

**MECHANISTIC INSIGHTS INTO THE BIOACTIVITY AND
BIOAVAILABILITY OF NATURAL COMPOUNDS AGAINST CANCER**

SEYAD SHEFRIN N



**DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

APRIL 2024

© Indian Institute of Technology Delhi (IITD), New Delhi, 2024

**MECHANISTIC INSIGHTS INTO THE BIOACTIVITY AND
BIOAVAILABILITY OF NATURAL COMPOUNDS AGAINST CANCER**

by

Seyad Shefrin N

Department of Biochemical Engineering and Biotechnology

Submitted

in the fulfilment of the requirements of the degree of Doctor of Philosophy

to the



Indian Institute of Technology Delhi

APRIL 2024

CERTIFICATE

This is to certify that the thesis entitled '**Mechanistic insights into the bioactivity and bioavailability of natural compounds against cancer**' being submitted by Mr. Seyad Shefrin N to the Indian Institute of Technology Delhi for the award of the degree of '**Doctor of Philosophy**', is a record of the bonafide research work carried out by him, which has been prepared under my supervision in conformity with the rules and regulations of Indian Institute of Technology Delhi. The research reports and the results presented in the thesis have not been submitted for any degree or diploma in any other University or Institute.

Prof. D. Sundar

Institute Chair Professor

Department of Biochemical Engineering and Biotechnology

Indian Institