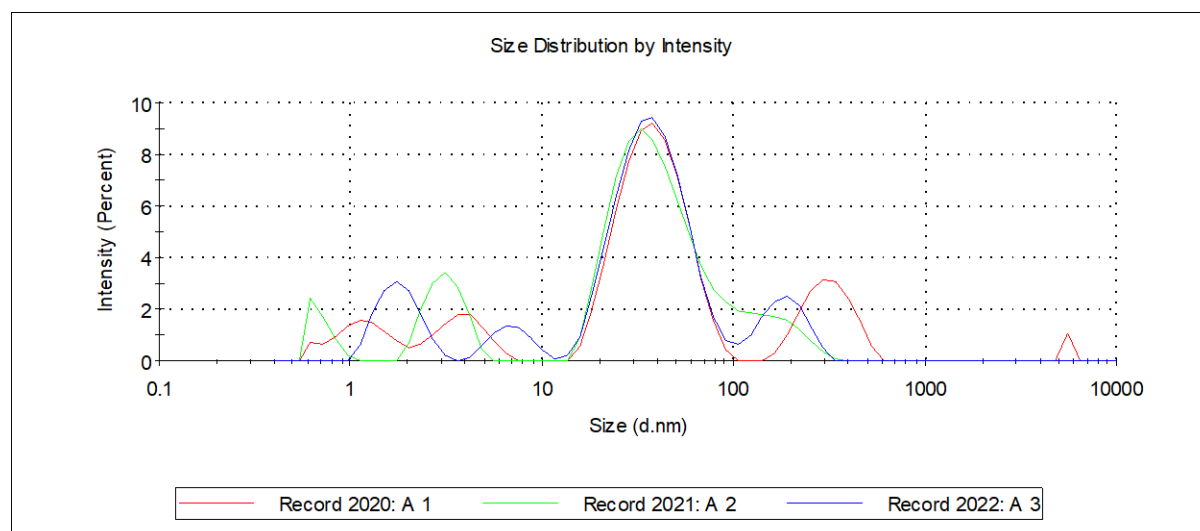


Figure 5.6(a) Size distribution of AuNPs among various records. Most of the nanoparticles correspond to a size range of 10 nm to 100 nm. The peaks of intensity represent the relative abundance of particles around given size ranges.

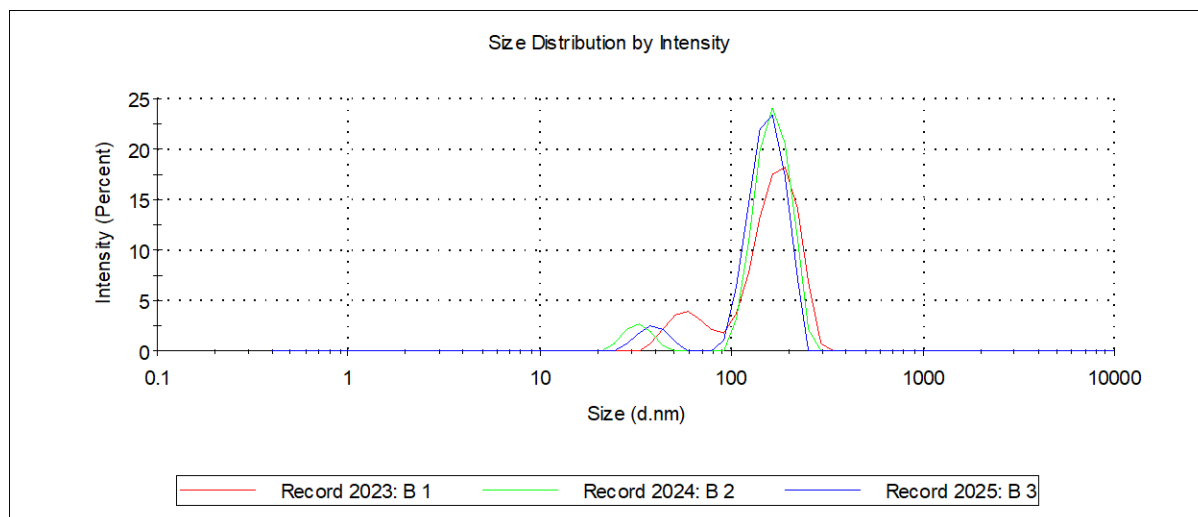


Figure 5.6(b) Size Distribution by Intensity of Gallic Acid-Loaded Gold Nanoparticles. The DLS graph shows the size distribution by intensity for gallic acid-loaded gold nanoparticles (Records 2023: B1, 2024: B2, and 2025: B3). The peaks in size distribution vary, indicating different ranges of nanoparticle sizes compared to free gold nanoparticles. This distribution suggests successful loading of gallic acid onto the gold nanoparticles, affecting their size characteristics.

In DLS graph of AuNP (Figure 5.6(a)) the red line is for Record 2020: A1, where there is a strong peak around 10 nm to 100 nm; a weaker peak is near 1000 nm.

The green line also indicates a main peak in the 10–100 nm range but with a more pronounced secondary peak around 100 nm. The blue line shows a similar pattern, with one large peak in the same range, and some minor oscillations (Record 2022: A 3). In figure 5.6(b) the size distribution of GA-AuNPs is demonstrated across different records. The intensity peak indicates the relative abundance of GA-AuNP complex particles within particular size ranges. Size distribution of GA-AuNPs at different records: 2023: B 1; 2024: B 2; and 2025: B 3. The peaks of the intensity curves show the relative abundance of particles with a certain size range. The peak of the red line, record 2023: B 1, has just one major peak around 90 nm to 100 nm. The green line then indicates a similar peak in the same size range but is a bit displaced towards larger sizes (Record 2024: B 2).

The blue line also shows almost a similar pattern, peaking at the centre around 100 nm. This is because the size of the particles in the sample affects the amount of light that will be scattered, where more light will be scattered by larger particles than smaller ones. In Dynamic Light Scattering, the size of particles is determined by calculating their diffusion coefficient, D , through the central Stokes-Einstein equation. The diffusion coefficient, D , for gold nanoparticles of radius 48.2 nm was about $4.54 \times 10^{-12} \text{ m}^2/\text{s}$. For gallic acid-loaded gold nanoparticles of radius 94.5 nm, the value of the diffusion coefficient was about $0.0299 \text{ m}^2/\text{s}$. Again, these were values obtained while considering other known parameters from temperature in Kelvin and Boltzmann's constant to dynamic viscosity. In this respect, the obtained results further support how effective the Stokes-Einstein equation is in explaining the dynamics of particle size during DLS measurements.

The size distribution is, therefore, consistent for all of the records from 10 nm to 100 nm, indicating a stable and uniform synthesis process of gold nanoparticles and their gallic acid-loaded counterparts. Peaks at higher sizes could indicate some aggregation or other large complexes that might exist in solution. The wavelength at which free gallic acid absorbs is

between 212 nm to 250 nm. These observations helped us confirm that the gallic acid molecules were successfully attached to the gold nanoparticle surface. Other studies suggest that interaction of gallic acid with metallic nanoparticles like silver and gold nanoparticle typically results in shift in the absorption spectra due to complex formation of gallic acid with nanoparticle. This absorption peak shift can be accredited to the SPR effect of the gold nanoparticle which is sensitive to the presence and refractive index of the molecule attached upon loading.

It has been also demonstrated that same kind of shifts in absorption spectrum occur upon loading and conjugation of various other drugs to gold nanoparticles. For instance, a study involving adsorption of doxorubicin on citrate-capped AuNP (Curry et al., 2015) stated that the attachment of doxorubicin to gold nanoparticles resulted in a shift of the absorption maximum suggestive of drug loading effectively. Similarly, another study demonstrated that shifts in the UV-Visible absorption peak when the drug quercetin was conjugated to gold nanoparticles, reflecting the versatility of gold nanoparticle in drug delivery applications (Balakrishnan et al., 2017). The mechanism behind the attachment of gallic acid to gold nanoparticle surface involves the interaction between the hydroxyl groups present on surface of both, gallic acid and gold nanoparticles. Gallic acid, a phenolic compound, can act as both a reducing and stabilizing agent in the synthesis of AuNPs. The hydroxyl groups in gallic acid facilitate the reduction of gold ions (Au^{3+}) to elemental gold (Au^0), leading to the formation of AuNPs. Subsequently, these hydroxyl groups can adsorb onto the nanoparticle surface, providing stability and preventing aggregation. This interaction is crucial for the formation and stabilization of AuNPs in various biomedical applications (Park et al., 2016). Hydrogen bonding, coordination bonds involving lone pairs of electrons on the oxygen atoms of gallic acid interact with the atoms on the gold nanoparticle surface. The absorption peak observed at 320-370 nm in the UV-Vis spectra of GA-AuNP is a result of formation of such interactions. Furthermore, stability of the Gallic acid-gold nanoparticle complex can be conditional from the consistent absorption maximum at 280 nm. The formation of this peak specifies that the complex maintains its structural integrity upon binding, which is an important factor in potential therapeutic application of this conjugated natural compound-nanoparticle structure.

Au-NPs were synthesized by the reduction of the corresponding Au^{3+} metal ions using GA as a natural molecule at room temperature. The choice of GA bifunctional molecule was done so as it contains both reducing and stabilizing groups; hence, in situ synthesis can be provided. According to that, the oxidation potential of GA and Au is 0.50 and 1.4 V, respectively. Therefore, the reduction of Au metal ions according to the following reaction is thermodynamically favourable at room temperature.

The hydroxyl groups in ortho and para positions seem to reduce Au ions. In a case when there are no agents to stabilize the formed metal nanoparticles, they interact with the strong force of reducing surface energy, thus aggregating irreversibly. The presence of an electron-releasing group ($-\text{COOH}$) in the para position and electron donor group ($-\text{OH}$) in the GA structure provides the same kind of interaction between GA and formed AuNPs that stabilized the AuNPs in the aqueous medium. Accordingly, one of the main advantages of the present approach is a possibility to synthesize nanoparticles just by mixing two reagents without generating any toxic by-products, while obtained nanoparticles are stable for several months. Gallic acid is the ideal candidate for coupling with AuNPs to enhance delivery and controlled release, taking advantage of their known antioxidant, anti-inflammatory, and anti-cancer properties. Being biocompatible and easy to functionalize, AuNPs are considered a perfect candidate to be used in coupling for the targeted drug delivery system. Our study next aimed

at assess the in vitro release kinetics of gallic acid from the GA-AuNP complex under physiological conditions. In vitro and in vivo studies on the therapeutic efficacy and safety of the GA-AuNP formulation are also duly needed. The drug loading efficiency and stability of the complex of the drug with the nanoparticle was further enhanced by the optimization of conditions of loading with respect to parameters such as pH and temperature.

For free AuNPs, major distribution of AuNPs size ranged between 10 nm and 100 nm and showed sharp peaks, which indicated ranges of relatively smaller sizes.

On the other hand, GA-AuNPs have drifted towards a larger size, with most of the particles dynamically being centred at around 100 nm. This could suggest successful loading of gallic acid onto the nanoparticles, thereby going on to increase the effective size.

It is noted that the peak for GA-AuNPs is more toward the centre at about 100 nm in comparison with free AuNPs, which indicates an attachment of gallic acid molecules that increases hydrodynamic diameter. This is consistent with previous studies in which the loading of nanoparticles with biomolecules results in an increase in size due to added molecular weight and surface modifications. In this study, the size distribution observed for GA-AuNPs was within the limit and is concurrent with results in other findings. For example, Liu et al. (2017) recorded a similar shift in size while loading therapeutic agents onto gold nanoparticles. The size distribution changed from 10-80 nm for free AuNPs to a shift at 90-110 nm for loaded nanoparticles. It is attributed to an additional molecular layer on the surface, which adds to the overall diameter of the nanoparticle. Another study conducted by Kim et al. has also reported a size increase from 20-60 nm to 80-120 nm for gold nanoparticles with different drug loadings (C.-S. Lee et al., 2017). This matches the findings of other studies and thus consequently lends credibility to our synthesis and loading methods by re-affirming that gallic acid was loaded onto the AuNPs.

Notably, the results of the current study corroborate (Al-Khedhairy & Wahab, 2022) in the respect that DLS measures the effective size of nanoparticles in solution with the hydrated shell and attached molecules, which is an important factor to help explain their behavior in biological systems.

DLS analysis of GA-AuNPs, in comparison with free AuNPs revealed a successful load of gallic acid, evidenced by the size increase from the nanoparticle size range. This increase in size is also in agreement with literature reports of other studies, thus showing that the tested nanoparticle synthesis and loading protocols are reliable and effective. Further, this uniform size distribution could be a pointer toward stability and efficacy in drug delivery applications, therefore making GA-AuNPs a potential drug carrier.

5.3.7 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

The FTIR analysis was conducted to obtain the infrared spectrum of emission, absorption and photoconductivity of Gallic acid, gold nanoparticle and gallic acid loaded gold nanoparticle. These spectra were studied to identify the presence of functional groups and to gather the interactions between nanoparticles and the bioactive natural compound gallic acid and is displayed in figure 5.7. The spectrum of the citrate-capped AuNPs acts as a baseline and shows characteristic absorption bands. A strong peak at 1625 cm^{-1} corresponds to the C=O stretch of the capping agent citrate.

5.3.7.1 Gallic Acid:

The spectrum of pure gallic acid shows clear peaks matching its functional groups. The major peaks can be assignable to hydroxyl, O-H, stretching at 3629 cm^{-1} , carbonyl, C=O, stretching at 1633 cm^{-1} , and aromatic ring vibrations.(Venkatesan et al., 2024)(Pandi et al., 2024).

Moreover, stretching vibrations in the region from 1293 cm^{-1} to 1207 cm^{-1} and at 945 cm^{-1} specify the presence of phenolic groups. These vibrations relate to the C-O bonds within the phenolic groups and the out-of-plane bending vibration of aromatic C-H bonds vicinal to the phenolic hydroxyl group.

5.3.7.2 Gallic Acid-Loaded Gold Nanoparticles (GA-AuNPs):

The spectrum of GA-AuNPs, in red, manifests changes compared with both pure AuNPs and gallic acid, which approves the successful loading of gallic acid onto the nanoparticles.

Such a typical peak of C=O stretching can also be observed in the GA-AuNPs at 1633 cm^{-1} for gallic acid due to the carbonyl group conjugation of gallic acid with the nanoparticles (figure 5.7). The phenolic O-H vibration for gallic acid can also be located in GA-AuNPs at 3629 cm^{-1} , further confirming the successful conjugation of gallic acid onto the gold nanoparticle surface.

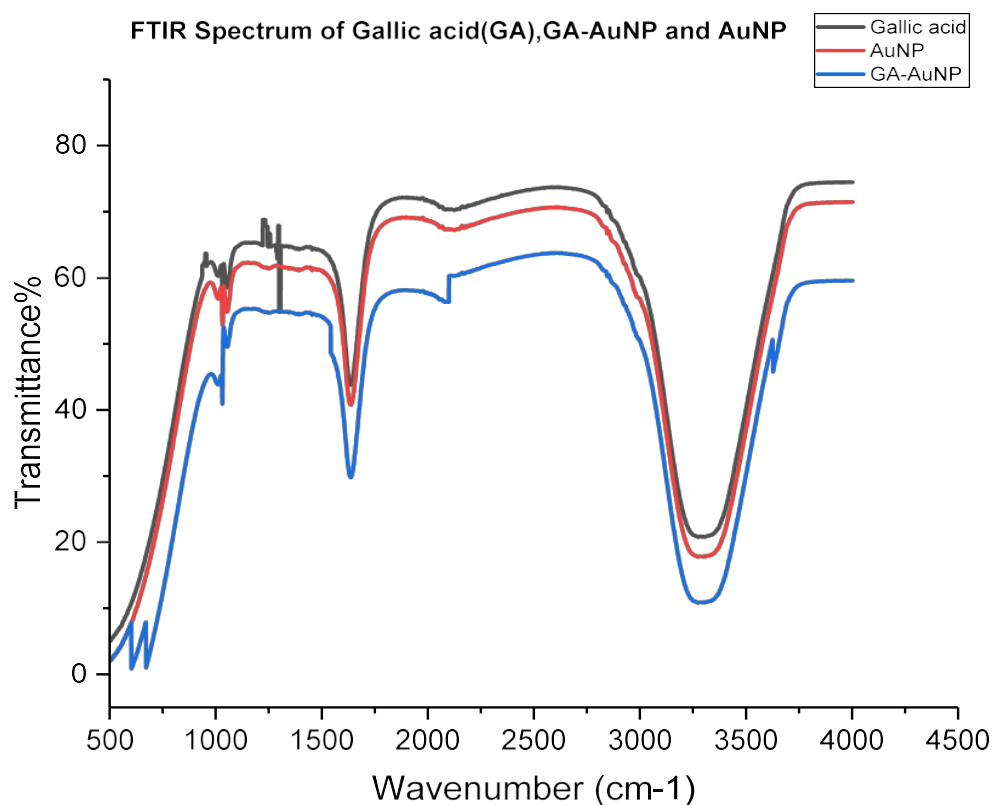


Figure 5.7 FTIR spectra for citrate-capped gold nanoparticles, gallic acid, and gallic acid-loaded gold nanoparticles. Characteristic peaks for gallic acid were observed, which then shifted or varied in intensity upon loading onto gold nanoparticles, thus proving that gallic acid has interacted and bound with the nanoparticle surface. The stretching peaks of phenolic O-H and C=O in GA-AuNPs further proved the conjugation of gallic acid onto the gold nanoparticles.

5.3.7.3 Characteristic Peaks and Functional Groups

C=O Stretching:

In the FTIR spectrum of GA-AuNPs, a distinctive stretching peak at 1633 cm^{-1} for C=O, very characteristics for carbonyl functionality of gallic acid, was prominently observed in this region. This peak confirms the presence of the carbonyl group from gallic acid on the surface of the nanoparticle; hence, conjugation is confirmed.

In contrast, the spectrum of AuNPs capped with citrate showed only one peak at 1625 cm^{-1} , which corresponded to the C=O stretch of the capping agent citrate. This difference in the spectral position of the peak for C=O stretching makes it easy to distinguish citrate-capped nanoparticles from gallic acid-loaded nanoparticles.

Phenolic OH Vibrations:

In the FTIR spectrum of GA-AuNPs, a phenolic OH vibration of gallic acid was picked up at 3629 cm^{-1} . This peak will turn out to be an excellent indicator of the presence of phenolic groups and mainly holds relevance with respect to the conjugation process. The detection of this vibration proves that the phenolic OH group is retained post-conjugation and hence supports the successful attachment of gallic acid onto the gold nanoparticles.

5.3.7.4 Stretching Vibrations of Phenolic Groups

The different peaks noticed for the stretching vibrations in the region of 1293 cm^{-1} to 1207 cm^{-1} , and the one at 945 cm^{-1} in the FTIR spectrum, confirms the existence of groups in gallic acid. Here, usually the stretching vibrations of C-O are found in the bond sections of the phenolic groups, -ArOH that are present in gallic acid, are obtained in this range. This peak is the occurrence of functional groups in the GA-AuNPs FTIR spectrum, indicating that these functional groups remain whole and may partake in the conjugations process.

The stretching vibration occurring at about 945 cm^{-1} is so mainly attributed to the presence of the phenolic groups and corresponds to the out-of-plane bending vibration for the C-H aromatic ring bonds adjacent to the phenolic hydroxyl group. Both these vibrations hence become characteristic of the structure of the aromatic ring in phenolic compounds such as gallic acid.

Other reports on gallic acid-loaded nanoparticles also record the presence of characteristic peaks representing C=O stretching and phenolic OH stretching in FTIR spectra (Espina et al., 2022) (Sandhiya & Ubaidulla, 2022). From these studies, hydrogen bonding and coordination interactions are demonstrated to be the major forces that help to stabilize the drug at the nanoparticle surface. In this study, the spectral features are consistent with the reports in literature, which further validate the successful conjugation of gallic acid onto gold nanoparticles.

5.3.8 DRUG RELEASE PROFILES

In the present study, gradually released profile was observed having release kinetics that were similar to some other studies. The fact that findings on this aspect are mainly in concert across different studies underlines once more that an optimal nanoparticle design is absolutely necessary for successful targeted delivery applications.

Detailed analysis of the FTIR spectra, the DLS data, and the kinetics of drug release delivers substantial information related to the physicochemical properties of GA-AuNPs. The successful conjugation of gallic acid to AuNPs, evidenced by FTIR and DLS, underlines the potential of GA-AuNPs to become a very effective drug delivery system. The drug release kinetics from nanoparticles are significantly influenced by both nanoparticle design and

environmental conditions. Factors such as particle size, surface charge, and composition play crucial roles in determining the release profile of encapsulated drugs. Additionally, environmental conditions like pH, temperature, and the presence of specific enzymes can affect drug release rates. Understanding these interactions is essential for optimizing targeted drug delivery systems (Pandey et al., 2022). More importantly, the kinetic profile of drug release observed and the factors influencing it have receptors that suggest that nanoparticle design and environmental conditions are indeed important in targeting drug delivery applications. The fact that it is corroborated by other studies further underscores the high quality of methods and great reliability of results.

This is the linear release profile characteristic of zero-order kinetics, in which the rate of drug release is constant and is not affected by the drug's concentration. The slope value 21.73913 points that the cumulative release of gallic acid increased by 21.74 units (approximately) per unit of time, on average. The value of the intercept is close to zero, suggesting that the initial cumulative release at time zero should be minimal, which aligns with the expectation for an initial lag phase in drug release. This rate reflects the efficacy of the gallic acid loaded gold nanoparticles in releasing gallic acid over the experimental setting. The linear release profile analysed is indicative of zero-order kinetics model, in which the rate of drug release is constant and independent of the concentration of the drug. There are various factors that affect drug release dynamics. Particle size and surface area, surface chemistry and functionalization, interaction between drug and nanoparticle, drug loading density etc. which are to be kept in the optimal conditions for a sustained drug release. Beside these intrinsic factors there are few extrinsic factors like pH which in our experimental setup was 7.4. pH of the medium can affect the rate of drug release as basic or acidic conditions can highly influence the ionization state of gallic acid or gold nanoparticle changing solubility and the way they interact with each other. For instance, one of the reports on doxorubicin-loaded gold nanoparticles demonstrated an increased rate of drug release at pH 5.5, simulating the tumor microenvironment in comparison with a neutral pH of 7.4 (Aryal et al., 2009) (Madhusudhan et al., 2014).

Temperatures therefore influenced the gold nanoparticles and, hence, modulated the rates of drug release; at higher temperatures, higher rates of release could be observed. Drug release in this manner is of beneficial for maintaining therapeutic levels of drug over extended durations providing a consistent dosage to the target site. The correlation Pearson's $r=1$ and high R^2 value further validate the stability of our drug release model.

The minimal residual sum of squares further aligns with the accuracy of the linear fit. We further used this complex for studying its anti-cancer effect by performing cell viability assay.

5.3.9 DRUG RELEASE KINETICS

Release of gallic acid by gold nanoparticle was studied in PBS which exhibited a time-dependent profile, with absorbance readings demonstrating a gradual increase in concentration of drug released. The drug release kinetics possibly controlled by numerous factors like diffusion of gallic acid through the nanoparticle matrix, interactions between drug and surrounding medium as well as the degradation or disintegration of the nanoparticle carrier. The release percentage of gallic acid in PBS ranged from 20.65% to 41.3% over the 48-hour duration, indicating constant and precise drug release characteristics of the gold nanoparticle formulation.

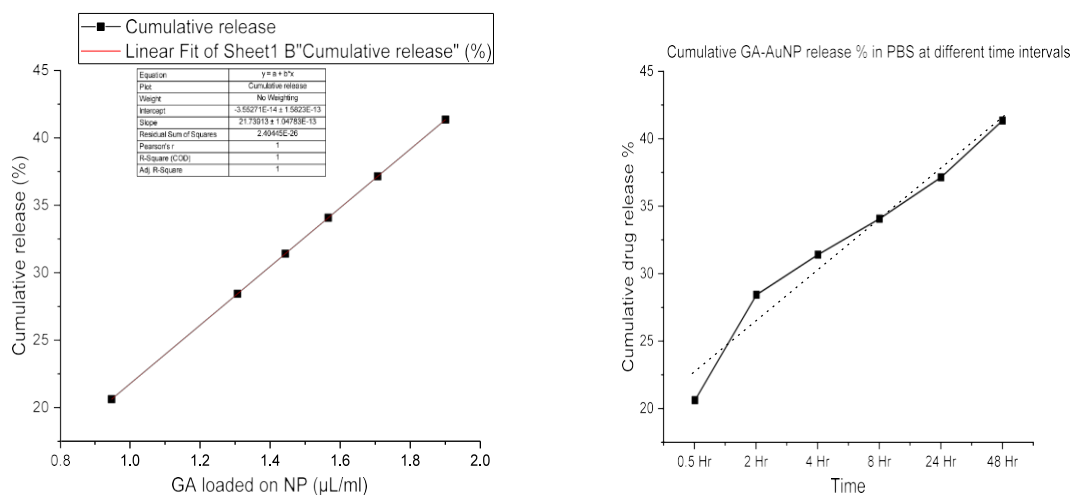


Figure 5.8 Linear regression analysis of the cumulative release of gallic acid from gold nanoparticles (GA-AuNPs) with time. A graph to show very strong linearity, as indicated by $R^2 = 1$. The related graph shows a consistent and controlled drug release profile. The slope of the linear regression (211.73913) depicts the rate at which gallic acid release occurred, and the minimal residual sum of squares (2.40445E-26) further checks accuracy of the model fit.

Figure 5.3 represents the drug release profile of gallic acid by gold nanoparticle over time, (0.5 hour to 48 hour), the cumulative of drug release. The graph demonstrates a linear relationship between cumulative release of gallic acid and amount of gallic acid loaded (over time). This linearity suggests a consistent and regulated release from the nanoparticle over a time period of 48 hours. The regression analysis demonstrated by figure 5.8 indicates the equation of the line $y = a + bx$, where y is the cumulative release, a is the intercept, b is the slope and x is time. The parameters mentioned on the graph in figure 4.8 specify a perfect fit for the linear model, with the R^2 value of 1, further suggesting that about 100% of the variability in drug release can be explained by time. Table 4.2 has detailed drug release profile of gallic acid by gold nanoparticle over 48 hours-time interval, mentioning the absorbance, concentration of drug release in 1 ml and 50 ml PBS, along with the cumulative percentages of release. The data specifies a gradual increase in the percentage and amount of gallic acid released, touching a maximum release of 41.31% at 48th hour.

Table 5.2 Cumulative Release Profile of Gallic Acid (GA) Loaded on Gold Nanoparticles (AuNP) in PBS Over Time

Time	Absorbance	Concentration of Drug	Concentration of Drug in 1ml	1 cumulative ml	Concentration of Drug in 50 ml	50 Cumulative Ml	GA Amount loaded on AuNP in ml	GA Released in PBS	% Release Of GA in PBS
0.5 hr	3.7	57	19	325	950	950	0.95	0.206522	20.65217
2 hr	3.9	59	19.66667	344.6667	983.3333	1308.333	1.308333	0.28442	28.44203
4 hr	4.6	66	22	366.6667	1100	1444.667	1.444667	0.314058	31.4058
8 hr	5.2	72	24	390.6667	1200	1566.667	1.566667	0.34058	34.05797
24 hr	5.9	79	26.33333	417	1316.667	1707.333	1.707333	0.371159	37.11594
48 hr	3.7	89	29.66667	446.6667	1483.333	1900.333	1.900333	0.413116	41.31159

5.3.10 TIME AND DOSE DEPENDENT EFFECT OF GALLIC ACID- IN VITRO STUDY

In the present study, the time- and dose-dependent effects of gallic acid were evaluated on the viability of colon cancer cell line for 12, 24, and 48 hours. At 12 hours of treatment, cell viability showed a progressive decrease from 91.63% at a concentration of 10 $\mu\text{g/mL}$ down to 46.8% at 60 $\mu\text{g/mL}$ of gallic acid (Figure 4.9a). There was a further dose-dependent progressive reduction in viability at 24 hours, where the cell viability decreased from 86.81% at a concentration of 10 $\mu\text{g/mL}$ down to 25.24% at 100 $\mu\text{g/mL}$. The steepest drop showed up after 48 hours, ranging from 84.07% at 10 $\mu\text{g/mL}$ all the way down to merely 16.18% at 100 $\mu\text{g/mL}$. These results indicated that gallic acid showed very potent cytotoxic effects on these cells and that exposure time and concentration were critical factors for reducing cell viability (Subramanian et al., 2015) (Wang Et Al., 2016) (Paolini Et Al., 2015).

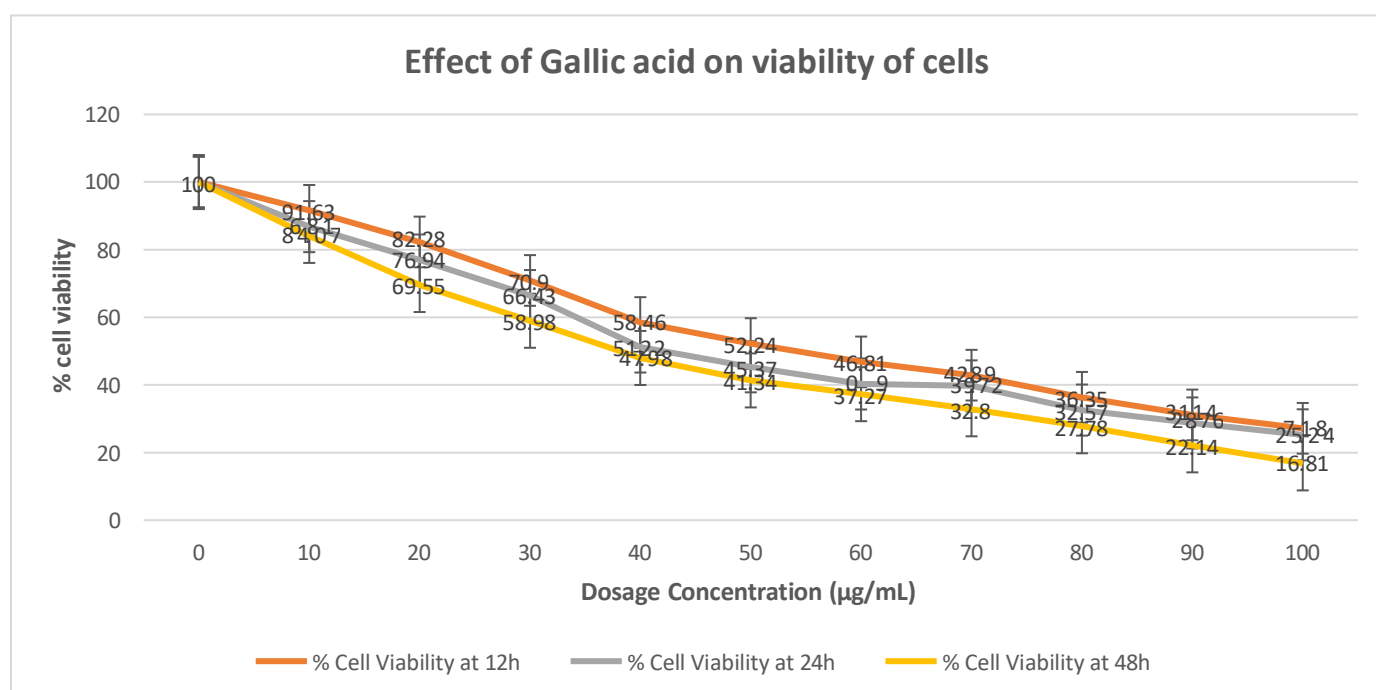


Figure 5.9(a) Time- and Dose-Dependent Cytotoxic Effects of Gallic Acid on Cancer Cell Viability as studied by MTT assay.

5.3.10.1 TIME AND DOSE-DEPENDENT EFFECT OF AUNP.

In our study, the cytotoxic effects of gold nanoparticles were investigated using the MTT assay on colon cancer cell lines and changes in the viability of treated cells was observed over 12, 24, and 48 hours. Cell viability decreased with increasing concentrations of gold nanoparticle after 12 hours of treatment, from about 94.38% at lower concentrations to about 50 percent at higher doses (Figure 4.9b). Viability dropped significantly to about 58.4% for the 70 $\mu\text{g/mL}$ concentration and less than 40 percent as test concentrations were further increased. The most pronounced decrease was recorded after 48-hour treatment, where, under the highest test concentration conditions, it has been reported that cell viability has drastically fallen to nearly 39%. These results underline the potent cytotoxic effects of gold nanoparticles and clearly show that an increased time of exposure or higher concentrations drastically bring down the viability of cancer cells.

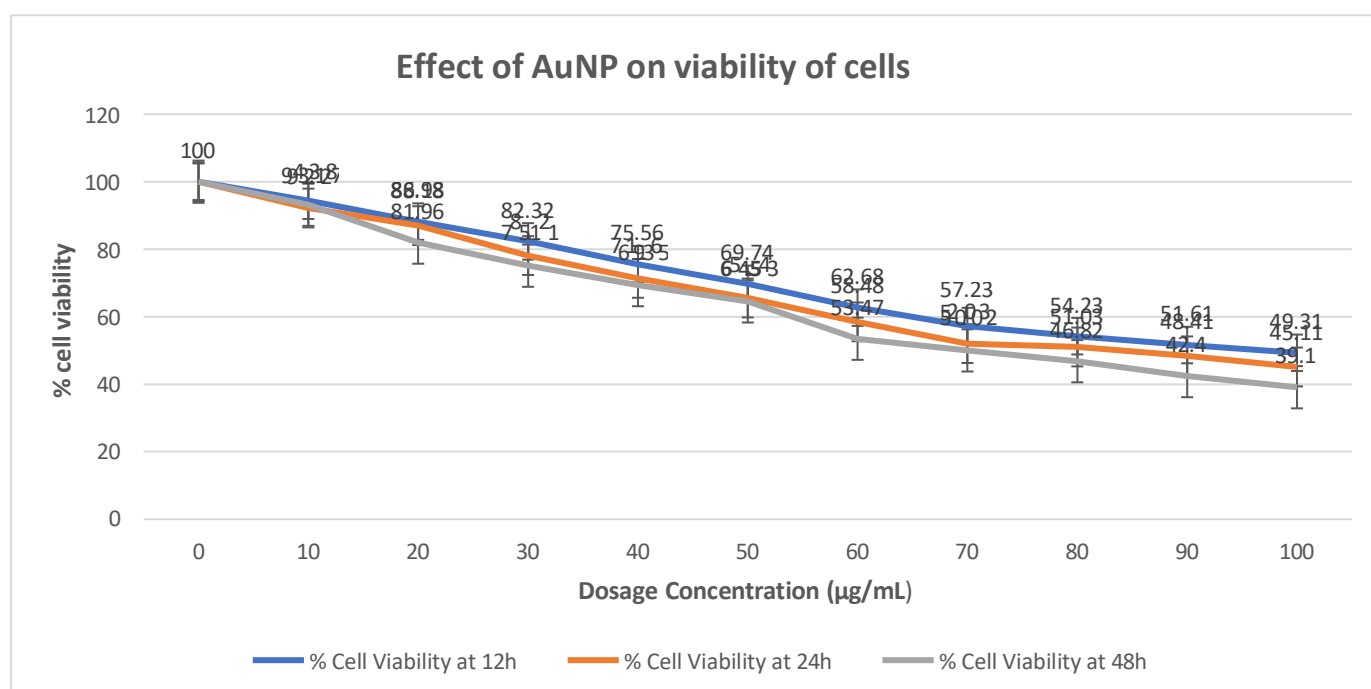


Figure 5.9(b) This graph illustrates the time- and dose-dependent effects of gold nanoparticles on the viability of cancer cells at 12, 24, and 48 hours. The viability decreases progressively with increasing concentrations of gallic acid, demonstrating its potent cytotoxic effects on the cells.

5.3.10.2 GA-AUNP EXHIBITS A DOSE- AND TIME-DEPENDENT EFFECT.

Gold nanoparticle conjugated with gallic acid was investigated for its time- and dose-dependent effects on the viability of cancer cells over a period of 12, 24, and 48 hours. At 12 hours of treatment, cell viability was relatively constant at the lower concentrations; it showed an initial cell viability of approximately 77% and gradually decreased with increased dosage. Viability at 24 hours decreased appreciably, from around 70% at low concentrations to about 20% at high concentrations (Figure 4.9c). This was more marked 48 hours later, with cell viability under 20% at the greatest concentrations tested. The findings revealed a strong cytotoxic effect of gallic acid and gallic acid-loaded gold nanoparticles. The extent of cancer cell death was significantly influenced by both the duration and dosage of the treatment.

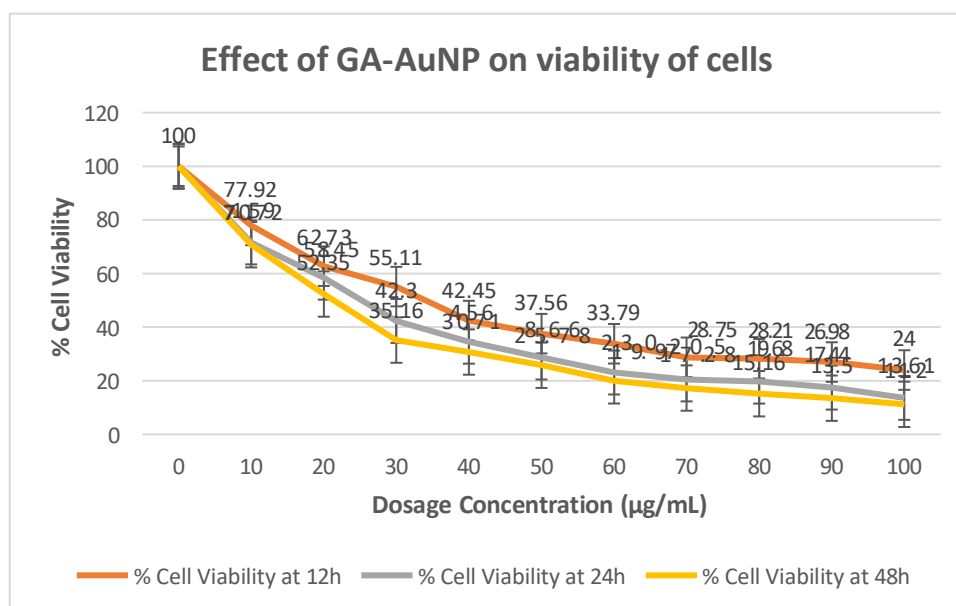


Figure 5.9(c) This graph depicts the percentage of viable cancer cells following treatment with various concentrations of gallic acid loaded nanoparticle over 12, 24, and 48 hours. The data show a significant reduction in cell viability with increasing concentrations of gallic acid and longer exposure times, highlighting the potent cytotoxic effects of gallic acid conjugated with gold nanoparticles.

Table 5.3 Half maximal inhibitory concentration (IC50)

Time	Gallic acid (µg/mL)	Gold nanoparticle(µg/mL)	GA-AuNP (µg/mL)
12 hours	57.21	70.63	30.27
24 hours	42.53	59.57	19.23
48 hours	35.12	48.29	18.35

After 12 hours, the IC₅₀ value for the GA-AuNPs (gallic acid loaded gold nanoparticles) was found to be 30.27 µg/mL (table 4.3, Figure 4.9c). It was significantly lower than that of gallic acid and AuNPs, whose IC₅₀ were recorded at 57.21 and 70.63 µg/mL, respectively. This is suggestive of the fact that GA-AuNPs were more effective inhibiting cell viability within a shorter period. This was due to synergistic action, as the gallic acid and gold nanoparticles facilitated better cellular uptake and targeted delivery. The higher efficacy continued at the 24-hour mark, with GA-AuNPs showing an IC₅₀ of 19.23 µg/mL, much more significant compared to an IC₅₀ value for free gallic acid at 42.53 µg/mL and AuNPs at 59.57 µg/mL. Such findings prove an increase in therapeutic effects that result from the sustained release and subsequent prolonged action of gallic acid from the nanoparticles over time. At 48 hours, the GA-AuNPs continued to show higher efficacy by its IC₅₀ value of 18.35 µg/mL. In contrast, free gallic acid and AuNPs manifested IC₅₀ values of 35.12 µg/mL and 48.29 µg/mL, respectively. Continued efficacy over 48 hours therefore proves the potential of GA-AuNPs for long-term therapeutic applications since a sustained release mechanism will ensure the availability of the drug and its subsequent constant action.

Chavda et. Al (Chavda et al., 2022) concluded that gold nanoparticles potentiate the bioavailability and cellular uptake of conjugated bioactive compounds, leading to enhanced therapeutic efficacy. The conjugation of gallic acid to AuNPs must have brought about such synergistic effects, enhancing its anticancer properties. Numerous studies have showed that drug-loaded nanoparticles exhibited controlled and more sustained drug release, which improved the *in vivo* therapeutic efficacy over longer periods (Yusuf et al., 2023). We have seen from our studies that the gallic acid from GA-AuNPs showed the sustained release, in line with these findings, proving once again the importance of nanoparticle-mediated drug delivery systems so far as the prolonged therapeutic effects are concerned. According to Zhang et al. (Y. Zhang et al., 2019), nanoparticles promote better cellular uptake of drugs due to reduction in their size and increase in surface area, which would result in increased amount at the cell level and improved efficacy. The GA-AuNPs showed improved IC₅₀ values, which could be attributed to the enhanced cellular uptake of gallic acid facilitated by the gold nanoparticles.

The conjugation of gallic acid to the surface of gold nanoparticles increases its solubility and stability, enhancing its bioavailability and hence its therapeutic potential. Of course, this holds especially true against a background of poorly soluble gallic acid in aqueous environments. Gold nanoparticles are very good carriers for targeted drug delivery and help in ensuring preferential accumulation of gallic acid within cancer cells. This means the targeted delivery minimizes the off-target effects, thereby enhancing the therapeutic efficacy of the drug. With a controlled release mechanism embedded within the nanoparticle matrix, gallic acid would have a continuous, sustained release with time to ensure the maintenance of the therapeutic drug concentration at the target site, thus improving treatment efficacy. This likely results in the synergy between gold nanoparticles and gallic acid, enhancing the anticancer properties of the latter. Gold nanoparticles are known to cause oxidative stress, hence disturbing cellular homeostasis, and would further potentiate the anticancer activity of gallic acid.

CHAPTER 6 Conclusion, Future Scope and Social Impact

Cancer continues to pose a significant challenge within the healthcare domain owing to its inherent resistance to current therapeutic modalities and its capacity to adapt to adverse conditions, which includes the evasion of programmed cell death mechanisms such as anoikis.

Anoikis resistance is of paramount importance for progression of cancer metastasis. This metastatic transition is dependent on the regulation of multiple factors like apoptosis inhibition, dysregulation of detachment induced apoptosis, EMT, autophagy modulation etc. The coordination of these processes enables cancer cells to withstand detachment-induced stress, evade programmed cell death, and establish secondary tumors, underscoring the complexity of metastasis. Therapeutic strategies targeting anoikis resistance are being explored to inhibit metastasis. These include targeting survival pathways like inhibiting key signaling pathways that confer anoikis resistance, such as the PI3K/Akt and MAPK pathways, can sensitize cancer cells to detachment-induced apoptosis. Another example is modulating autophagy by regulating autophagy through pharmacological agents can influence cancer cell survival during detachment, offering potential therapeutic avenues. By understanding and targeting the mechanisms underlying anoikis resistance, these therapeutic approaches aim to prevent metastasis and improve cancer treatment outcomes.

This investigation emphasizes the paramount importance of anoikis resistance in the processes of cancer progression and metastasis, thereby illuminating the prospects for therapeutic interventions aimed at targeting this particular phenomenon. Our study has illustrated the efficacy of amalgamating computational and experimental methodologies to elucidate the molecular pathways and genes that are implicated in anoikis, apoptosis, autophagy, EMT and metastasis. Through the examination of 102 natural compounds recognized for their anti-tumor attributes, we have identified several bioactive molecules, such as Dioscin, nitidine chloride, gastrodin, chenodeoxycholic acid (CDCA), bilobalide, and gallic acid, as potential modulators of pivotal proteins and genes associated with anoikis resistance. Remarkably, Dioscin exhibited favorable interactions with Bcl-2, whereas nitidine chloride, bilobalide, CDCA, and gastrodin demonstrated synergistic effects in modulating gene expression profiles pertinent to anoikis resistance. The full potential of Dioscin, a natural compound isolated from traditional Chinese herbs, as a potent inhibitor of anti-apoptotic protein Bcl-2, was investigated in this study. In this respect, molecular docking and molecular dynamics simulation techniques combined with detailed bioinformatic analysis have shown that Dioscin binds efficiently to Bcl-2, similar to the well-known Bcl-2 inhibitor ABT-737. According to molecular dynamics simulations, Dioscin revealed a higher conformational flexibility compared with ABT-737 but showed stable interactions with the key residues of Bcl-2 within a simulation time of 100 ns.

The free energy calculations of binding further reiterate the potential strong affinity of Dioscin with Bcl-2 and hence its possible therapeutic application in cancer therapy by the downregulation of Bcl-2, a promoter of cell death through apoptosis. It was further assessed by gene expression studies that Dioscin had significantly downregulated Bcl-2 while upregulating the pro-apoptotic protein, BAX, leading to apoptosis in cancer cells. Overexpression of BCL-2 in cancer cells can suppress anoikis, enabling these cells to survive without proper attachment, thereby facilitating metastasis. By binding to and neutralizing pro-apoptotic proteins, BCL-2 prevents the activation of apoptotic pathways that would normally lead to cell death upon detachment. This suppression of anoikis contributes to the metastatic potential of cancer cells, allowing them to disseminate and establish secondary tumors.

Differential gene expression analysis revealed that anti-apoptotic effects of Dioscin make it a potential therapeutic agent in multi-targeted cancer therapies. The study also explained how Dioscin could modulate different biological pathways associated with the progression of cancer and hence gave insights into its mechanism of therapy beyond the inhibition of Bcl-2.

The overall outcome that Dioscin emerges as a potential candidate for further development into an anticancer agent is its origin from a natural product, and multifaceted pharmacological properties are useful in combating cancer cell survival and proliferation effectively. Our study provides a comprehensive analysis of the therapeutic potential of bioactive natural compounds in cancer treatment, focusing on their multitargeting properties and their effects on gene expression profiles associated with cancer progression and anoikis resistance. By modulating multiple signaling pathways, these compounds can influence key processes such as apoptosis, EMT, and autophagy, which are critical in cancer metastasis.

A combination of bioactive natural compounds—gastrodin, nitidine chloride, chenodeoxycholic acid, and bilobalide—emerged from our stringent analysis of differential gene expression patterns over the different time intervals and treatments as very promising candidates against prostate cancer. These bioactive compounds regulated the key genes participating in critical processes, such as apoptosis, modulation of immune response, and signalling pathways required for tumor progression, under differential conditions. It had been shown that these natural compounds could exert multitargeting capabilities, wherein the multiple pathways were affected contemporaneously. This is one critical approach in addressing the heterogeneous nature of cancer and in overcoming mechanisms of drug resistance often developed in response to single-target therapies.

Our analysis of selected bioactive natural compounds revealed synergistic interactions, indicating that their combined use may enhance therapeutic efficacy while minimizing potential side effects. This synergy underscores the importance of exploring combination therapies in cancer treatment to maximize patient benefits.

In cancer therapy, utilizing synergistic combinations of natural compounds enables the concurrent targeting of multiple pathways involved in tumor progression. This strategy enhances therapeutic efficacy and allows for the use of lower doses, thereby minimizing potential side effects. Consequently, adopting a therapeutic approach that integrates multitargeting, synergy, and reduced dosages holds significant promise for improving patient outcomes.

Specifically, among the genes important for the survival and proliferation of cancer cells, a set was consistently targeted and regulated by the studied compounds. For instance, the genes controlling apoptosis, such as PDLIM5 and ZSCAN12; immune response, like SLC22A23; and cell polarity, like PARD6B, are greatly downregulated and may form some of the mechanisms through which these compounds exert their antitumor activities.

The findings show that they offer promising avenues of clinical translation, in which these natural compounds are integrated into extant therapeutic regimes or explored as adjuncts to the therapy. Their potentials and capabilities in modulating various biological pathways associated with cancer made them a very good fit to complement conventional treatments, such that there will be an overall improvement in patient outcomes. Moreover, further research is needed to validate these findings in clinical trials as well as pre-clinical models. Explicitly, elucidating the optimal combinations This will not only detail the optimum combinations, dosages, and regimes of administration for the compounds but also aid in maximizing their therapeutic benefits against minimal side effects.

Finally, further insight into the mechanisms underlying the observed synergistic effects and their impact on tumor microenvironment dynamics will go a long way in opening up the therapeutic potential behind these compounds.

The present study also aimed at determining the dynamic regulation of gene expression associated with anoikis resistance in cancer cells upon treatment at different time intervals with gallic acid. By analysing RNA sequencing data from the GSE158788 dataset, we found changes in the 53 mapped DEGs at different temporal points. These observations underline the treatment potential of gallic acid in circumventing mechanisms of resistance in cancer cells through multifaceted effects on crucial bioprocesses.

Gallic acid exhibits time-dependent effects on gene expression profiles associated with cancer cell death mechanisms. At early intervals—specifically the 2nd, 4th, and 6th hours—39 out of 53 DEGs are differentially regulated. By the 9th hour, 37 genes show differential expression. This dynamic interaction highlights gallic acid's influence on cancer cell pathways related to apoptosis, cell polarity, and cellular homeostasis.

Several of these genes, critically associated with cancer progression and resistance, were significantly affected by gallic acid treatment. This includes genes involved in regulating cytoskeletal organization and cell survival, such as PDLIM5, whose downregulation may be the possible mechanism behind gallic acid's ability to inhibit tumor growth and survival. Again, genes like ZSCAN12, PARD6B, TRIM36, and SLC22A23 whose expression was consistently modulated across all time intervals, their roles, therefore, mediating the anticancer activities of gallic acid.

Temporal studies on gallic acid revealed that it activates events, such as caspase activation and mitochondrial release of cytochrome c-GFP, nuclear condensation, and plasma membrane rupture at different time intervals. These molecular events support gallic acid-induced cytotoxicity towards cancer cells yet sparing normal cells, underscoring its potential as a targeted therapeutic agent. We further used gallic acid for in-vitro studies. In this study, the gold nanoparticles (AuNP) synthesized by the citrate reduction method were used to carry gallic acid for studying drug loading and drug release efficiency by this complex. The attachment of gallic acid molecules onto the AuNP surface was evident from UV-visible spectra which exhibited different absorption peaks at 280 nm and 320–370 nm indicating GA conjugation. Dynamic light scattering (DLS) showed a change in the particle size distribution after loading with GA; GA-AuNPs mostly had a size between 100 nm and 250 nm, which confirmed the successful loading.

FTIR spectra corroborated the attachment of GA and revealed that these were typically associated with characteristic peaks for C=O stretching and phenolic OH vibrations in the GA-AuNPs spectra. The kinetics of drug release and the stability of GA-AuNPs were also studied and showed a controlled and effective profile over 48 h at physiological conditions with zero-order kinetics. The slow and sustained release pattern would indicate GA-AuNPs to be a potent candidate for an effective and reliable drug release system.

The results demonstrate the successful conjugation of gallic acid with gold nanoparticles for the use of their combined properties in biomedical applications. These conditions for enhanced drug delivery efficiency should be optimized in further research with tests in in vitro and in vivo studies toward therapeutic efficacy evaluations and safety.

This study, therefore, brings an invaluable contribution related to the designing and characterization of GA-AuNPs, underpinning the promising role in targeted drug delivery systems as nanocarriers.

Gallic acid, received from common dietary sources, has been evaluated against a wide spectrum of cancers showing anticancer and antioxidant properties. The minimal adverse effects observed in studies suggest that these treatments warrant further investigation for rigorous clinical application as safe and effective options. This is particularly relevant in combination therapies aimed at enhancing treatment responses and mitigating resistance mechanisms. These results form a basis for more detailed researches into the molecular mechanisms of actions of gallic acid on gene expression and induction of cancer cell death pathways. How gallic acid interacts synergistically with other therapeutic agents will have to be understood, which can then be subsequently validated in preclinical and clinical setups for translation into clinics.

In cancer therapy, combining natural compounds with conventional treatments can produce synergistic effects, allowing for the use of lower doses while maintaining or enhancing therapeutic efficacy. This approach not only reduces potential side effects but also targets multiple pathways involved in tumor progression, improving overall treatment outcomes. Therefore, employing combination therapies that leverage the synergistic properties of natural compounds offers a promising strategy for effective and safer cancer treatment. Furthermore, combining gene therapy with nanoplatforms offers strategies to induce cancer cell apoptosis and inhibit tumorigenesis, providing a multifunctional approach to cancer treatment.

In conclusion, it can be determined that gallic acid, Dioscin, CDCA, nitidine chloride, gastrodin and bilobalide among bioactive natural compounds are promising candidate for targeting anoikis resistance in cancer therapy. By elucidating the dynamic effects of natural compounds on gene expression profiles, this study enhances our understanding of their significant therapeutic potential. These findings pave the way for future research into personalized cancer treatment strategies, emphasizing the importance of tailoring therapies to individual genetic and epigenetic landscapes. These insights underscore the potential of integrating natural compounds into personalized cancer treatment regimens, offering a promising avenue for enhancing therapeutic outcomes and overcoming resistance mechanisms.

References

- Abdollahiyan, P., Oroojalian, F., Baradaran, B., de la Guardia, M., & Mokhtarzadeh, A. (2021). Advanced mechanotherapy: Biotensegrity for governing metastatic tumor cell fate via modulating the extracellular matrix. *Journal of Controlled Release*, 335, 596–618. <https://doi.org/10.1016/j.jconrel.2021.06.002>
- Adasme, M. F., Linnemann, K. L., Bolz, S. N., Kaiser, F., Salentin, S., Haupt, V. J., & Schroeder, M. (2021). PLIP 2021: expanding the scope of the protein–ligand interaction profiler to DNA and RNA. *Nucleic Acids Research*, 49(W1), W530–W534. <https://doi.org/10.1093/nar/gkab294>
- Adeshakin, F. O., Adeshakin, A. O., Afolabi, L. O., Yan, D., Zhang, G., & Wan, X. (2021). Mechanisms for Modulating Anoikis Resistance in Cancer and the Relevance of Metabolic Reprogramming. *Frontiers in Oncology*, 11. <https://doi.org/10.3389/fonc.2021.626577>
- Ainsley, J., Mulholland, A. J., Black, G. W., Sparagano, O., Christov, C. Z., & Karabancheva-Christova, T. G. (2018). Structural Insights from Molecular Dynamics Simulations of Tryptophan 7-Halogenase and Tryptophan 5-Halogenase. *ACS Omega*, 3(5), 4847–4859. <https://doi.org/10.1021/acsomega.8b00385>
- Akhtar, M. F., Saleem, A., Rasul, A., Faran Ashraf Baig, M. M., Bin-Jumah, M., & Abdel Daim, M. M. (2020). Anticancer natural medicines: An overview of cell signaling and other targets of anticancer phytochemicals. *European Journal of Pharmacology*, 888, 173488. <https://doi.org/10.1016/j.ejphar.2020.173488>
- Al-Khedhairy, A. A., & Wahab, R. (2022). Size-Dependent Cytotoxic and Molecular Study of the Use of Gold Nanoparticles against Liver Cancer Cells. *Applied Sciences*, 12(2), 901. <https://doi.org/10.3390/app12020901>
- Almansour, N. M., Allemailem, K. S., Abd El Aty, A. A., Ismail, E. I. F., & Ibrahim, M. A. A. (2023). In Silico Mining of Natural Products Atlas (NPAtlas) Database for Identifying Effective Bcl-2 Inhibitors: Molecular Docking, Molecular Dynamics, and Pharmacokinetics Characteristics. *Molecules*, 28(2), 783. <https://doi.org/10.3390/molecules28020783>
- Aly, S. H., Abulsoud, A. I., Moustafa, Y. M., Abdel Mageed, S. S., Abdelmaksoud, N. M., El-Dakrouy, W. A., Mohammed, O. A., Abdel-Reheim, M. A., Zaki, M. B., Rizk, N. I., Elshafei, A., Elimam, H., Ashraf, A., & Doghish, A. S. (2024). Harnessing natural compounds to modulate miRNAs in breast cancer therapy. *Functional & Integrative Genomics*, 24(6), 211. <https://doi.org/10.1007/s10142-024-01489-7>
- Anantram, A., Kundaikar, H., Degani, M., & Prabhu, A. (2019). Molecular dynamic simulations on an inhibitor of anti-apoptotic Bcl-2 proteins for insights into its interaction mechanism for anti-cancer activity. *Journal of Biomolecular Structure and Dynamics*, 37(12), 3109–3121. <https://doi.org/10.1080/07391102.2018.1508371>
- Armstrong, C. M., & Gao, A. C. (2015). Drug resistance in castration resistant prostate cancer: resistance mechanisms and emerging treatment strategies. *American Journal of Clinical and Experimental Urology*, 3(2), 64–76.
- Arneth, B. (2020). Tumor microenvironment. *Medicina (Lithuania)*, 56(1).

<https://doi.org/10.3390/MEDICINA56010015>

- Aryal, S., Grailer, J. J., Pilla, S., Steeber, D. A., & Gong, S. (2009). Doxorubicin conjugated gold nanoparticles as water-soluble and pH-responsive anticancer drug nanocarriers. *Journal of Materials Chemistry*, 19(42), 7879. <https://doi.org/10.1039/b914071a>
- Attwood, M. M., Fabbro, D., Sokolov, A. V., Knapp, S., & Schiöth, H. B. (2021). Trends in kinase drug discovery: targets, indications and inhibitor design. *Nature Reviews Drug Discovery*, 20(11), 839–861. <https://doi.org/10.1038/s41573-021-00252-y>
- Aumsuwan, P., Khan, S. I., Khan, I. A., Walker, L. A., & Dasmahapatra, A. K. (2016). Gene expression profiling and pathway analysis data in MCF-7 and MDA-MB-231 human breast cancer cell lines treated with dioscin. *Data in Brief*, 8, 272–279. <https://doi.org/10.1016/j.dib.2016.05.040>
- Ayla, S., & Karahüseyinoğlu, S. (2019). Cancer stem cells, their microenvironment and anoikis. *Critical Reviews in Oncogenesis*, 24(1), 27–34. <https://doi.org/10.1615/CritRevOncog.2018029433>
- Babaei, G., Aziz, S. G.-G., & Jaghi, N. Z. Z. (2021). EMT, cancer stem cells and autophagy; The three main axes of metastasis. *Biomedicine & Pharmacotherapy*, 133, 110909. <https://doi.org/10.1016/j.biopha.2020.110909>
- Badadani, M. (2012). Autophagy Mechanism, Regulation, Functions, and Disorders. *International Scholarly Research Network ISRN Cell Biology*, 2012, 11. <https://doi.org/10.5402/2012/927064>
- Baghban, R., Roshangar, L., Jahanban-Esfahlan, R., Seidi, K., Ebrahimi-Kalan, A., Jaymand, M., Kolahian, S., Javaheri, T., & Zare, P. (2020). Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Communication and Signaling*, 18(1), 59. <https://doi.org/10.1186/s12964-020-0530-4>
- Bahadar, N., Bahadar, S., Sajid, A., Wahid, M., Ali, G., Alghamdi, A., Zada, H., Khan, T., Ullah, S., & Sun, Q. (2024). Epigallocatechin gallate and curcumin inhibit Bcl-2: a pharmacophore and docking based approach against cancer. *Breast Cancer Research*, 26(1), 114. <https://doi.org/10.1186/s13058-024-01868-9>
- Balakrishnan, S., Mukherjee, S., Das, S., Bhat, F. A., Raja Singh, P., Patra, C. R., & Arunakaran, J. (2017). Gold nanoparticles–conjugated quercetin induces apoptosis via inhibition of EGFR/PI3K/Akt–mediated pathway in breast cancer cell lines (MCF-7 and MDA-MB-231). *Cell Biochemistry and Function*, 35(4), 217–231. <https://doi.org/10.1002/cbf.3266>
- Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., Marshall, K. A., Phillippy, K. H., Sherman, P. M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C. L., Serova, N., Davis, S., & Soboleva, A. (2012). NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Research*, 41(D1), D991–D995. <https://doi.org/10.1093/nar/gks1193>
- Barriere, G., Fici, P., Gallerani, G., Fabbri, F., & Rigaud, M. (2015). Epithelial Mesenchymal Transition: a double-edged sword. *Clinical and Translational Medicine*, 4(1). <https://doi.org/10.1186/s40169-015-0055-4>
- Baulida, J. (2017). Epithelial-to-mesenchymal transition transcription factors in cancer-associated fibroblasts. *Molecular Oncology*, 11(7), 847–859.

<https://doi.org/10.1002/1878-0261.12080>

- Bayne, L. J., Beatty, G. L., Jhala, N., Clark, C. E., Rhim, A. D., Stanger, B. Z., & Vonderheide, R. H. (2012). Tumor-Derived Granulocyte-Macrophage Colony-Stimulating Factor Regulates Myeloid Inflammation and T Cell Immunity in Pancreatic Cancer. *Cancer Cell*, 21(6), 822–835. <https://doi.org/10.1016/j.ccr.2012.04.025>
- Begemann, D., Anastos, H., & Kyprianou, N. (2018). Cell death under epithelial–mesenchymal transition control in prostate cancer therapeutic response. *International Journal of Urology*, 25(4), 318–326. <https://doi.org/10.1111/iju.13505>
- Benvenuto, M., Albonici, L., Focaccetti, C., Ciuffa, S., Fazi, S., Cifaldi, L., Miele, M. T., De Maio, F., Tresoldi, I., Manzari, V., Modesti, A., Masuelli, L., & Bei, R. (2020). Polyphenol-Mediated Autophagy in Cancer: Evidence of In Vitro and In Vivo Studies. *International Journal of Molecular Sciences*, 21(18), 6635. <https://doi.org/10.3390/ijms21186635>
- Bergers, G., & Fendt, S.-M. (2021). The metabolism of cancer cells during metastasis. *Nature Reviews Cancer*, 21(3), 162–180. <https://doi.org/10.1038/s41568-020-00320-2>
- Berthelet, J., & Dubrez, L. (2013). Regulation of Apoptosis by Inhibitors of Apoptosis (IAPs). *Cells*, 2(1), 163–187. <https://doi.org/10.3390/cells2010163>
- Betin, V. M. S., & Lane, J. D. (2009). Atg4D at the interface between autophagy and apoptosis. *Autophagy*, 5(7), 1057–1059. <https://doi.org/10.4161/auto.5.7.9684>
- Binienda, A., Ziolkowska, S., & Pluciennik, E. (2020). The Anticancer Properties of Silibinin: Its Molecular Mechanism and Therapeutic Effect in Breast Cancer. *Anti-Cancer Agents in Medicinal Chemistry*, 20(15), 1787–1796. <https://doi.org/10.2174/1871520620666191220142741>
- Birkinshaw, R. W., Gong, J., Luo, C. S., Lio, D., White, C. A., Anderson, M. A., Blombery, P., Lessene, G., Majewski, I. J., Thijssen, R., Roberts, A. W., Huang, D. C. S., Colman, P. M., & Czabotar, P. E. (2019). Structures of BCL-2 in complex with venetoclax reveal the molecular basis of resistance mutations. *Nature Communications*, 10(1), 2385. <https://doi.org/10.1038/s41467-019-10363-1>
- Bohg, C., Öster, C., Utesch, T., Bischoff, S., Lange, S., Shi, C., Sun, H., & Lange, A. (2021). A combination of solid-state NMR and MD simulations reveals the binding mode of a rhomboid protease inhibitor. *Chemical Science*, 12(38), 12754–12762. <https://doi.org/10.1039/D1SC02146J>
- Boppart, M. D., & Mahmassani, Z. S. (2019). Integrin signaling: linking mechanical stimulation to skeletal muscle hypertrophy. *American Journal of Physiology-Cell Physiology*, 317(4), C629–C641. <https://doi.org/10.1152/ajpcell.00009.2019>
- Bouchier-Hayes, L., & Green, D. R. (2012). Caspase-2: the orphan caspase. *Cell Death & Differentiation*, 19(1), 51–57. <https://doi.org/10.1038/cdd.2011.157>
- Braicu, C., Zanoaga, O., Zimta, A.-A., Tigau, A. B., Kilpatrick, K. L., Bishayee, A., Nabavi, S. M., & Berindan-Neagoe, I. (2022). Natural compounds modulate the crosstalk between apoptosis- and autophagy-regulated signaling pathways: Controlling the uncontrolled expansion of tumor cells. *Seminars in Cancer Biology*, 80, 218–236. <https://doi.org/10.1016/j.semcancer.2020.05.015>
- Cao, W., Lan, J., Zeng, Z., Yu, W., & Lei, S. (2023). Gastrodin Induces Ferroptosis of Glioma

- Cells via Upregulation of Homeobox D10. *Molecules*, 28(24), 8062. <https://doi.org/10.3390/molecules28248062>
- Cao, Y. (2011). Mechanisms of the apoptosis induced by CD176 antibody in human leukemic cells. *International Journal of Oncology*. <https://doi.org/10.3892/ijo.2011.992>
- Cao, Z., & Kyprianou, N. (2015). Mechanisms navigating the TGF- β pathway in prostate cancer. *Asian Journal of Urology*, 2(1), 11–18. <https://doi.org/10.1016/j.ajur.2015.04.011>
- Cao, Z., Livas, T., & Kyprianou, N. (2016). Anoikis and EMT: Lethal “Liaisons” during Cancer Progression. *Critical ReviewsTM in Oncogenesis*, 21(3–4), 155–168. <https://doi.org/10.1615/CritRevOncog.2016016955>
- Carneiro, B. A., & El-Deiry, W. S. (2020). Targeting apoptosis in cancer therapy. *Nature Reviews Clinical Oncology*, 17(7), 395–417. <https://doi.org/10.1038/s41571-020-0341-y>
- Cavalcante, G. C., Schaan, A. P., Cabral, G. F., Santana-da-Silva, M. N., Pinto, P., Vidal, A. F., & Ribeiro-dos-Santos, Â. (2019). A Cell’s Fate: An Overview of the Molecular Biology and Genetics of Apoptosis. *International Journal of Molecular Sciences*, 20(17). <https://doi.org/10.3390/ijms20174133>
- Cetraro, P., Plaza-Diaz, J., Mackenzie, A., & Abadía-Molina, F. (2022). A Review of the Current Impact of Inhibitors of Apoptosis Proteins and Their Repression in Cancer. *Cancers*, 14(7), 1–25. <https://doi.org/10.3390/cancers14071671>
- Chamberlin, S. R., Blucher, A., Wu, G., Shinto, L., Choonoo, G., Kulesz-Martin, M., & McWeeney, S. (2019). Natural Product Target Network Reveals Potential for Cancer Combination Therapies. *Frontiers in Pharmacology*, 10. <https://doi.org/10.3389/fphar.2019.00557>
- Chandrasekar, T., Yang, J. C., Gao, A. C., & Evans, C. P. (2015). Mechanisms of resistance in castration-resistant prostate cancer (CRPC). *Translational Andrology and Urology*, 4(3), 365–380. <https://doi.org/10.3978/j.issn.2223-4683.2015.05.02>
- Chatterjee, S., Chakraborty, R., & Hasija, Y. (2022). Polymorphisms at site 469 of B-Raf protein associated with skin melanoma may be correlated with dabrafenib resistance: An in silico study. *Journal of Biomolecular Structure and Dynamics*, 40(21), 10862–10877. <https://doi.org/10.1080/07391102.2021.1950571>
- Chaurasia, M., Gupta, S., Das, A., Dwarakanath, B. S., Simonsen, A., & Sharma, K. (2019). Radiation induces EIF2AK3/PERK and ERN1/IRE1 mediated pro-survival autophagy. *Autophagy*, 15(8), 1391–1406. <https://doi.org/10.1080/15548627.2019.1582973>
- Chavda, V. P., Patel, A. B., Mistry, K. J., Suthar, S. F., Wu, Z.-X., Chen, Z.-S., & Hou, K. (2022). Nano-Drug Delivery Systems Entrapping Natural Bioactive Compounds for Cancer: Recent Progress and Future Challenges. *Frontiers in Oncology*, 12. <https://doi.org/10.3389/fonc.2022.867655>
- Chen, C.-Y., Lin, Y.-S., Chen, C.-H., & Chen, Y.-J. (2018). Annexin A2-mediated cancer progression and therapeutic resistance in nasopharyngeal carcinoma. *Journal of Biomedical Science*, 25(1), 30. <https://doi.org/10.1186/s12929-018-0430-8>
- Chen, D., Fan, W., Lu, Y., Ding, X., Chen, S., & Zhong, Q. (2012). A Mammalian Autophagosome Maturation Mechanism Mediated by TECPR1 and the Atg12-Atg5 Conjugate. *Molecular Cell*, 45(5), 629–641. <https://doi.org/10.1016/j.molcel.2011.12.036>

- Chen, J., Wang, J., Lin, L., He, L., Wu, Y., Zhang, L., Yi, Z., Chen, Y., Pang, X., & Liu, M. (2012). Inhibition of STAT3 Signaling Pathway by Nitidine Chloride Suppressed the Angiogenesis and Growth of Human Gastric Cancer. *Molecular Cancer Therapeutics*, 11(2), 277–287. <https://doi.org/10.1158/1535-7163.MCT-11-0648>
- Chen, N., & Debnath, J. (2013). IκB kinase complex (IKK) triggers detachment-induced autophagy in mammary epithelial cells independently of the PI3K-AKT-MTORC1 pathway. *Autophagy*, 9(8), 1214–1227. <https://doi.org/10.4161/auto.24870>
- Chen, Q., Kang, J., & Fu, C. (2018). The independence of and associations among apoptosis, autophagy, and necrosis. *Signal Transduction and Targeted Therapy*, 3(1), 18. <https://doi.org/10.1038/s41392-018-0018-5>
- Chen, S., Gu, J., Zhang, Q., Hu, Y., & Ge, Y. (2021). Development of Biomarker Signatures Associated with Anoikis to Predict Prognosis in Endometrial Carcinoma Patients. *Journal of Oncology*, 2021, 1–12. <https://doi.org/10.1155/2021/3375297>
- Choi, H.-S., & Baek, K.-H. (2022). Pro-apoptotic and anti-apoptotic regulation mediated by deubiquitinating enzymes. *Cellular and Molecular Life Sciences*, 79(2), 117. <https://doi.org/10.1007/s00018-022-04132-5>
- Choi, Y. H., Im, E. O., Suh, H., Jin, Y., Yoo, Y. H., & Kim, N. D. (2003). Apoptosis and modulation of cell cycle control by synthetic derivatives of ursodeoxycholic acid and chenodeoxycholic acid in human prostate cancer cells. *Cancer Letters*, 199(2), 157–167. [https://doi.org/10.1016/S0304-3835\(03\)00351-3](https://doi.org/10.1016/S0304-3835(03)00351-3)
- Chunhacha, P., Pongrakhananon, V., Rojanasakul, Y., & Chanvorachote, P. (2012). Caveolin-1 regulates Mcl-1 stability and anoikis in lung carcinoma cells. *American Journal of Physiology-Cell Physiology*, 302(9), C1284–C1292. <https://doi.org/10.1152/ajpcell.00318.2011>
- Corkery, D. P., Clarke, L. E., Gebremeskel, S., Salsman, J., Pinder, J., Le Page, C., Meunier, L., Xu, Z., Mes-Masson, A.-M., Berman, J. N., Johnston, B., & Dellaire, G. (2018). Loss of PRP4K drives anoikis resistance in part by dysregulation of epidermal growth factor receptor endosomal trafficking. *Oncogene*, 37(2), 174–184. <https://doi.org/10.1038/onc.2017.318>
- Cox, A. D., & Der, C. J. (2010). Ras history. *Small GTPases*, 1(1), 2–27. <https://doi.org/10.4161/sgtp.1.1.12178>
- Cragg, G. M., & Pezzuto, J. M. (2016). Natural Products as a Vital Source for the Discovery of Cancer Chemotherapeutic and Chemopreventive Agents. *Medical Principles and Practice*, 25(Suppl. 2), 41–59. <https://doi.org/10.1159/000443404>
- Criollo, A., Senovilla, L., Authier, H., Maiuri, M. C., Morselli, E., Vitale, I., Kepp, O., Tadmair, E., Galluzzi, L., Shen, S., Tailler, M., Delahaye, N., Tesniere, A., De Stefano, D., Younes, A. Ben, Harper, F., Pierron, G., Lavandro, S., Zitvogel, L., ... Kroemer, G. (2010). The IKK complex contributes to the induction of autophagy. *The EMBO Journal*, 29(3), 619–631. <https://doi.org/10.1038/emboj.2009.364>
- Cui, Y., Wu, L., Cao, R., Xu, H., Xia, J., Wang, Z. P., & Ma, J. (2020). Antitumor functions and mechanisms of nitidine chloride in human cancers. *Journal of Cancer*, 11(5), 1250–1256. <https://doi.org/10.7150/jca.37890>
- Curry, D., Cameron, A., MacDonald, B., Nganou, C., Scheller, H., Marsh, J., Beale, S., Lu,

- M., Shan, Z., Kaliaperumal, R., Xu, H., Servos, M., Bennett, C., MacQuarrie, S., Oakes, K. D., Mkandawire, M., & Zhang, X. (2015). Adsorption of doxorubicin on citrate-capped gold nanoparticles: insights into engineering potent chemotherapeutic delivery systems. *Nanoscale*, 7(46), 19611–19619. <https://doi.org/10.1039/C5NR05826K>
- Dadsena, S., King, L. E., & García-Sáez, A. J. (2021). Apoptosis regulation at the mitochondria membrane level. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1863(12), 183716. <https://doi.org/10.1016/j.bbamem.2021.183716>
- Dai, Y., & Grant, S. (2015). BCL2L1/Bim as a dual-agent regulating autophagy and apoptosis in drug resistance. *Autophagy*, 11(2), 416–418. <https://doi.org/10.1080/15548627.2014.998892>
- Dalal, V., Dhankhar, P., Singh, V., Singh, V., Rakhaminov, G., Golemi-Kotra, D., & Kumar, P. (2021). Structure-Based Identification of Potential Drugs Against FmtA of *Staphylococcus aureus*: Virtual Screening, Molecular Dynamics, MM-GBSA, and QM/MM. *The Protein Journal*, 40(2), 148–165. <https://doi.org/10.1007/s10930-020-09953-6>
- de Jesus, A. J., & Allen, T. W. (2013). The role of tryptophan side chains in membrane protein anchoring and hydrophobic mismatch. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1828(2), 864–876. <https://doi.org/10.1016/j.bbamem.2012.09.009>
- De Silva, F., & Alcorn, J. (2022). A Tale of Two Cancers: A Current Concise Overview of Breast and Prostate Cancer. *Cancers*, 14(12), 2954. <https://doi.org/10.3390/cancers14122954>
- Dehelean, C. A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., Cretu, O. M., & Pinzaru, I. (2021). Plant-Derived Anticancer Compounds as New Perspectives in Drug Discovery and Alternative Therapy. *Molecules*, 26(4), 1109. <https://doi.org/10.3390/molecules26041109>
- Del Prete, A., Schioppa, T., Tiberio, L., Stabile, H., & Sozzani, S. (2017). Leukocyte trafficking in tumor microenvironment. *Current Opinion in Pharmacology*, 35, 40–47. <https://doi.org/10.1016/J.COPH.2017.05.004>
- Delgado, C., Mendez-Callejas, G., & Celis, C. (2021). Caryophyllene Oxide, the Active Compound Isolated from Leaves of *Hymenaea courbaril* L. (Fabaceae) with Antiproliferative and Apoptotic Effects on PC-3 Androgen-Independent Prostate Cancer Cell Line. *Molecules*, 26(20), 6142. <https://doi.org/10.3390/molecules26206142>
- Delgado, M., & Tesfaigzi, Y. (2014). Is BMF central for anoikis and autophagy? *Autophagy*, 10(1), 168–169. <https://doi.org/10.4161/auto.26759>
- Deng, Z., Wang, H., Liu, J., Deng, Y., & Zhang, N. (2021). Comprehensive understanding of anchorage-independent survival and its implication in cancer metastasis. *Cell Death & Disease*, 12(7), 629. <https://doi.org/10.1038/s41419-021-03890-7>
- Denk, D., & Greten, F. R. (2022). Inflammation: the incubator of the tumor microenvironment. *Trends in Cancer*, 8(11), 901–914. <https://doi.org/10.1016/j.trecan.2022.07.002>
- Desgrosellier, J. S., & Cheresch, D. A. (2010). Integrins in cancer: biological implications and therapeutic opportunities. *Nature Reviews Cancer*, 10(1), 9–22. <https://doi.org/10.1038/nrc2748>
- Dipalo, M., McGuire, A. F., Lou, H.-Y., Caprettini, V., Melle, G., Bruno, G., Lubrano, C.,

- Matino, L., Li, X., De Angelis, F., Cui, B., & Santoro, F. (2018). Cells Adhering to 3D Vertical Nanostructures: Cell Membrane Reshaping without Stable Internalization. *Nano Letters*, 18(9), 6100–6105. <https://doi.org/10.1021/acs.nanolett.8b03163>
- Doldi, V., Pennati, M., Forte, B., Gandellini, P., & Zaffaroni, N. (2016). Dissecting the role of microRNAs in prostate cancer metastasis: implications for the design of novel therapeutic approaches. *Cellular and Molecular Life Sciences*, 73(13), 2531–2542. <https://doi.org/10.1007/s00018-016-2176-3>
- Dong, W., Li, Y., Gao, M., Hu, M., Li, X., Mai, S., Guo, N., Yuan, S., & Song, L. (2012). IKK α contributes to UVB-induced VEGF expression by regulating AP-1 transactivation. *Nucleic Acids Research*, 40(7), 2940–2955. <https://doi.org/10.1093/nar/gkr1216>
- Dower, C. M., Wills, C. A., Frisch, S. M., & Wang, H.-G. (2018a). Mechanisms and context underlying the role of autophagy in cancer metastasis. *Autophagy*, 14(7), 1110–1128. <https://doi.org/10.1080/15548627.2018.1450020>
- Dower, C. M., Wills, C. A., Frisch, S. M., & Wang, H. G. (2018b). Mechanisms and context underlying the role of autophagy in cancer metastasis. *Autophagy*, 14(7), 1110–1128. <https://doi.org/10.1080/15548627.2018.1450020>
- Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J., & Schreiber, R. D. (2002). Cancer immunoediting: from immunosurveillance to tumor escape. *Nature Immunology*, 3(11), 991–998. <https://doi.org/10.1038/ni1102-991>
- Edlich, F. (2018). BCL-2 proteins and apoptosis: Recent insights and unknowns. *Biochemical and Biophysical Research Communications*, 500(1), 26–34. <https://doi.org/https://doi.org/10.1016/j.bbrc.2017.06.190>
- Elgendy, M., Sheridan, C., Brumatti, G., & Martin, S. J. (2011). Oncogenic Ras-Induced Expression of Noxa and Beclin-1 Promotes Autophagic Cell Death and Limits Clonogenic Survival. *Molecular Cell*, 42(1), 23–35. <https://doi.org/10.1016/j.molcel.2011.02.009>
- Emi, M., Kim, R., Tanabe, K., Uchida, Y., & Toge, T. (2005). Targeted therapy against Bcl-2-related proteins in breast cancer cells. *Breast Cancer Research*, 7(6), R940. <https://doi.org/10.1186/bcr1323>
- Espina, A., Sanchez-Cortes, S., & Jurašková, Z. (2022). Vibrational Study (Raman, SERS, and IR) of Plant Gallnut Polyphenols Related to the Fabrication of Iron Gall Inks. *Molecules*, 27(1), 279. <https://doi.org/10.3390/molecules27010279>
- Fan, Y.-X., Bian, X.-H., Qian, P.-D., Chen, Z.-Z., Wen, J., Luo, Y.-H., Yan, P.-W., & Zhang, Q. (2018). MicroRNA-125b inhibits cell proliferation and induces cell apoptosis in esophageal squamous cell carcinoma by targeting BMF. *Oncology Reports*. <https://doi.org/10.3892/or.2018.6413>
- Fang, Z., Tang, Y., Jiao, W., Xing, Z., Guo, Z., Wang, W., Shi, B., Xu, Z., & Liu, Z. (2013). Nitidine chloride inhibits renal cancer cell metastasis via suppressing AKT signaling pathway. *Food and Chemical Toxicology*, 60, 246–251. <https://doi.org/10.1016/j.fct.2013.07.062>
- Farahani, E., Patra, H. K., Jangamreddy, J. R., Rashedi, I., Kawalec, M., Rao Pariti, R. K., Batakis, P., & Wiechec, E. (2014). Cell adhesion molecules and their relation to (cancer) cell stemness. *Carcinogenesis*, 35(4), 747–759. <https://doi.org/10.1093/carcin/bgu045>
- Fatemipour, M., Nahand, J. S., Fard Azar, M. E., Baghi, H. B., Taghizadieh, M., Sorayyayi, S.,

- Hussen, B. M., Mirzaei, H., Moghoofei, M., & Bokharaei-Salim, F. (2021). Human papillomavirus and prostate cancer: The role of viral expressed proteins in the inhibition of anoikis and induction of metastasis. *Microbial Pathogenesis*, 152, 104576. <https://doi.org/10.1016/j.micpath.2020.104576>
- Ferraresi, A., Girone, C., Esposito, A., Vidoni, C., Vallino, L., Secomandi, E., Dhanasekaran, D. N., & Isidoro, C. (2020). How Autophagy Shapes the Tumor Microenvironment in Ovarian Cancer. *Frontiers in Oncology*, 10. <https://doi.org/10.3389/fonc.2020.599915>
- Fonseka, P., Pathan, M., Chitti, S. V., Kang, T., & Mathivanan, S. (2021). FunRich enables enrichment analysis of OMICs datasets. *Journal of Molecular Biology*, 433(11), 166747. <https://doi.org/10.1016/j.jmb.2020.166747>
- Formaggio, N., Rubin, M. A., & Theurillat, J.-P. (2021). Loss and revival of androgen receptor signaling in advanced prostate cancer. *Oncogene*, 40(7), 1205–1216. <https://doi.org/10.1038/s41388-020-01598-0>
- Frazzi, R. (2021). BIRC3 and BIRC5: multi-faceted inhibitors in cancer. *Cell & Bioscience*, 11(1), 8. <https://doi.org/10.1186/s13578-020-00521-0>
- Garg, A., Agrawal, R., & Deshmukh, R. (2024). Pharmacology of polygala tenuifolia and its significance in traditional Chinese medicine. *Pharmacological Research - Modern Chinese Medicine*, 10, 100341. <https://doi.org/10.1016/j.prmcm.2023.100341>
- Georgous, J., AlSawaftah, N., Abuwatfa, W. H., & Hussein, G. A. (2024). Review of Gold Nanoparticles: Synthesis, Properties, Shapes, Cellular Uptake, Targeting, Release Mechanisms and Applications in Drug Delivery and Therapy. *Pharmaceutics*, 16(10), 1332. <https://doi.org/10.3390/pharmaceutics16101332>
- Ghaemi, S., Arefian, E., Rezazadeh Valojerdi, R., Soleimani, M., Moradimotlagh, A., & Jamshidi Adegani, F. (2020). Inhibiting the expression of anti-apoptotic genes BCL2L1 and MCL1, and apoptosis induction in glioblastoma cells by microRNA-342. *Biomedicine & Pharmacotherapy*, 121, 109641. <https://doi.org/10.1016/j.biopha.2019.109641>
- Ghahremanian, S., Rashidi, M. M., Raeisi, K., & Toghraie, D. (2022). Molecular dynamics simulation approach for discovering potential inhibitors against SARS-CoV-2: A structural review. *Journal of Molecular Liquids*, 354, 118901. <https://doi.org/10.1016/j.molliq.2022.118901>
- Ghosh, S., Khanam, R., & Acharya Chowdhury, A. (2021). The Evolving Roles of Bacopa monnieri as Potential Anti-Cancer Agent: A Review. *Nutrition and Cancer*, 73(11–12), 2166–2176. <https://doi.org/10.1080/01635581.2020.1841248>
- Giannoni, E., Fiaschi, T., Ramponi, G., & Chiarugi, P. (2009). Redox regulation of anoikis resistance of metastatic prostate cancer cells: Key role for Src and EGFR-mediated pro-survival signals. *Oncogene*, 28(20), 2074–2086. <https://doi.org/10.1038/onc.2009.77>
- Girnius, N., & Davis, R. J. (2017). JNK Promotes Epithelial Cell Anoikis by Transcriptional and Post-translational Regulation of BH3-Only Proteins. *Cell Reports*, 21(7), 1910–1921. <https://doi.org/10.1016/j.celrep.2017.10.067>
- González-Llorente, L., Santacatterina, F., García-Aguilar, A., Nuevo-Tapióles, C., González-García, S., Tirpakova, Z., Toribio, M. L., & Cuezva, J. M. (2020). Overexpression of mitochondrial ifl prevents metastatic disease of colorectal cancer by enhancing anoikis and tumor infiltration of NK cells. *Cancers*, 12(1), 6–8.

<https://doi.org/10.3390/cancers12010022>

- Gradzka, S., Thomas, O. S., Kretz, O., Haimovici, A., Vasilikos, L., Wong, W. W.-L., Häcker, G., & Gentle, I. E. (2018). Inhibitor of apoptosis proteins are required for effective fusion of autophagosomes with lysosomes. *Cell Death & Disease*, 9(5), 529. <https://doi.org/10.1038/s41419-018-0508-y>
- Grimm, D., Schulz, H., Krüger, M., Cortés-Sánchez, J. L., Egli, M., Kraus, A., Sahana, J., Corydon, T. J., Hemmersbach, R., Wise, P. M., Infanger, M., & Wehland, M. (2022). The Fight against Cancer by Microgravity: The Multicellular Spheroid as a Metastasis Model. *International Journal of Molecular Sciences*, 23(6), 3073. <https://doi.org/10.3390/ijms23063073>
- Grisoni, F., Merk, D., Friedrich, L., & Schneider, G. (2019). Design of Natural-Product-Inspired Multitarget Ligands by Machine Learning. *ChemMedChem*, 14(12), 1129–1134. <https://doi.org/10.1002/cmdc.201900097>
- Guadamillas, M. C., Cerezo, A., & Del Pozo, M. A. (2011). Overcoming anoikis--pathways to anchorage-independent growth in cancer. *Journal of Cell Science*, 124(Pt 19), 3189–3197. <https://doi.org/10.1242/jcs.072165>
- Guilford, J. M., & Pezzuto, J. M. (2008). Natural products as inhibitors of carcinogenesis. *Expert Opinion on Investigational Drugs*, 17(9), 1341–1352. <https://doi.org/10.1517/13543784.17.9.1341>
- Guo, C., Xu, L., Li, H., Wang, W., Guo, J., Jia, M., Jia, R., & Jia, J. (2019). Transcriptomic study of the mechanism of anoikis resistance in head and neck squamous carcinoma. *PeerJ*, 7, e6978. <https://doi.org/10.7717/peerj.6978>
- Guo, Q., Cao, H., Qi, X., Li, H., Ye, P., Wang, Z., Wang, D., & Sun, M. (2017). Research Progress in Reversal of Tumor Multi-drug Resistance via Natural Products. *Anti-Cancer Agents in Medicinal Chemistry*, 17(11). <https://doi.org/10.2174/1871520617666171016105704>
- Guo, W., & Giancotti, F. G. (2004). Integrin signalling during tumour progression. *Nature Reviews Molecular Cell Biology*, 5(10), 816–826. <https://doi.org/10.1038/nrm1490>
- Gupta, D. Das, Mishra, S., Verma, S. S., Shekher, A., Rai, V., Awasthee, N., Das, T. J., Paul, D., Das, S. K., Tag, H., Chandra Gupta, S., & Hui, P. K. (2021). Evaluation of antioxidant, anti-inflammatory and anticancer activities of diosgenin enriched Paris polyphylla rhizome extract of Indian Himalayan landraces. *Journal of Ethnopharmacology*, 270, 113842. <https://doi.org/10.1016/j.jep.2021.113842>
- Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144(5), 646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
- Harris, K. D. M. (2022). NMR Crystallography as a Vital Tool in Assisting Crystal Structure Determination from Powder XRD Data. *Crystals*, 12(9), 1277. <https://doi.org/10.3390/cryst12091277>
- Hayashida, T., Jinno, H., Kitagawa, Y., & Kitajima, M. (2011). Cooperation of cancer stem cell properties and epithelial-mesenchymal transition in the establishment of breast cancer metastasis. In *Journal of Oncology*. <https://doi.org/10.1155/2011/591427>
- He, S., Yang, J., Hong, S., Huang, H., Zhu, Q., Ye, L., Li, T., Zhang, X., Wei, Y., & Gao, Y. (2020). Dioscin Promotes Prostate Cancer Cell Apoptosis and Inhibits Cell Invasion by

- Increasing SHP1 Phosphorylation and Suppressing the Subsequent MAPK Signaling Pathway. *Frontiers in Pharmacology*, 11. <https://doi.org/10.3389/fphar.2020.01099>
- He, Z. J., Zhu, F. Y., Li, S. S., Zhong, L., Tan, H. Y., & Wang, K. (2017). Inhibiting ROS-NF- κ B-dependent autophagy enhanced brazilin-induced apoptosis in head and neck squamous cell carcinoma. *Food and Chemical Toxicology*, 101, 55–66. <https://doi.org/10.1016/J.FCT.2017.01.002>
- Heerboth, S., Housman, G., Leary, M., Longacre, M., Byler, S., Lapinska, K., Willbanks, A., & Sarkar, S. (2015). EMT and tumor metastasis. *Clinical and Translational Medicine*, 4(1). <https://doi.org/10.1186/s40169-015-0048-3>
- Herishanu, Y., Pérez-Galán, P., Liu, D., Biancotto, A., Pittaluga, S., Vire, B., Gibellini, F., Njuguna, N., Lee, E., Stennett, L., Raghavachari, N., Liu, P., McCoy, J. P., Raffeld, M., Stetler-Stevenson, M., Yuan, C., Sherry, R., Arthur, D. C., Maric, I., ... Wiestner, A. (2011). The lymph node microenvironment promotes B-cell receptor signaling, NF- κ B activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood*, 117(2), 563–574. <https://doi.org/10.1182/blood-2010-05-284984>
- Ho, T. T., Tran, Q. T., & Chai, C. L. (2018). The polypharmacology of natural products. *Future Medicinal Chemistry*, 10(11), 1361–1368. <https://doi.org/10.4155/fmc-2017-0294>
- Huang, A., Garraway, L. A., Ashworth, A., & Weber, B. (2020). Synthetic lethality as an engine for cancer drug target discovery. *Nature Reviews Drug Discovery*, 19(1), 23–38. <https://doi.org/10.1038/s41573-019-0046-z>
- Huang, J., & MacKerell, A. D. (2013). CHARMM36 all-atom additive protein force field: Validation based on comparison to NMR data. *Journal of Computational Chemistry*, 34(25), 2135–2145. <https://doi.org/10.1002/jcc.23354>
- Inge, L. J., Barwe, S. P., D'Ambrosio, J., Gopal, J., Lu, K., Ryazantsev, S., Rajasekaran, S. A., & Rajasekaran, A. K. (2011). Soluble E-cadherin promotes cell survival by activating epidermal growth factor receptor. *Experimental Cell Research*, 317(6), 838–848. <https://doi.org/10.1016/j.yexcr.2010.12.025>
- Jahanban-Esfahlan, R., Seidi, K., Manjili, M. H., Jahanban-Esfahlan, A., Javaheri, T., & Zare, P. (2019). Tumor Cell Dormancy: Threat or Opportunity in the Fight against Cancer. *Cancers*, 11(8), 1207. <https://doi.org/10.3390/cancers11081207>
- Jain, S., Hirst, D. G., & O'Sullivan, J. M. (2012). Gold nanoparticles as novel agents for cancer therapy. *The British Journal of Radiology*, 85(1010), 101–113. <https://doi.org/10.1259/bjr/59448833>
- Jang, Y.-G., Ko, E.-B., & Choi, K.-C. (2020). Gallic acid, a phenolic acid, hinders the progression of prostate cancer by inhibition of histone deacetylase 1 and 2 expression. *The Journal of Nutritional Biochemistry*, 84, 108444. <https://doi.org/10.1016/j.jnutbio.2020.108444>
- Jeong, K. I., Kim, S.-G., Go, D.-S., & Kim, D. K. (2020). Inhibition of cell growth and induction of apoptosis by bilobalide in FaDu human pharyngeal squamous cell carcinoma. *International Journal of Oral Biology*, 45(1), 8–14. <https://doi.org/10.11620/IJOB.2020.45.1.8>
- Jiang, M., Li, J., Peng, Q., Liu, Y., Liu, W., Luo, C., Peng, J., Li, J., Yung, K. K. L., & Mo, Z. (2014). Neuroprotective effects of bilobalide on cerebral ischemia and reperfusion injury

- are associated with inhibition of pro-inflammatory mediator production and down-regulation of JNK1/2 and p38 MAPK activation. *Journal of Neuroinflammation*, 11(1), 167. <https://doi.org/10.1186/s12974-014-0167-6>
- Jing, X., Yang, F., Shao, C., Wei, K., Xie, M., Shen, H., & Shu, Y. (2019). Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Molecular Cancer*, 18(1), 157. <https://doi.org/10.1186/s12943-019-1089-9>
- Kale, J., Kutuk, O., Brito, G. C., Andrews, T. S., Leber, B., Letai, A., & Andrews, D. W. (2018). Phosphorylation switches Bax from promoting to inhibiting apoptosis thereby increasing drug resistance. *EMBO Reports*, 19(9), e45235. <https://doi.org/https://doi.org/10.15252/embr.201745235>
- Kaloni, D., Diepstraten, S. T., Strasser, A., & Kelly, G. L. (2023). BCL-2 protein family: attractive targets for cancer therapy. *Apoptosis*, 28(1–2), 20–38. <https://doi.org/10.1007/s10495-022-01780-7>
- Kamarajugadda, S., Stemboroski, L., Cai, Q., Simpson, N. E., Nayak, S., Tan, M., & Lu, J. (2012). Glucose Oxidation Modulates Anoikis and Tumor Metastasis. *Molecular and Cellular Biology*, 32(10), 1893–1907. <https://doi.org/10.1128/MCB.06248-11>
- Kang, R., Zeh, H. J., Lotze, M. T., & Tang, D. (2011). The Beclin 1 network regulates autophagy and apoptosis. *Cell Death & Differentiation*, 18(4), 571–580. <https://doi.org/10.1038/cdd.2010.191>
- Kapoor, I., Bodo, J., Hill, B. T., Hsi, E. D., & Almasan, A. (2020). Targeting BCL-2 in B-cell malignancies and overcoming therapeutic resistance. *Cell Death & Disease*, 11(11), 941. <https://doi.org/10.1038/s41419-020-03144-y>
- Kepp, O., Tesniere, A., Zitvogel, L., & Kroemer, G. (2009). The immunogenicity of tumor cell death. *Current Opinion in Oncology*, 21(1), 71–76. <https://doi.org/10.1097/CCO.0b013e32831bc375>
- Kesharwani, P., Ma, R., Sang, L., Fatima, M., Sheikh, A., Abourehab, M. A. S., Gupta, N., Chen, Z.-S., & Zhou, Y. (2023). Gold nanoparticles and gold nanorods in the landscape of cancer therapy. *Molecular Cancer*, 22(1), 98. <https://doi.org/10.1186/s12943-023-01798-8>
- Khan, S. U., Fatima, K., & Malik, F. (2022). Understanding the cell survival mechanism of anoikis-resistant cancer cells during different steps of metastasis. *Clinical & Experimental Metastasis*, 39(5), 715–726. <https://doi.org/10.1007/s10585-022-10172-9>
- Kim, J. Y., Lee, N., Kim, Y.-J., Cho, Y., An, H., Oh, E., Cho, T.-M., Sung, D., & Seo, J. H. (2017). Disulfiram induces anoikis and suppresses lung colonization in triple-negative breast cancer via calpain activation. *Cancer Letters*, 386, 151–160. <https://doi.org/10.1016/j.canlet.2016.11.022>
- Kim, L.-H., Khadka, S., Shin, J.-A., Jung, J.-Y., Ryu, M.-H., Yu, H.-J., Lee, H. N., Jang, B., Yang, I.-H., Won, D.-H., Kwon, H.-J., Jeong, J. H., Hong, S. D., Cho, N.-P., & Cho, S.-D. (2017). Nitidine chloride acts as an apoptosis inducer in human oral cancer cells and a nude mouse xenograft model via inhibition of STAT3. *Oncotarget*, 8(53), 91306–91315. <https://doi.org/10.18632/oncotarget.20444>
- Kim, Y.-N., Koo, K. H., Sung, J. Y., Yun, U.-J., & Kim, H. (2012). Anoikis Resistance: An Essential Prerequisite for Tumor Metastasis. *International Journal of Cell Biology*, 2012,

1–11. <https://doi.org/10.1155/2012/306879>

- Kim, Y. N., Koo, K. H., Sung, J. Y., Yun, U. J., & Kim, H. (2012). Anoikis resistance: An essential prerequisite for tumor metastasis. In *International Journal of Cell Biology* (Vol. 2012). Int J Cell Biol. <https://doi.org/10.1155/2012/306879>
- Kinghorn, A. D. (2010). Pharmacognosy in the 21st century. *Journal of Pharmacy and Pharmacology*, 53(2), 135–148. <https://doi.org/10.1211/0022357011775334>
- Knight, T., Luedtke, D., Edwards, H., Taub, J. W., & Ge, Y. (2019). A delicate balance – The BCL-2 family and its role in apoptosis, oncogenesis, and cancer therapeutics. *Biochemical Pharmacology*, 162, 250–261. <https://doi.org/10.1016/j.bcp.2019.01.015>
- Koehler Leman, J., D’Avino, A. R., Bhatnagar, Y., & Gray, J. J. (2018). Comparison of NMR and crystal structures of membrane proteins and computational refinement to improve model quality. *Proteins: Structure, Function, and Bioinformatics*, 86(1), 57–74. <https://doi.org/10.1002/prot.25402>
- Kord, E., Flores, J. P., Posielski, N., Koenig, H., Ho, O., & Porter, C. (2022). Patient reported outcomes and health related quality of life in localized prostate cancer: A review of current evidence. *Urologic Oncology: Seminars and Original Investigations*, 40(7), 304–314. <https://doi.org/10.1016/j.urolonc.2022.04.008>
- Kovermann, M., Rogne, P., & Wolf-Watz, M. (2016). Protein dynamics and function from solution state NMR spectroscopy. *Quarterly Reviews of Biophysics*, 49, e6. <https://doi.org/10.1017/S0033583516000019>
- Krishna, S., Kumar, S. B., Murthy, T. P. K., & Murahari, M. (2021). Structure-based design approach of potential BCL-2 inhibitors for cancer chemotherapy. *Computers in Biology and Medicine*, 134, 104455. <https://doi.org/10.1016/j.compbiomed.2021.104455>
- Krishnan, V., & Rupp, B. (2012). Macromolecular Structure Determination: Comparison of X-ray Crystallography and ^1H NMR Spectroscopy. In *eLS*. Wiley. <https://doi.org/10.1002/9780470015902.a0002716.pub2>
- Kumar, M., Irungbam, K., & Kataria, M. (2018). Depletion of membrane cholesterol compromised caspase-8 imparts in autophagy induction and inhibition of cell migration in cancer cells. *Cancer Cell International*, 18(1), 23. <https://doi.org/10.1186/s12935-018-0520-4>
- Kumar, S., & Das, A. (2023). A Cocktail of Natural Compounds Holds Promise for New Immunotherapeutic Potential in Head and Neck Cancer. *Chinese Journal of Integrative Medicine*. <https://doi.org/10.1007/s11655-023-3694-0>
- Kumari, N., Dalal, V., Kumar, P., & Rath, S. N. (2022). Antagonistic interaction between TTA-A2 and paclitaxel for anti-cancer effects by complex formation with T-type calcium channel. *Journal of Biomolecular Structure and Dynamics*, 40(6), 2395–2406. <https://doi.org/10.1080/07391102.2020.1839558>
- Kumari, R., Rathi, R., Pathak, S. R., & Dalal, V. (2022). Structural-based virtual screening and identification of novel potent antimicrobial compounds against YsxC of *Staphylococcus aureus*. *Journal of Molecular Structure*, 1255, 132476. <https://doi.org/10.1016/j.molstruc.2022.132476>
- Kumari, S., Badana, A. K., Murali Mohan, G., Shailender, G., & Malla, R. R. (2018). Reactive Oxygen Species: A Key Constituent in Cancer Survival. *Biomarker Insights*, 13.

<https://doi.org/10.1177/1177271918755391>

- Le, J., Fu, Y., Han, Q., Ma, Y., Ji, H., Wei, X., Chen, Y., Sun, Y., Gao, Y., & Wu, H. (2021). Transcriptome Analysis of the Inhibitory Effect of Sennoside A on the Metastasis of Hepatocellular Carcinoma Cells. *Frontiers in Pharmacology*, 11. <https://doi.org/10.3389/fphar.2020.566099>
- Leão, R., Domingos, C., Figueiredo, A., Hamilton, R., Tabori, U., & Castelo-Branco, P. (2017). Cancer Stem Cells in Prostate Cancer: Implications for Targeted Therapy. *Urologia Internationalis*, 99(2), 125–136. <https://doi.org/10.1159/000455160>
- Lee, C.-S., Kim, H., Yu, J., Yu, S. H., Ban, S., Oh, S., Jeong, D., Im, J., Baek, M. J., & Kim, T. H. (2017). Doxorubicin-loaded oligonucleotide conjugated gold nanoparticles: A promising in vivo drug delivery system for colorectal cancer therapy. *European Journal of Medicinal Chemistry*, 142, 416–423. <https://doi.org/10.1016/j.ejmech.2017.08.063>
- Lee, K.-H. (2010). Discovery and Development of Natural Product-Derived Chemotherapeutic Agents Based on a Medicinal Chemistry Approach. *Journal of Natural Products*, 73(3), 500–516. <https://doi.org/10.1021/np900821e>
- Lee, Y.-K., & Lee, J.-A. (2016). Role of the mammalian ATG8/LC3 family in autophagy: differential and compensatory roles in the spatiotemporal regulation of autophagy. *BMB Reports*, 49(8), 424–430. <https://doi.org/10.5483/BMBRep.2016.49.8.081>
- Li, X., He, S., & Ma, B. (2020). Autophagy and autophagy-related proteins in cancer. *Molecular Cancer*, 19(1), 12. <https://doi.org/10.1186/s12943-020-1138-4>
- Li, X., & Hu, Y. (2021). Attribution of NF- κ B Activity to CHUK/IKK α -Involved Carcinogenesis. *Cancers*, 13(6), 1411. <https://doi.org/10.3390/cancers13061411>
- Li, Z., Zou, J., Cao, D., & Ma, X. (2020). Pharmacological basis of tanshinone and new insights into tanshinone as a multitarget natural product for multifaceted diseases. *Biomedicine & Pharmacotherapy*, 130, 110599. <https://doi.org/10.1016/j.biopha.2020.110599>
- Lin, D., Shen, L., Luo, M., Zhang, K., Li, J., Yang, Q., Zhu, F., Zhou, D., Zheng, S., Chen, Y., & Zhou, J. (2021). Circulating tumor cells: biology and clinical significance. *Signal Transduction and Targeted Therapy*, 6(1), 404. <https://doi.org/10.1038/s41392-021-00817-8>
- Liu, H., Ong, S.-E., Badu-Nkansah, K., Schindler, J., White, F. M., & Hynes, R. O. (n.d.). CUB-domain-containing protein 1 (CDCP1) activates Src to promote melanoma metastasis. *National Acad Sciences*. <https://doi.org/10.1073/pnas.1017228108>
- Liu, L., Shi, D., Xia, Z., Wang, B., Wang, X., Wang, X., Wang, G., Li, M., Zheng, Q., Li, D., & Li, B. (2023). Gamabufotalin Induces Apoptosis and Cytoprotective Autophagy through the mTOR Signaling Pathway in Hepatocellular Carcinoma. *Journal of Natural Products*, 86(4), 966–978. <https://doi.org/10.1021/acs.jnatprod.2c01155>
- Liu, M., Jiang, L., Fu, X., Wang, W., Ma, J., Tian, T., Nan, K., & Liang, X. (2018). Cytoplasmic liver kinase B1 promotes the growth of human lung adenocarcinoma by enhancing autophagy. *Cancer Science*, 109(10), 3055–3067. <https://doi.org/10.1111/cas.13746>
- Liu, Y., Gao, J., Peng, M., Meng, H., Ma, H., Cai, P., Xu, Y., Zhao, Q., & Si, G. (2018). A Review on Central Nervous System Effects of Gastrodin. *Frontiers in Pharmacology*, 9. <https://doi.org/10.3389/fphar.2018.00024>

- Livas, L., Hasani, S., & Kyprianou, N. (2020). *Integrated Therapeutic Targeting of the Prostate Tumor Microenvironment* (pp. 183–198). https://doi.org/10.1007/978-3-030-59038-3_11
- Logothetis, C. J., & Lin, S.-H. (2005). Osteoblasts in prostate cancer metastasis to bone. *Nature Reviews Cancer*, 5(1), 21–28. <https://doi.org/10.1038/nrc1528>
- Loh, C.-Y., Chai, J., Tang, T., Wong, W., Sethi, G., Shanmugam, M., Chong, P., & Looi, C. (2019). The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. *Cells*, 8(10), 1118. <https://doi.org/10.3390/cells8101118>
- Long, X., Zhou, Y., Lan, M., Huang, S., Liu, Z., & Shu, Y. (2019). Valosin-containing protein promotes metastasis of osteosarcoma through autophagy induction and anoikis inhibition via the ERK/NF- κ B/beclin-1 signaling pathway. *Oncology Letters*. <https://doi.org/10.3892/ol.2019.10716>
- Lopez, A., Reyna, D. E., Gitego, N., Kopp, F., Zhou, H., Miranda-Roman, M. A., Nordstrøm, L. U., Narayanagari, S.-R., Chi, P., Vilar, E., Tsirigos, A., & Gavathiotis, E. (2022). Co-targeting of BAX and BCL-XL proteins broadly overcomes resistance to apoptosis in cancer. *Nature Communications*, 13(1), 1199. <https://doi.org/10.1038/s41467-022-28741-7>
- Lossi, L. (2022). The concept of intrinsic versus extrinsic apoptosis. *Biochemical Journal*, 479(3), 357–384. <https://doi.org/10.1042/BCJ20210854>
- Luo, J.-L., Kamata, H., & Karin, M. (2005). IKK/NF-kappaB signaling: balancing life and death--a new approach to cancer therapy. *The Journal of Clinical Investigation*, 115(10), 2625–2632. <https://doi.org/10.1172/JCI26322>
- Luo, Q., Liu, Q., Tang, K., Zhong, B., Yang, S., & Li, X. (2024). Phillyrin improves myocardial remodeling in salt-sensitive hypertensive mice by reducing endothelin1 signaling. *Journal of Pharmacy and Pharmacology*, 76(6), 672–680. <https://doi.org/10.1093/jpp/rgae018>
- Luo, T., Fu, J., Xu, A., Su, B., Ren, Y., Li, N., Zhu, J., Zhao, X., Dai, R., Cao, J., Wang, B., Qin, W., Jiang, J., Li, J., Wu, M., Feng, G., Chen, Y., & Wang, H. (2016). PSMD10/gankyrin induces autophagy to promote tumor progression through cytoplasmic interaction with ATG7 and nuclear transactivation of ATG7 expression. *Autophagy*, 12(8), 1355–1371. <https://doi.org/10.1080/15548627.2015.1034405>
- Lyu, L., Cui, H., Shao, M., Fu, Y., Zhao, R., & Chen, Q. (2022). Computational Medicine: Past, Present and Future. *Chinese Journal of Integrative Medicine*, 28(5), 453–462. <https://doi.org/10.1007/s11655-021-3453-z>
- Madhusudhan, A., Reddy, G., Venkatesham, M., Veerabhadram, G., Kumar, D., Natarajan, S., Yang, M.-Y., Hu, A., & Singh, S. (2014). Efficient pH Dependent Drug Delivery to Target Cancer Cells by Gold Nanoparticles Capped with Carboxymethyl Chitosan. *International Journal of Molecular Sciences*, 15(5), 8216–8234. <https://doi.org/10.3390/ijms15058216>
- Maksimov, E. G., Protasova, E. A., Tsoraev, G. V., Yaroshevich, I. A., Maydykovskiy, A. I., Shirshin, E. A., Gostev, T. S., Jelzow, A., Moldenhauer, M., Slonimskiy, Y. B., Sluchanko, N. N., & Friedrich, T. (2020). Probing of carotenoid-tryptophan hydrogen bonding dynamics in the single-tryptophan photoactive Orange Carotenoid Protein. *Scientific Reports*, 10(1), 11729. <https://doi.org/10.1038/s41598-020-68463-8>

- Malagobadan, S., & Nagoor, H. (2018). *Anoikis*. <https://doi.org/10.1016/B978-0-12-801238-3.65021-3>
- Mandal, R., Barrón, J. C., Kostova, I., Becker, S., & Strebhardt, K. (2020). Caspase-8: The double-edged sword. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 1873(2), 188357. <https://doi.org/10.1016/j.bbcan.2020.188357>
- Mann, J. (2002). Natural products in cancer chemotherapy: past, present and future. *Nature Reviews Cancer*, 2(2), 143–148. <https://doi.org/10.1038/nrc723>
- Mao, X., Xu, J., Wang, W., Liang, C., Hua, J., Liu, J., Zhang, B., Meng, Q., Yu, X., & Shi, S. (2021). Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. *Molecular Cancer*, 20(1), 131. <https://doi.org/10.1186/s12943-021-01428-1>
- Maurizi, A., Ciocca, M., Giuliani, C., Di Carlo, I., & Teti, A. (2022). Role of Neural (N)-Cadherin in Breast Cancer Cell Stemness and Dormancy in the Bone Microenvironment. *Cancers*, 14(5), 1317. <https://doi.org/10.3390/cancers14051317>
- McDonald, T. O., Cheng, Y.-C., Graser, C., Nicol, P. B., Temko, D., & Michor, F. (2023). Computational approaches to modelling and optimizing cancer treatment. *Nature Reviews Bioengineering*, 1(10), 695–711. <https://doi.org/10.1038/s44222-023-00089-7>
- Micalizzi, D. S., Maheswaran, S., & Haber, D. A. (2017). A conduit to metastasis: circulating tumor cell biology. *Genes & Development*, 31(18), 1827–1840. <https://doi.org/10.1101/gad.305805.117>
- Miller, K. D., Nogueira, L., Mariotto, A. B., Rowland, J. H., Yabroff, K. R., Alfano, C. M., Jemal, A., Kramer, J. L., & Siegel, R. L. (2019). Cancer treatment and survivorship statistics, 2019. *CA: A Cancer Journal for Clinicians*, 69(5), 363–385. <https://doi.org/10.3322/caac.21565>
- Miyamoto, D. T., Zheng, Y., Wittner, B. S., Lee, R. J., Zhu, H., Broderick, K. T., Desai, R., Fox, D. B., Brannigan, B. W., Trautwein, J., Arora, K. S., Desai, N., Dahl, D. M., Sequist, L. V., Smith, M. R., Kapur, R., Wu, C.-L., Shioda, T., Ramaswamy, S., ... Haber, D. A. (2015). RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Science*, 349(6254), 1351–1356. <https://doi.org/10.1126/science.aab0917>
- Mohamad Anuar, N. N., Nor Hisam, N. S., Liew, S. L., & Ugusman, A. (2020). Clinical Review: Navitoclax as a Pro-Apoptotic and Anti-Fibrotic Agent. *Frontiers in Pharmacology*, 11. <https://doi.org/10.3389/fphar.2020.564108>
- Moldoveanu, T., & Czabotar, P. E. (2020). BAX, BAK, and BOK: A coming of age for the BCL-2 family effector proteins. *Cold Spring Harbor Perspectives in Biology*, 12(4). <https://doi.org/10.1101/cshperspect.a036319>
- Morgan, D., Garg, M., Tergaonkar, V., Tan, S. Y., & Sethi, G. (2020). Pharmacological significance of the non-canonical NF-κB pathway in tumorigenesis. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 1874(2), 188449. <https://doi.org/https://doi.org/10.1016/j.bbcan.2020.188449>
- Mortensen, M. M., Høyer, S., Lynnerup, A.-S., Ørntoft, T. F., Sørensen, K. D., Borre, M., & Dyrskjød, L. (2015). Expression profiling of prostate cancer tissue delineates genes associated with recurrence after prostatectomy. *Scientific Reports*, 5, 16018.

<https://doi.org/10.1038/srep16018>

- Muniraj, N., Siddharth, S., & Sharma, D. (2019). Bioactive Compounds: Multi-Targeting Silver Bullets for Preventing and Treating Breast Cancer. *Cancers*, 11(10), 1563. <https://doi.org/10.3390/cancers11101563>
- Nabizadeh, F., Momtaz, S., Ghanbari-Movahed, M., Qalekhani, F., Mohsenpour, H., Aneva, I. Y., Bishayee, A., Farzaei, M. H., & Bishayee, A. (2022). Pediatric acute lymphoblastic leukemia management using multitargeting bioactive natural compounds: A systematic and critical review. *Pharmacological Research*, 177, 106116. <https://doi.org/10.1016/j.phrs.2022.106116>
- Nagaprashantha, L., Vartak, N., Awasthi, S., Awasthi, S., & Singhal, S. S. (2012). Novel Anti-cancer Compounds for Developing Combinatorial Therapies to Target Anoikis-Resistant Tumors. *Pharmaceutical Research*, 29(3), 621–636. <https://doi.org/10.1007/s11095-011-0645-9>
- Nakazawa, M., & Kyprianou, N. (2017). Epithelial-mesenchymal-transition regulators in prostate cancer: Androgens and beyond. *The Journal of Steroid Biochemistry and Molecular Biology*, 166, 84–90. <https://doi.org/10.1016/j.jsbmb.2016.05.007>
- Nejati, K., Dadashpour, M., Gharibi, T., Mellatyar, H., & Akbarzadeh, A. (2022). Biomedical Applications of Functionalized Gold Nanoparticles: A Review. *Journal of Cluster Science*, 33(1), 1–16. <https://doi.org/10.1007/s10876-020-01955-9>
- Nepali, P. R., & Kyprianou, N. (2023). Anoikis in phenotypic reprogramming of the prostate tumor microenvironment. *Frontiers in Endocrinology*, 14. <https://doi.org/10.3389/fendo.2023.1160267>
- Neuendorf, H. M., Simmons, J. L., & Boyle, G. M. (2023). Therapeutic targeting of anoikis resistance in cutaneous melanoma metastasis. *Frontiers in Cell and Developmental Biology*, 11. <https://doi.org/10.3389/fcell.2023.1183328>
- Nieszporek, A., Skrzypek, K., Adamek, G., & Majka, M. (2019). Molecular mechanisms of epithelial to mesenchymal transition in tumor metastasis. *Acta Biochimica Polonica*. https://doi.org/10.18388/abp.2019_2899
- Nikolaou, M., Pavlopoulou, A., Georgakilas, A. G., & Kyrodimos, E. (2018). The challenge of drug resistance in cancer treatment: a current overview. *Clinical & Experimental Metastasis*, 35(4), 309–318. <https://doi.org/10.1007/s10585-018-9903-0>
- Niu, Q., Deng, H., Zhang, Z., Xu, Q., Luan, S., Huang, M., Liu, D., & Zhao, L. (2021). Design, synthesis and biological evaluation of dual Bcl-2/Mcl-1 inhibitors bearing 2-(1H-indol-4-yl)benzoic acid scaffold. *Bioorganic & Medicinal Chemistry Letters*, 47, 128215. <https://doi.org/10.1016/j.bmcl.2021.128215>
- Niu, T., Tian, Y., Wang, G., Guo, G., Tong, Y., & Shi, Y. (2020). Inhibition of ROS-NF-κB-dependent autophagy enhances Hypocrellin A united LED red light-induced apoptosis in squamous carcinoma A431 cells. *Cellular Signalling*, 69, 109550. <https://doi.org/10.1016/J.CELLSIG.2020.109550>
- No Title. (n.d.-a). <https://doi.org/10.5281/zenodo.3732170>
- No Title. (n.d.-b). <https://doi.org/https://doi.org/10.5281/zenodo.7588710>
- Noel, B., Singh, S. K., Lillard, J. W. J., & Singh, R. (2020). Role of natural compounds in

- preventing and treating breast cancer. *Frontiers in Bioscience (Scholar Edition)*, 12(1), 137–160. <https://doi.org/10.2741/S544>
- Norman, J. M., Cohen, G. M., & Bampton, E. T. W. (2010). The in vitro cleavage of the hAtg proteins by cell death proteases. *Autophagy*, 6(8), 1042–1056. <https://doi.org/10.4161/auto.6.8.13337>
- O’Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, 3(1), 33. <https://doi.org/10.1186/1758-2946-3-33>
- Oh, Y.-T., & Sun, S.-Y. (2021). Regulation of Cancer Metastasis by TRAIL/Death Receptor Signaling. *Biomolecules*, 11(4), 499. <https://doi.org/10.3390/biom11040499>
- Ohlendorf, D., Wanke, E. M., Braun, M., Oremek, G. M., & Groneberg, D. A. (2017). Prostataspezifisches Antigen (PSA). *Zentralblatt Für Arbeitsmedizin, Arbeitsschutz Und Ergonomie*, 67(4), 221–223. <https://doi.org/10.1007/s40664-017-0195-z>
- Ojha, R., Ishaq, M., & Singh, S. (2015). Caspase-mediated crosstalk between autophagy and apoptosis: Mutual adjustment or matter of dominance. *Journal of Cancer Research and Therapeutics*, 11(3), 514. <https://doi.org/10.4103/0973-1482.163695>
- Oloruntobi Famuyiwa, T. (2017). Prostate Cancer Cells Produce Exosomes Modulating Metastasis to the Bones. *Journal of Cancer Prevention & Current Research*, 8(2). <https://doi.org/10.15406/jcpcr.2017.08.00269>
- Oltersdorf, T., Elmore, S. W., Shoemaker, A. R., Armstrong, R. C., Augeri, D. J., Belli, B. A., Bruncko, M., Deckwerth, T. L., Dinges, J., Hajduk, P. J., Joseph, M. K., Kitada, S., Korsmeyer, S. J., Kunzer, A. R., Letai, A., Li, C., Mitten, M. J., Nettesheim, D. G., Ng, S., ... Rosenberg, S. H. (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature*, 435(7042), 677–681. <https://doi.org/10.1038/nature03579>
- Orrenius, S., Gogvadze, V., & Zhivotovsky, B. (2015). Calcium and mitochondria in the regulation of cell death. *Biochemical and Biophysical Research Communications*, 460(1), 72–81. <https://doi.org/10.1016/j.bbrc.2015.01.137>
- Pagliarini, V., Wirawan, E., Romagnoli, A., Ciccocanti, F., Lisi, G., Lippens, S., Cecconi, F., Fimia, G. M., Vandenabeele, P., Corazzari, M., & Piacentini, M. (2012). Proteolysis of Ambra1 during apoptosis has a role in the inhibition of the autophagic pro-survival response. *Cell Death & Differentiation*, 19(9), 1495–1504. <https://doi.org/10.1038/cdd.2012.27>
- Palorini, R., Votta, G., Pirola, Y., De Vitto, H., De Palma, S., Airoidi, C., Vasso, M., Ricciardiello, F., Lombardi, P. P., Cirulli, C., Rizzi, R., Nicotra, F., Hiller, K., Gelfi, C., Alberghina, L., & Chiaradonna, F. (2016). Protein Kinase A Activation Promotes Cancer Cell Resistance to Glucose Starvation and Anoikis. *PLOS Genetics*, 12(3), e1005931. <https://doi.org/10.1371/journal.pgen.1005931>
- Pandey, P., Patel, J. K., & Kumar, S. (2022). Nanoparticle Properties Affecting the Drug Release, Absorption, and Pharmacokinetics of Nanoparticulate Drug Delivery Systems. In *Pharmacokinetics and Pharmacodynamics of Nanoparticulate Drug Delivery Systems* (pp. 25–40). Springer International Publishing. https://doi.org/10.1007/978-3-030-83395-4_2
- Pandi, E., Proskhan, B. F., Kunjiappan, S., Sundar, K., & Balakrishnan, V. (2024). Fabrication,

- Characterization and Evaluation of Gallic Acid-Encapsulated Curdlan Gum Nanoparticles with Potential Application for Breast Cancer Treatment. *Journal of Polymers and the Environment*, 32(7), 3071–3088. <https://doi.org/10.1007/s10924-023-03139-8>
- Paoli, P., Giannoni, E., & Chiarugi, P. (2013a). Anoikis molecular pathways and its role in cancer progression. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1833(12), 3481–3498. <https://doi.org/10.1016/j.bbamcr.2013.06.026>
- Paoli, P., Giannoni, E., & Chiarugi, P. (2013b). Anoikis molecular pathways and its role in cancer progression. In *Biochimica et Biophysica Acta - Molecular Cell Research* (Vol. 1833, Issue 12, pp. 3481–3498). Biochim Biophys Acta. <https://doi.org/10.1016/j.bbamcr.2013.06.026>
- PAOLINI, A., CURTI, V., PASI, F., MAZZINI, G., NANO, R., & CAPELLI, E. (2015). Gallic acid exerts a protective or an anti-proliferative effect on glioma T98G cells via dose-dependent epigenetic regulation mediated by miRNAs. *International Journal of Oncology*, 46(4), 1491–1497. <https://doi.org/10.3892/ijo.2015.2864>
- Park, J., Cha, S.-H., Cho, S., & Park, Y. (2016). Green synthesis of gold and silver nanoparticles using gallic acid: catalytic activity and conversion yield toward the 4- nitrophenol reduction reaction. *Journal of Nanoparticle Research*, 18(6), 166. <https://doi.org/10.1007/s11051-016-3466-2>
- Patil, T., Gambhir, R., Vibhute, A., & Tiwari, A. P. (2023). Gold Nanoparticles: Synthesis Methods, Functionalization and Biological Applications. *Journal of Cluster Science*, 34(2), 705–725. <https://doi.org/10.1007/s10876-022-02287-6>
- Paul, A., Edwards, J., Pepper, C., & Mackay, S. (2018). Inhibitory- κ B kinase (IKK) α and nuclear factor- κ B (NF κ B)-inducing kinase (NIK) as anti-cancer drug targets. *Cells*, 7(10). <https://doi.org/10.3390/cells7100176>
- Perlmutter, M. A., & Lepor, H. (2007). Androgen deprivation therapy in the treatment of advanced prostate cancer. *Reviews in Urology*, 9 Suppl 1(Suppl 1), S3-8. <http://www.ncbi.nlm.nih.gov/pubmed/17387371>
- Poillet-Perez, L., Sarry, J.-E., & Joffre, C. (2021). Autophagy is a major metabolic regulator involved in cancer therapy resistance. *Cell Reports*, 36(7), 109528. <https://doi.org/https://doi.org/10.1016/j.celrep.2021.109528>
- Pungsrinont, T., Kallenbach, J., & Baniahmad, A. (2021). Role of PI3K-AKT-mTOR Pathway as a Pro-Survival Signaling and Resistance-Mediating Mechanism to Therapy of Prostate Cancer. *International Journal of Molecular Sciences*, 22(20), 11088. <https://doi.org/10.3390/ijms222011088>
- Qiao, X., Wu, X., Chen, S., Niu, M.-M., Hua, H., & Zhang, Y. (2024). Discovery of novel and potent dual-targeting AXL/HDAC2 inhibitors for colorectal cancer treatment via structure-based pharmacophore modelling, virtual screening, and molecular docking, molecular dynamics simulation studies, and biological evaluation. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 39(1). <https://doi.org/10.1080/14756366.2023.2295241>
- Radziejewska, I., Supruniuk, K., & Bielawska, A. (2021). Anti-cancer effect of combined action of anti-MUC1 and rosmarinic acid in AGS gastric cancer cells. *European Journal of Pharmacology*, 902, 174119. <https://doi.org/10.1016/j.ejphar.2021.174119>

- Raeisi, M., Zehtabi, M., Velaei, K., Fayyazpour, P., Aghaei, N., & Mehdizadeh, A. (2022). Anoikis in cancer: The role of lipid signaling. *Cell Biology International*, 46(11), 1717–1728. <https://doi.org/10.1002/cbin.11896>
- Rahal, R., Frick, M., Romero, R., Korn, J. M., Kridel, R., Chan, F. C., Meissner, B., Bhang, H., Ruddy, D., Kauffmann, A., Farsidjani, A., Derti, A., Rakiec, D., Naylor, T., Pfister, E., Kovats, S., Kim, S., Dietze, K., Dörken, B., ... Stegmeier, F. (2014). Pharmacological and genomic profiling identifies NF- κ B-targeted treatment strategies for mantle cell lymphoma. *Nature Medicine*, 20(1), 87–92. <https://doi.org/10.1038/nm.3435>
- Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1863(12), 2977–2992. <https://doi.org/10.1016/j.bbamcr.2016.09.012>
- Reza, K. K., Dey, S., Wuethrich, A., Jing Wang, Behren, A., Antaw, F., Wang, Y., Sina, A. A. I., & Trau, M. (2021). In Situ Single Cell Proteomics Reveals Circulating Tumor Cell Heterogeneity during Treatment. *ACS Nano*, 15(7), 11231–11243. <https://doi.org/10.1021/acsnano.0c10008>
- Rocchetti, M. T., Bellanti, F., Zadorozhna, M., Fiocco, D., & Mangieri, D. (2023). Multi-Faceted Role of Luteolin in Cancer Metastasis: EMT, Angiogenesis, ECM Degradation and Apoptosis. *International Journal of Molecular Sciences*, 24(10), 8824. <https://doi.org/10.3390/ijms24108824>
- Rouschop, K. M. A., van den Beucken, T., Dubois, L., Niessen, H., Bussink, J., Savelkoul, K., Keulers, T., Mujcic, H., Landuyt, W., Voncken, J. W., Lambin, P., van der Kogel, A. J., Koritzinsky, M., & Wouters, B. G. (2010). The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *Journal of Clinical Investigation*, 120(1), 127–141. <https://doi.org/10.1172/JCI40027>
- Roy, S. K., Srivastava, R. K., & Shankar, S. (2010). Inhibition of PI3K/AKT and MAPK/ERK pathways causes activation of FOXO transcription factor, leading to cell cycle arrest and apoptosis in pancreatic cancer. *Journal of Molecular Signaling*, 5, 10. <https://doi.org/10.1186/1750-2187-5-10>
- RUSSO, M. A., PAOLILLO, M., SANCHEZ-HERNANDEZ, Y., CURTI, D., CIUSANI, E., SERRA, M., COLOMBO, L., & SCHINELLI, S. (2013). A small-molecule RGD-integrin antagonist inhibits cell adhesion, cell migration and induces anoikis in glioblastoma cells. *International Journal of Oncology*, 42(1), 83–92. <https://doi.org/10.3892/ijo.2012.1708>
- Sadik, S. B. S., Sivaprakasam, P., Ramasami, N., & Pandurangan, A. K. (2022). *The Molecular Mechanisms Involved in Suppressing Triple Negative Breast Cancer Using Natural Agents* (pp. 45–71). <https://doi.org/10.4018/978-1-7998-9258-8.ch003>
- Safa, A. R. (2022). Drug and apoptosis resistance in cancer stem cells (CSCs): A puzzle with many pieces. *Cancer Drug Resistance*, 5(4), 850–872. <https://doi.org/10.20517/cdr.2022.20>
- Sakamoto, S., & Kyprianou, N. (2010a). Targeting anoikis resistance in prostate cancer metastasis. In *Molecular Aspects of Medicine* (Vol. 31, Issue 2, pp. 205–214). NIH Public Access. <https://doi.org/10.1016/j.mam.2010.02.001>
- Sakamoto, S., & Kyprianou, N. (2010b). Targeting anoikis resistance in prostate cancer metastasis. *Molecular Aspects of Medicine*, 31(2), 205–214.

<https://doi.org/10.1016/j.mam.2010.02.001>

- Saleem, S. (2021). Apoptosis, Autophagy, Necrosis and Their Multi Galore Crosstalk in Neurodegeneration. *Neuroscience*, 469, 162–174. <https://doi.org/10.1016/j.neuroscience.2021.06.023>
- Sandhiya, V., & Ubaidulla, U. (2022). Enhancing cellular uptake and membrane permeability of gallic acid for breast cancer therapy via folate-tagged PEGylated iron oxide nanoparticles has theronastic agent. *Bulletin of the National Research Centre*, 46(1), 234. <https://doi.org/10.1186/s42269-022-00909-7>
- Sarosiek, K. A., & Wood, K. C. (2023). Endogenous and imposed determinants of apoptotic vulnerabilities in cancer. *Trends in Cancer*, 9(2), 96–110. <https://doi.org/10.1016/j.trecan.2022.10.004>
- Sattari Fard, F., Jalilzadeh, N., Mehdizadeh, A., Sajjadian, F., & Velaei, K. (2023a). Understanding and targeting anoikis in metastasis for cancer therapies. *Cell Biology International*, 47(4), 683–698. <https://doi.org/10.1002/cbin.11970>
- Sattari Fard, F., Jalilzadeh, N., Mehdizadeh, A., Sajjadian, F., & Velaei, K. (2023b). Understanding and targeting anoikis in metastasis for cancer therapies. *Cell Biology International*, 47(4), 683–698. <https://doi.org/10.1002/cbin.11970>
- Schwartz, M. A. (2010). Remembrance of dead cells past: Discovering that the extracellular matrix is a cell survival factor. In *Molecular Biology of the Cell* (Vol. 21, Issue 4, pp. 499–500). American Society for Cell Biology. <https://doi.org/10.1091/mbc.E09-07-0602>
- Seca, A., & Pinto, D. (2018). Plant Secondary Metabolites as Anticancer Agents: Successes in Clinical Trials and Therapeutic Application. *International Journal of Molecular Sciences*, 19(1), 263. <https://doi.org/10.3390/ijms19010263>
- Sekhoacha, M., Riet, K., Motloun, P., Gumenku, L., Adegoke, A., & Mashele, S. (2022). Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches. *Molecules*, 27(17), 5730. <https://doi.org/10.3390/molecules27175730>
- Seruga, B., Ocana, A., & Tannock, I. F. (2011). Drug resistance in metastatic castration-resistant prostate cancer. *Nature Reviews Clinical Oncology*, 8(1), 12–23. <https://doi.org/10.1038/nrclinonc.2010.136>
- Shahar, N., & Larisch, S. (2020). Inhibiting the inhibitors: Targeting anti-apoptotic proteins in cancer and therapy resistance. *Drug Resistance Updates*, 52, 100712. <https://doi.org/10.1016/j.drug.2020.100712>
- Shalini, S., Dorstyn, L., Dawar, S., & Kumar, S. (2015). Old, new and emerging functions of caspases. *Cell Death & Differentiation*, 22(4), 526–539. <https://doi.org/10.1038/cdd.2014.216>
- Shamas-Din, A., Kale, J., Leber, B., & Andrews, D. W. (2013). Mechanisms of Action of Bcl-2 Family Proteins. *Cold Spring Harbor Perspectives in Biology*, 5(4), a008714–a008714. <https://doi.org/10.1101/cshperspect.a008714>
- Shen, D. (n.d.). *CDCA attenuates LUAD pathogenesis progression via integrin $\alpha 5 \beta 1$ / FAK / p53 signalling pathway.*
- Shen, D., Zeng, Y., Zhang, W., Li, Y., Zhu, J., Liu, Z., Yan, Z., & Huang, J. (2022). Chenodeoxycholic acid inhibits lung adenocarcinoma progression via the integrin

- $\alpha 5\beta 1$ /FAK/p53 signaling pathway. *European Journal of Pharmacology*, 923, 174925. <https://doi.org/10.1016/j.ejphar.2022.174925>
- Shi, C., Wu, F., Yew, D. T., Xu, J., & Zhu, Y. (2010). Bilobalide prevents apoptosis through activation of the PI3K/Akt pathway in SH-SY5Y cells. *Apoptosis*, 15(6), 715–727. <https://doi.org/10.1007/s10495-010-0492-x>
- Shu, G., Yang, T., Wang, C., Su, H., & Xiang, M. (2013). Gastrodin stimulates anticancer immune response and represses transplanted H22 hepatic ascitic tumor cell growth: Involvement of NF- κ B signaling activation in CD4⁺ T cells. *Toxicology and Applied Pharmacology*, 269(3), 270–279. <https://doi.org/10.1016/j.taap.2013.02.019>
- Siddique, S., & Chow, J. C. L. (2020). Gold Nanoparticles for Drug Delivery and Cancer Therapy. *Applied Sciences*, 10(11), 3824. <https://doi.org/10.3390/app10113824>
- Simpson, C. D., Anyiwe, K., & Schimmer, A. D. (2008). Anoikis resistance and tumor metastasis. In *Cancer Letters* (Vol. 272, Issue 2, pp. 177–185). Elsevier Ireland Ltd. <https://doi.org/10.1016/j.canlet.2008.05.029>
- Smyth, G. K. (2004). Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments. *Statistical Applications in Genetics and Molecular Biology*, 3(1), 1–25. <https://doi.org/10.2202/1544-6115.1027>
- Souers, A. J., Levenson, J. D., Boghaert, E. R., Ackler, S. L., Catron, N. D., Chen, J., Dayton, B. D., Ding, H., Enschede, S. H., Fairbrother, W. J., Huang, D. C. S., Hymowitz, S. G., Jin, S., Khaw, S. L., Kovar, P. J., Lam, L. T., Lee, J., Maecker, H. L., Marsh, K. C., ... Elmore, S. W. (2013). ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nature Medicine*, 19(2), 202–208. <https://doi.org/10.1038/nm.3048>
- Spill, F., Reynolds, D. S., Kamm, R. D., & Zaman, M. H. (2016). Impact of the physical microenvironment on tumor progression and metastasis. *Current Opinion in Biotechnology*, 40, 41–48. <https://doi.org/10.1016/J.COPBIO.2016.02.007>
- Stanislovas, J., & Kermorgant, S. (2022). c-Met-integrin cooperation: Mechanisms, tumorigenic effects, and therapeutic relevance. *Frontiers in Cell and Developmental Biology*, 10. <https://doi.org/10.3389/fcell.2022.994528>
- Strauss, S. J., Ng, T., Mendoza-Naranjo, A., Whelan, J., & Sorensen, P. H. B. (2010). Understanding Micrometastatic Disease and Anoikis Resistance in Ewing Family of Tumors and Osteosarcoma. *The Oncologist*, 15(6), 627–635. <https://doi.org/10.1634/theoncologist.2010-0093>
- Subramanian, A. P., John, A. A., Vellayappan, M. V., Balaji, A., Jaganathan, S. K., Supriyanto, E., & Yusof, M. (2015). Gallic acid: prospects and molecular mechanisms of its anticancer activity. *RSC Advances*, 5(45), 35608–35621. <https://doi.org/10.1039/C5RA02727F>
- Sui, P., Liu, X., Zhong, C., & Sha, Z. (2024). Integrated single-cell and bulk RNA-Seq analysis enhances prognostic accuracy of PD-1/PD-L1 immunotherapy response in lung adenocarcinoma through necroptotic anoikis gene signatures. *Scientific Reports*, 14(1), 10873. <https://doi.org/10.1038/s41598-024-61629-8>
- Sun, B. L. (2021). Immunotherapy in treatment of metastatic prostate cancer: An approach to circumvent immunosuppressive tumor microenvironment. *The Prostate*, 81(15), 1125–1134. <https://doi.org/10.1002/pros.24213>

- Sun, Z., Mao, Y., Zhang, X., Lu, S., Wang, H., Zhang, C., Xiao, C., Cao, Y., Qing, Y., Wang, Y., & Li, K. (2021). Identification of ARHGEF38, NETO2, GOLM1, and SAPCD2 Associated With Prostate Cancer Progression by Bioinformatic Analysis and Experimental Validation. *Frontiers in Cell and Developmental Biology*, 9. <https://doi.org/10.3389/fcell.2021.718638>
- Suvarna, V., Singh, V., & Murahari, M. (2019). Current overview on the clinical update of Bcl-2 anti-apoptotic inhibitors for cancer therapy. *European Journal of Pharmacology*, 862, 172655. <https://doi.org/https://doi.org/10.1016/j.ejphar.2019.172655>
- Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, 51(D1), D638–D646. <https://doi.org/10.1093/nar/gkac1000>
- Taddei, M., Giannoni, E., Fiaschi, T., & Chiarugi, P. (2012). Anoikis: an emerging hallmark in health and diseases. *The Journal of Pathology*, 226(2), 380–393. <https://doi.org/10.1002/path.3000>
- Tan, K., Goldstein, D., Crowe, P., & Yang, J.-L. (2013). Uncovering a key to the process of metastasis in human cancers: a review of critical regulators of anoikis. *Journal of Cancer Research and Clinical Oncology*, 139(11), 1795–1805. <https://doi.org/10.1007/s00432-013-1482-5>
- Tan, K., & Naylor, M. J. (2022). Tumour Microenvironment-Immune Cell Interactions Influencing Breast Cancer Heterogeneity and Disease Progression. *Frontiers in Oncology*, 12. <https://doi.org/10.3389/fonc.2022.876451>
- Tan, Q., Zou, S., Jin, R., Hu, Y., Xu, H., Wang, H., Ding, M., Hu, M., Wei, C., & Song, L. (2020). Selective degradation of IKK α by autophagy is essential for arsenite-induced cancer cell apoptosis. *Cell Death & Disease*, 11(4), 222. <https://doi.org/10.1038/s41419-020-2420-5>
- Tang, Z., Kang, B., Li, C., Chen, T., & Zhang, Z. (2019). GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Research*, 47(W1), W556–W560. <https://doi.org/10.1093/nar/gkz430>
- Taylor, W. F., Yanez, M., Moghadam, S. E., Moridi Farimani, M., Soroury, S., Ebrahimi, S. N., Tabefam, M., & Jabbarzadeh, E. (2019). 7-epi-Clusianone, a Multi-Targeting Natural Product with Potential Chemotherapeutic, Immune-Modulating, and Anti-Angiogenic Properties. *Molecules*, 24(23), 4415. <https://doi.org/10.3390/molecules24234415>
- Thiery, J. P., Acloque, H., Huang, R. Y. J., & Nieto, M. A. (2009). Epithelial-Mesenchymal Transitions in Development and Disease. *Cell*, 139(5), 871–890. <https://doi.org/10.1016/j.cell.2009.11.007>
- Thompson-Elliott, B., Johnson, R., & Khan, S. A. (2021). Alterations in TGF β signaling during prostate cancer progression. *American Journal of Clinical and Experimental Urology*, 9(4), 318–328. <http://www.ncbi.nlm.nih.gov/pubmed/34541030>
- Tiwari, D., Narang, R., Sudhakar, K., Singh, V., Lal, S., & Devgun, M. (2022). 1,3,4-oxadiazole derivatives as potential antimicrobial agents. *Chemical Biology & Drug Design*, 100(6), 1086–1121. <https://doi.org/10.1111/cbdd.14100>

- Tiwari, M., Sharma, L. K., Vanegas, D., Callaway, D. A., Bai, Y., Lechleiter, J. D., & Herman, B. (2014). A nonapoptotic role for CASP2/caspase 2. *Autophagy*, 10(6), 1054–1070. <https://doi.org/10.4161/auto.28528>
- TOREN, P., & ZOUBEIDI, A. (2014). Targeting the PI3K/Akt pathway in prostate cancer: Challenges and opportunities (Review). *International Journal of Oncology*, 45(5), 1793–1801. <https://doi.org/10.3892/ijo.2014.2601>
- Tutumlu, G., Dogan, B., Avsar, T., Orhan, M. D., Calis, S., & Durdagi, S. (2020). Integrating Ligand and Target-Driven Based Virtual Screening Approaches With in vitro Human Cell Line Models and Time-Resolved Fluorescence Resonance Energy Transfer Assay to Identify Novel Hit Compounds Against BCL-2. *Frontiers in Chemistry*, 8. <https://doi.org/10.3389/fchem.2020.00167>
- Uekita, T., & Sakai, R. (2011). Roles of CUB domain-containing protein 1 signaling in cancer invasion and metastasis. *Wiley Online Library*, 102(11), 1943–1948. <https://doi.org/10.1111/j.1349-7006.2011.02052.x>
- Usman, S., Waseem, N. H., Nguyen, T. K. N., Mohsin, S., Jamal, A., Teh, M.-T., & Waseem, A. (2021). Vimentin Is at the Heart of Epithelial Mesenchymal Transition (EMT) Mediated Metastasis. *Cancers*, 13(19), 4985. <https://doi.org/10.3390/cancers13194985>
- Vachon, P. H. (2011). Integrin Signaling, Cell Survival, and Anoikis: Distinctions, Differences, and Differentiation. *Journal of Signal Transduction*, 2011. <https://doi.org/10.1155/2011/738137>
- van Roy, F. (2014). Beyond E-cadherin: roles of other cadherin superfamily members in cancer. *Nature Reviews Cancer*, 14(2), 121–134. <https://doi.org/10.1038/nrc3647>
- Venkatesan, R., Dhilipkumar, T., Shankar, K. V., Almutairi, T. M., & Kim, S.-C. (2024). Eco-friendly food packaging: gallic acid as a cross-linking agent in PBAT/cellulose composite films. *Cellulose*, 31(13), 8105–8125. <https://doi.org/10.1007/s10570-024-06046-w>
- Vijayalakshmi, P., Indu, S., Ireen, C., Manjunathan, R., & Rajalakshmi, M. (2023). Octyl Gallate and Gallic Acid Isolated from Terminalia bellirica Circumvent Breast Cancer Progression by Enhancing the Intrinsic Apoptotic Signaling Pathway and Elevating the Levels of Anti-oxidant Enzymes. *Applied Biochemistry and Biotechnology*, 195(12), 7214–7235. <https://doi.org/10.1007/s12010-023-04450-9>
- Vitto, V. A. M., Bianchin, S., Zolondick, A. A., Pelliolo, G., Rimessi, A., Chianese, D., Yang, H., Carbone, M., Pinton, P., Giorgi, C., & Patergnani, S. (2022). Molecular Mechanisms of Autophagy in Cancer Development, Progression, and Therapy. *Biomedicines*, 10(7), 1596. <https://doi.org/10.3390/biomedicines10071596>
- Walczak, M., & Martens, S. (2013). Dissecting the role of the Atg12–Atg5–Atg16 complex during autophagosome formation. *Autophagy*, 9(3), 424–425. <https://doi.org/10.4161/auto.22931>
- Wang, D., Berglund, A., Kenchappa, R. S., Forsyth, P. A., Mulé, J. J., & Etame, A. B. (2016). BIRC3 is a novel driver of therapeutic resistance in Glioblastoma. *Scientific Reports*, 6(1), 21710. <https://doi.org/10.1038/srep21710>
- Wang, J., Luo, Z., Lin, L., Sui, X., Yu, L., Xu, C., Zhang, R., Zhao, Z., Zhu, Q., An, B., Wang, Q., Chen, B., Leung, E. L.-H., & Wu, Q. (2022). Anoikis-Associated Lung Cancer Metastasis: Mechanisms and Therapies. *Cancers*, 14(19), 4791.

<https://doi.org/10.3390/cancers14194791>

- Wang, P., Gao, Y., Yang, G., Zhao, Y., Zhao, Z., Gao, G., Zhao, L., & Li, S. (2023). Enhancing the inhibition of cell proliferation and induction of apoptosis in H22 hepatoma cells through biotransformation of notoginsenoside R1 by *Lactiplantibacillus plantarum* S165 into 20(S / R)-notoginsenoside R2. *RSC Advances*, 13(42), 29773–29783. <https://doi.org/10.1039/D3RA06029B>
- WANG, R., MA, L., WENG, D., YAO, J., LIU, X., & JIN, F. (2016). Gallic acid induces apoptosis and enhances the anticancer effects of cisplatin in human small cell lung cancer H446 cell line via the ROS-dependent mitochondrial apoptotic pathway. *Oncology Reports*, 35(5), 3075–3083. <https://doi.org/10.3892/or.2016.4690>
- Wang, Y., Liu, Z., Shu, S., Cai, J., Tang, C., & Dong, Z. (2020). AMPK/mTOR Signaling in Autophagy Regulation During Cisplatin-Induced Acute Kidney Injury. *Frontiers in Physiology*, 11. <https://doi.org/10.3389/fphys.2020.619730>
- Warren, C. F. A., Wong-Brown, M. W., & Bowden, N. A. (2019). BCL-2 family isoforms in apoptosis and cancer. *Cell Death & Disease*, 10(3), 177. <https://doi.org/10.1038/s41419-019-1407-6>
- Was, H., Krol, S. K., Rotili, D., Mai, A., Wojtas, B., Kaminska, B., & Maleszewska, M. (2019). Histone deacetylase inhibitors exert anti-tumor effects on human adherent and stem-like glioma cells. *Clinical Epigenetics*, 11(1), 11. <https://doi.org/10.1186/s13148-018-0598-5>
- Wei, Y., Cao, Y., Sun, R., Cheng, L., Xiong, X., Jin, X., He, X., Lu, W., & Zhao, M. (2020). Targeting Bcl-2 Proteins in Acute Myeloid Leukemia. *Frontiers in Oncology*, 10. <https://doi.org/10.3389/fonc.2020.584974>
- Westaby, D., Jimenez-Vacas, J. M., Padilha, A., Varkaris, A., Balk, S. P., de Bono, J. S., & Sharp, A. (2021). Targeting the Intrinsic Apoptosis Pathway: A Window of Opportunity for Prostate Cancer. *Cancers*, 14(1), 51. <https://doi.org/10.3390/cancers14010051>
- Whelan, K. A., Caldwell, S. A., Shahriari, K. S., Jackson, S. R., Franchetti, L. D., Johannes, G. J., & Reginato, M. J. (2010). Hypoxia Suppression of Bim and Bmf Blocks Anoikis and Luminal Clearing during Mammary Morphogenesis. *Molecular Biology of the Cell*, 21(22), 3829–3837. <https://doi.org/10.1091/mbc.e10-04-0353>
- White, E., & DiPaola, R. S. (2009). The double-edged sword of autophagy modulation in cancer. In *Clinical Cancer Research* (Vol. 15, Issue 17, pp. 5308–5316). Clin Cancer Res. <https://doi.org/10.1158/1078-0432.CCR-07-5023>
- Wilson, W. H., O'Connor, O. A., Czuczman, M. S., LaCasce, A. S., Gerecitano, J. F., Leonard, J. P., Tulpule, A., Dunleavy, K., Xiong, H., Chiu, Y.-L., Cui, Y., Busman, T., Elmore, S. W., Rosenberg, S. H., Krivoshik, A. P., Enschede, S. H., & Humerickhouse, R. A. (2010). Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. *The Lancet Oncology*, 11(12), 1149–1159. [https://doi.org/10.1016/S1470-2045\(10\)70261-8](https://doi.org/10.1016/S1470-2045(10)70261-8)
- Wolf, P., Schoeniger, A., & Edlich, F. (2022). Pro-apoptotic complexes of BAX and BAK on the outer mitochondrial membrane. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1869(10), 119317. <https://doi.org/https://doi.org/10.1016/j.bbamcr.2022.119317>

- Wong, T. S., Gao, W., & Chan, J. Y. W. (2014). Interactions between E-cadherin and microRNA deregulation in head and neck cancers: The potential interplay. In *BioMed Research International* (Vol. 2014). Hindawi Publishing Corporation. <https://doi.org/10.1155/2014/126038>
- Xu, H.-D., & Qin, Z.-H. (2019). *Beclin 1, Bcl-2 and Autophagy* (pp. 109–126). https://doi.org/10.1007/978-981-15-0602-4_5
- Xu, H.-D., Wu, D., Gu, J.-H., Ge, J.-B., Wu, J.-C., Han, R., Liang, Z.-Q., & Qin, Z.-H. (2013). The Pro-Survival Role of Autophagy Depends on Bcl-2 Under Nutrition Stress Conditions. *PLoS ONE*, 8(5), e63232. <https://doi.org/10.1371/journal.pone.0063232>
- Xu, L.-Z., Li, S.-S., Zhou, W., Kang, Z.-J., Zhang, Q.-X., Kamran, M., Xu, J., Liang, D.-P., Wang, C.-L., Hou, Z.-J., Wan, X.-B., Wang, H.-J., Lam, E. W.-F., Zhao, Z.-W., & Liu, Q. (2017). p62/SQSTM1 enhances breast cancer stem-like properties by stabilizing MYC mRNA. *Oncogene*, 36(3), 304–317. <https://doi.org/10.1038/onc.2016.202>
- Xu, X., Zhang, C., Xu, H., Wu, L., Hu, M., & Song, L. (2020). Autophagic feedback-mediated degradation of IKK α requires CHK1/p300/CBP-dependent acetylation of p53. *Journal of Cell Science*. <https://doi.org/10.1242/jcs.246868>
- Yadav, A., Kumar, B., Yu, J.-G., Old, M., Teknos, T. N., & Kumar, P. (2015). Tumor-Associated Endothelial Cells Promote Tumor Metastasis by Chaperoning Circulating Tumor Cells and Protecting Them from Anoikis. *PLOS ONE*, 10(10), e0141602. <https://doi.org/10.1371/journal.pone.0141602>
- Yadav, S. S., Stockert, J. A., Hackert, V., Yadav, K. K., & Tewari, A. K. (2018). Intratumor heterogeneity in prostate cancer. *Urologic Oncology: Seminars and Original Investigations*, 36(8), 349–360. <https://doi.org/10.1016/j.urolonc.2018.05.008>
- Yao, Y., Bian, L., Yang, P., Sui, Y., Li, R., Chen, Y., Sun, L., Ai, Q., Zhong, L., & Lu, D. (2019). Gastrodin attenuates proliferation and inflammatory responses in activated microglia through Wnt/ β -catenin signaling pathway. *Brain Research*, 1717, 190–203. <https://doi.org/10.1016/j.brainres.2019.04.025>
- Yin, X., Xin, H., Mao, S., Wu, G., & Guo, L. (2019). The Role of Autophagy in Sepsis: Protection and Injury to Organs. *Frontiers in Physiology*, 10. <https://doi.org/10.3389/fphys.2019.01071>
- Yin, Z., Pascual, C., & Klionsky, D. (2016). Autophagy: machinery and regulation. *Microbial Cell*, 3(12), 588–596. <https://doi.org/10.15698/mic2016.12.546>
- Yu, G., & Klionsky, D. J. (2022). Life and Death Decisions—The Many Faces of Autophagy in Cell Survival and Cell Death. *Biomolecules*, 12(7), 866. <https://doi.org/10.3390/biom12070866>
- Yu, H., Lin, L., Zhang, Z., Zhang, H., & Hu, H. (2020). Targeting NF- κ B pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduction and Targeted Therapy*, 5(1), 209. <https://doi.org/10.1038/s41392-020-00312-6>
- Yu, Y., Liu, B., Li, X., Lu, D., Yang, L., Chen, L., Li, Y., Cheng, L., Lv, F., Zhang, P., Song, Y., & Xing, Y. (2022a). ATF4/CEMIP/PKC α promotes anoikis resistance by enhancing protective autophagy in prostate cancer cells. *Cell Death & Disease*, 13(1), 46. <https://doi.org/10.1038/s41419-021-04494-x>
- Yu, Y., Liu, B., Li, X., Lu, D., Yang, L., Chen, L., Li, Y., Cheng, L., Lv, F., Zhang, P., Song,

- Y., & Xing, Y. (2022b). ATF4/CEMIP/PKC α promotes anoikis resistance by enhancing protective autophagy in prostate cancer cells. *Cell Death & Disease*, 13(1), 46. <https://doi.org/10.1038/s41419-021-04494-x>
- Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*, 21(5), 559. <https://doi.org/10.3390/molecules21050559>
- Yuan, J., Zhang, Q., Wu, S., Yan, S., Zhao, R., Sun, Y., Tian, X., & Zhou, K. (2021). MiRNA-223-3p Affects Mantle Cell Lymphoma Development by Regulating the CHUK/NF- κ B2 Signaling Pathway. *OncoTargets and Therapy*, 14, 1553–1564. <https://doi.org/10.2147/OTT.S283486>
- Yue, J., Sun, H., Liu, S., Yu, F., Wang, S., Wang, F., Shen, R., Zhu, F., Zhang, L., & Shao, C. (2018). Downregulation of NDR^1 contributes to metastasis of prostate cancer cells via activating epithelial-mesenchymal transition. *Cancer Medicine*, 7(7), 3200–3212. <https://doi.org/10.1002/cam4.1532>
- Yun, C. W., & Lee, S. H. (2018). The roles of autophagy in cancer. In *International Journal of Molecular Sciences* (Vol. 19, Issue 11). MDPI AG. <https://doi.org/10.3390/ijms19113466>
- Yusuf, A., Almotairy, A. R. Z., Henidi, H., Alshehri, O. Y., & Aldughaim, M. S. (2023). Nanoparticles as Drug Delivery Systems: A Review of the Implication of Nanoparticles' Physicochemical Properties on Responses in Biological Systems. *Polymers*, 15(7), 1596. <https://doi.org/10.3390/polym15071596>
- Zada, S., Hwang, J. S., Ahmed, M., Lai, T. H., Pham, T. M., Elashkar, O., & Kim, D. R. (2021). Cross talk between autophagy and oncogenic signaling pathways and implications for cancer therapy. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 1876(1), 188565. <https://doi.org/10.1016/j.bbcan.2021.188565>
- Zaman, S., Wang, R., & Gandhi, V. (2014). Targeting the apoptosis pathway in hematologic malignancies. *Leukemia & Lymphoma*, 55(9), 1980–1992. <https://doi.org/10.3109/10428194.2013.855307>
- ZHAI, H., HU, S., LIU, T., WANG, F., WANG, X., WU, G., ZHANG, Y., SUI, M., LIU, H., & JIANG, L. (2016). Nitidine chloride inhibits proliferation and induces apoptosis in colorectal cancer cells by suppressing the ERK signaling pathway. *Molecular Medicine Reports*, 13(3), 2536–2542. <https://doi.org/10.3892/mmr.2016.4827>
- Zhai, K., Siddiqui, M., Abdellatif, B., Liskova, A., Kubatka, P., & Büsselberg, D. (2021). Natural Compounds in Glioblastoma Therapy: Preclinical Insights, Mechanistic Pathways, and Outlook. *Cancers*, 13(10). <https://doi.org/10.3390/cancers13102317>
- Zhang, H.-S., Liu, M.-F., Ji, X.-Y., Jiang, C.-R., Li, Z.-L., & OuYang, B. (2019). Gastrodin combined with rhynchophylline inhibits cerebral ischaemia-induced inflammasome activation via upregulating miR-21–5p and miR-331–5p. *Life Sciences*, 239, 116935. <https://doi.org/10.1016/j.lfs.2019.116935>
- Zhang, P., Chen, L., Song, Y., Li, X., Sun, Y., Xiao, Y., & Xing, Y. (2016). Tetraiodothyroacetic acid and transthyretin silencing inhibit pro-metastatic effect of L-thyroxine in anoikis-resistant prostate cancer cells through regulation of MAPK/ERK pathway. *Experimental Cell Research*, 347(2), 350–359. <https://doi.org/10.1016/j.yexcr.2016.08.019>

- Zhang, P., Chen, L., Zhou, F., He, Z., Wang, G., & Luo, Y. (2023). NRP1 promotes prostate cancer progression via modulating EGFR-dependent AKT pathway activation. *Cell Death & Disease*, 14(2), 159. <https://doi.org/10.1038/s41419-023-05696-1>
- Zhang, P., Song, Y., Sun, Y., Li, X., Chen, L., Yang, L., & Xing, Y. (2018). AMPK/GSK3 β / β -catenin cascade-triggered overexpression of CEMIP promotes migration and invasion in anoikis-resistant prostate cancer cells by enhancing metabolic reprogramming. *The FASEB Journal*, 32(7), 3924–3935. <https://doi.org/10.1096/fj.201701078R>
- Zhang, Y., Lin, R., Li, H., He, W., Du, J., & Wang, J. (2019). Strategies to improve tumor penetration of nanomedicines through nanoparticle design. *WIREs Nanomedicine and Nanobiotechnology*, 11(1). <https://doi.org/10.1002/wnan.1519>
- Zhang, Y., Zhang, Y., Shi, X.-J., Li, J.-X., Wang, L.-H., Xie, C.-E., & Wang, Y.-L. (2022). Chenodeoxycholic Acid Enhances the Effect of Sorafenib in Inhibiting HepG2 Cell Growth Through EGFR/Stat3 Pathway. *Frontiers in Oncology*, 12. <https://doi.org/10.3389/fonc.2022.836333>
- Zhao, H., Wu, L., Yan, G., Chen, Y., Zhou, M., Wu, Y., & Li, Y. (2021). Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduction and Targeted Therapy*, 6(1), 263. <https://doi.org/10.1038/s41392-021-00658-5>
- Zhao, M., Kong, L., Liu, Y., & Qu, H. (2015). dbEMT: an epithelial-mesenchymal transition associated gene resource. *Scientific Reports*, 5, 11459. <https://doi.org/10.1038/srep11459>
- Zhao, M., Liu, Y., Zheng, C., & Qu, H. (2019). dbEMT 2.0: An updated database for epithelial-mesenchymal transition genes with experimentally verified information and precalculated regulation information for cancer metastasis. In *Journal of genetics and genomics = Yi chuan xue bao* (Vol. 46, Issue 12, pp. 595–597). <https://doi.org/10.1016/j.jgg.2019.11.010>
- Zhi, Z., Ouyang, Z., Ren, Y., Cheng, Y., Liu, P., Wen, Y., & Shao, Y. (2022). Non-canonical phosphorylation of Bmf by p38 MAPK promotes its apoptotic activity in anoikis. *Cell Death & Differentiation*, 29(2), 323–336. <https://doi.org/10.1038/s41418-021-00855-3>
- Zhong, X., & Rescorla, F. J. (2012). Cell surface adhesion molecules and adhesion-initiated signaling: Understanding of anoikis resistance mechanisms and therapeutic opportunities. *Cellular Signalling*, 24(2), 393–401. <https://doi.org/10.1016/j.cellsig.2011.10.005>
- Zhong, Z.-F., Tan, W., Tian, K., Yu, H., Qiang, W.-A., & Wang, Y.-T. (2017). Combined effects of furanodiene and doxorubicin on the migration and invasion of MDA-MB-231 breast cancer cells in vitro. *Oncology Reports*, 37(4), 2016–2024. <https://doi.org/10.3892/or.2017.5435>
- Zhou, Y., Wu, Q., Yu, W., Ye, F., Cao, Y., Akan, O. D., Wu, X., Xie, T., Lu, H., Cao, F., Luo, F., & Lin, Q. (2023). Gastrodin ameliorates exercise-induced fatigue via modulating Nrf2 pathway and inhibiting inflammation in mice. *Food Bioscience*, 51, 102262. <https://doi.org/10.1016/j.fbio.2022.102262>
- Zhu, L., Jiang, M., Wang, H., Sun, H., Zhu, J., Zhao, W., Fang, Q., Yu, J., Chen, P., Wu, S., Zheng, Z., & He, Y. (2021). A narrative review of tumor heterogeneity and challenges to tumor drug therapy. *Annals of Translational Medicine*, 9(16), 1351–1351. <https://doi.org/10.21037/atm-21-1948>
- Zhu, M.-L., Horbinski, C. M., Garzotto, M., Qian, D. Z., Beer, T. M., & Kyprianou, N. (2010).

- Tubulin-Targeting Chemotherapy Impairs Androgen Receptor Activity in Prostate Cancer. *Cancer Research*, 70(20), 7992–8002. <https://doi.org/10.1158/0008-5472.CAN-10-0585>
- Zhu, Y., Mo, M., Wei, Y., Wu, J., Pan, J., Freedland, S. J., Zheng, Y., & Ye, D. (2021). Epidemiology and genomics of prostate cancer in Asian men. *Nature Reviews Urology*, 18(5), 282–301. <https://doi.org/10.1038/s41585-021-00442-8>
- Zhu, Z., Fang, C., Xu, H., Yuan, L., Du, Y., Ni, Y., Xu, Y., Shao, A., Zhang, A., & Lou, M. (2022). Anoikis resistance in diffuse glioma: The potential therapeutic targets in the future. *Frontiers in Oncology*, 12. <https://doi.org/10.3389/fonc.2022.976557>
- Zia, Q., Azhar, A., Hassan, N., Jain, P., Singh, M., Mirza, M. A., Ali, A., Parveen, S., Hasan, S., Alothaim, A. S., & Jamal, A. (2021). Cell Death: a Molecular Perspective. *Current Molecular Biology Reports*, 7(4), 41–66. <https://doi.org/10.1007/s40610-021-00146-3>
- Zong, Y., & Goldstein, A. S. (2013). Adaptation or selection—mechanisms of castration-resistant prostate cancer. *Nature Reviews Urology*, 10(2), 90–98. <https://doi.org/10.1038/nrurol.2012.237>
- Zoppi, N., Chiarelli, N., Ritelli, M., & Colombi, M. (2018). Multifaced Roles of the $\alpha\text{v}\beta 3$ Integrin in Ehlers–Danlos and Arterial Tortuosity Syndromes' Dermal Fibroblasts. *International Journal of Molecular Sciences*, 19(4), 982. <https://doi.org/10.3390/ijms1904098>

nal_draft_thesis_2024 (1).docx

Ihi Technological University

Document Details

Submission ID	135 Pages	trn:oid:::27535:74949729	50,058 Words	Submission Date
	29, 2024, 5:13 PM GMT+5:30		302,722 Characters	
Upload Date	29, 2024, 5:17 PM GMT+5:30			
Name	final_draft_thesis_2024			
File	.docx			
Size	7 MB			

Match Groups

-  **63 Not Cited or Quoted 2%**
Matches with neither in-text citation nor quotation marks
-  **0 Missing Quotations 0%**
Matches that are still very similar to source material
-  **3 Missing Citation 0%**
Matches that have quotation marks, but no in-text citation
-  **0 Cited and Quoted 0%**
Matches with in-text citation present, but no quotation marks

Top Sources

- 0%  Internet sources
- 1%  Publications
- 1%  Submitted works (Student Papers)

Top Sources

% Overall Similarity

combined total of all matches, including overlapping sources, for each database.

tered from the Report

- Bibliography
- Cited Text
- Small Matches (less than 10 words)

Exclusions

- 2 Excluded Sources

Match Groups

-  **63 Not Cited or Quoted 2%**
Matches with neither in-text citation nor quotation marks
-  **0 Missing Quotations 0%**
Matches that are still very similar to source material
-  **3 Missing Citation 0%**
Matches that have quotation marks, but no in-text citation
-  **0 Cited and Quoted 0%**
Matches with in-text citation present, but no quotation marks

Top Sources

- 0%  Internet sources
- 1%  Publications
- 1%  Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

suspicious text manipulations found.

sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.
A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

LIST OF PUBLICATIONS

Research and review paper publications:

- **Combating anoikis resistance: Bioactive compounds transforming prostate cancer therapy. Anti-cancer Drug 2024**
- **Natural Compound Dioscin Targeting Multiple Cancer Pathways through its High Affinity Binding to B Cell Lymphoma-2.** Current computer aided drug-design 2024, DOI: <https://doi.org/10.2174/0115734099279130231211053542>
- **The Prognosis of Cancer Depends on the Interplay of Autophagy, Apoptosis, and Anoikis within the Tumor Microenvironment.** Cell Biochemistry and Biophysics 2023: DOI: <https://doi.org/10.1007/s12013-023-01179-4>
- **An insight into impact of Nanomaterials Toxicity on Human Health,** PeerJ, 12:e17807 2023, Impact Factor:2.7, DOI: <https://doi.org/10.7717/peerj.17807>

Book chapter publications

- **Bioremediation of PAHs using nanotechnology,** June 2023, DOI:10.1016/B978-0-323-91767-4.00012-4. In book: Nanobiotechnology for Bioremediation Fundamentals and Mechanisms (pp.249-271)Publisher: Elsevier
- **Sensors in Monitoring global deposition of Pollutants in Aquatic Ecosystems,**
- **Sensors for Marine Biosciences: Next-generation sensing approaches,** IOP Publishing, 2025, Pages- 2-1-2-32
- **Potentiality of Advanced Nanomaterial-Based on Microorganisms for Regenerative Medicine - in Advances in Microbial Nanotechnology**

Conference presentations:

- **Shweta Gulia, Asmita Das , Prakash Chandra** “Targeting cancer pathways: Licoagrodin as a potent Bcl-2 inhibitor,” presented at International World Cancer Congress, Magnivel International, and the editors of International Journals of Biomedical Research and Health Sciences
- **Shweta Gulia, Asmita Das, Prakash Chandra** “Computational insights into Punica Granatum Phytochemicals as HSP90 inhibitors”, presented at 12th International Conference on Contemporary Engineering and Technology, Organisation of Science and Innovative Engineering and Technology

Submitted work:

- **Dissecting the Effects of Gallic Acid on Anoikis Resistance through Temporal Gene Expression Profiling**
- **From Bulk to Single-Cell: A Review of Transcriptomic Technologies and Tools**

The Prognosis of Cancer Depends on the Interplay of Autophagy, Apoptosis, and Anoikis within the Tumor Microenvironment

Review Paper | [Published: 03 October 2023](#)

Volume 81, pages 621–658, (2023) [Cite this article](#)

[Shweta Gulia](#), [Prakash Chandra](#) & [Asmita Das](#) 

 533 Accesses  3 Citations [Explore all metrics](#) →

Abstract

Within the tumor microenvironment, the fight between the immune system and cancer influences tumor transformation. Metastasis formation is an important stage in the progression of cancer. This process is aided by cellular detachment and resistance to anoikis, which are achieved by altering intercellular signaling. Autophagy, specifically pro-survival autophagy, aids cancer cells in developing treatment resistance. Numerous studies have shown that autophagy promotes tumor growth and resistance to anoikis. To regulate protective autophagy, cancer-related genes phosphorylate both pro- and anti-apoptotic proteins. Apoptosis, a type of controlled cell death, eliminates damaged or unwanted cells. Anoikis is a type of programmed cell death in which cells lose contact with the extracellular matrix. The dysregulation of these cellular pathways promotes tumor growth and spread. Apoptosis, anoikis, and autophagy interact meticulously and differently depending on the cellular circumstances. For instance, autophagy can protect cancer cells from apoptosis by



Cite



Share



Favorites



Permissions

PRE-CLINICAL REPORTS

Combating anoikis resistance: bioactive compounds transforming prostate cancer therapy

Gulia, Shweta; Chandra, Prakash; Das, Asmita

[Author Information](#) ✓

Anti-Cancer Drugs 35(8):p 687-697, September 2024. | DOI: 10.1097/CAD.0000000000001616

BUY

SDC

Abstract

The study aims to discuss the challenges associated with treating prostate cancer (PCa), which is known for its complexity and drug resistance. It attempts to find differentially expressed genes (DEGs), such as those linked to anoikis resistance and circulating tumor cells, in PCa samples. This study involves analyzing the functional roles of these DEGs using gene enrichment analysis, and then screening of 102 bioactive compounds to identify a combination that can control the expression of the identified DEGs. In this study, 53 DEGs were identified from PCa samples including anoikis-resistant PCa cells and circulating tumor cells in PCa. Gene enrichment analysis with regards to functional enrichment of DEGs was performed. An inclusive screening process was carried out among 102 bioactive compounds to identify a combination capable of affecting and regulating the expression of selected DEGs. Eventually, gastrodin, nitidine chloride,

Natural Compound Dioscin Targeting Multiple Cancer Pathways through its High Affinity Binding to B Cell Lymphoma-2.

Gulia S¹ , Chandra P¹ , Das A¹ 

Author information ▶

Current Computer-aided Drug Design, 19 Feb 2024,

<https://doi.org/10.2174/0115734099279130231211053542> PMID: 38375836

Share this article    

Abstract

Objective

The study aimed to explore the crucial genes involved in cancer-related biological processes, including EMT, autophagy, apoptosis, anoikis, and metastasis. It also sought to identify common genes among the pathways linked to these biological processes, determine the level of Bcl-2 expression in various types of cancers, and find a potent inhibitor of Bcl-2 among natural compounds.

Methods

Common genes involved in the pathways related to EMT, autophagy, apoptosis, anoikis, and metastasis were explored, and the level of the most frequently overexpressed gene that was Bcl-2, in various types of cancers was analyzed by gene expression analysis. A set of 102 natural compounds was sorted according to their docking scores using molecular docking and filtering. The top-ranked molecule was chosen for additional molecular dynamics (MD) simulation for 100 ns. Differential gene expression analysis was performed for Dioscin using GEO2R.

Results

The study identified four common genes, Bcl-2, Bax, BIRC3, and CHUK, among the pathways linked to EMT, autophagy, apoptosis, anoikis, and metastasis. Bcl-2 was highly overexpressed in many cancers, including Acute Myeloid Leukemia, Diffuse large B cell lymphoma, and Thymoma. The Dioscin structure in the Bcl-2 binding site received the highest docking score and the most relevant interactions. Dioscin's determined binding free energy by MM/GBSA was -52.21 kcal/mol, while the same calculated by MM/PBSA was -9.18 kcal/mol. A p-value of less than 0.05 was used to determine the statistical significance of the analysis performed using GEO2R. It was observed that



PROFILE

I am a passionate and results-driven cancer biologist with a h.D. in Cancer Therapeutics from Delhi Technological University (2020–2024). My research focuses on overcoming anoikis resistance and addressing drug-resistant cancer pathways using natural bioactive compounds and gold nanoparticle-based delivery systems. I have a strong foundation in molecular biology, cancer immunology, cell signaling, and computational drug design.

As a published researcher with articles in high-impact, peer-reviewed journals, I bring extensive experience in both experimental oncology and bioinformatics-guided drug discovery.

I have also contributed to AI-driven biology education as a Subject Matter Expert at Soul AI, collaborating with interdisciplinary teams to develop intelligent learning platforms.

Currently, I serve as an Assistant Editor at Biothink, the official magazine of the National Academy of Biosciences, where I engage in critical editorial evaluation and peer review.

I am deeply committed to advancing translational oncology and personalized medicine through interdisciplinary innovation, scientific rigor, and a focus on clinical impact

CONTACT

- Phone: 9718971399
- Email: guliashweta9@gmail.com
- LinkedIn: <https://linkedin.com/in/shweta-gulia-76470028>
- GoogleScholar: <https://scholar.google.com/citations?user=JRR4wjoAAAAJ&hl=en>

EDITORIAL & DOMAIN CONTRIBUTIONS

- Currently serving as Assistant Editor at **Biothink**, the official journal of the **National Academy of Biosciences (2025-Present)**, where I handle manuscript screening, peer communication, and content curation.
- Peer reviewer for multiple reputed journals including: American Journal of Biomedical and Life Sciences, Phytotherapy Research, Cancer Cell International, and BMC Cancer.
- As a Subject Matter Expert (Biology) at Soul AI, I collaborated with engineers and data scientists to build AI-driven educational and healthcare platforms.
- I contributed scientific content, validated domain knowledge, and ensured biological accuracy across curriculum-aligned tutoring modules and intelligent assistants.

HONORS& AWARDS

- Research Excellence Award for publication in journal “Cell Biochemistry and Biophysics”, 2023
- Best Research Paper - World Cancer Congress, 2023

SHWETA GULIA

EDUCATION

Ph.D. Scholar- Department of Biotechnology, Delhi Technological university
Thesis Title: Targeting anoikis resistance in tumor cells using bioactive natural compound.

MSc Biotechnology- Symbiosis International University
Project Title: Biochar-based environmental remediation

B.Sc. (Honors) Biotechnology- Maharishi Dayanand University

WORK EXPERIENCE

Role	Organization	Work Description	Duration
Subject Matter Expert	Soul AI	Biology AI education tools	2025-Present
Teaching Assistant	DTU	Lab sessions & mentoring	2021-2024
Ph.D. Researcher	DTU	Cancer resistance & nanodrug design	2020-2024

Skills

Skill Area	Details
Molecular Biology	DNA/RNA Isolation, Gel Electrophoresis, DNA/RNA/Protein Quantification, Cell Culture, Cell Preservation
Bioinformatics	TCGA/GEO, Docking, Pathway Analysis
Software Tools	PyMOL, GraphPad, MATLAB, Discovery Studio
Communication	Grant Writing, Manuscript Drafting, Editing, Peer Review, Scientific Reviewing

Publications

Category	Title	Journal/Publisher	Year
Research Articles	Combating Anoikis Resistance: Bioactive Compounds in Prostate Cancer Therapy	Anticancer Drug	2024
	Dioscin-Mediated Regulation of Cancer Pathways	Current Computer-Aided Drug Design	2024
	Development of Sorbing Films for Air Pollution	Cardiometry	2022
Review Articles	Cancer Prognosis and Tumor Microenvironment	Cell Biochemistry and Biophysics	2023
	Nanomaterial Toxicity and Human Health	PeerJ	2023
Book Chapters	Bioremediation Using Nanotechnology	Elsevier	2023
	Sensors for Aquatic Pollutants	—	2025
	Microbial Nanomaterials in Regenerative Medicine	Advances in Microbial Nanotechnology	2025

PLAGIARISM VERIFICATION

of the Thesis Targeting Anoikis Resistance using bioactive
natural compounds Total P

Name of the Scholar Shweta Gulia Supervisor (s)

Dr. Asmita Das

Dr. Prakash Chandra (3)

ment of Biotechnology

to report that the above thesis was scanned for similarity detection. Process and outcome is given below:

re used: Turnitin Similarity Index: 2%, Total Word Count: 50,058

[Signature]
16/6/28

ate's Signature

[Signature]

Signature of Supervisor(s)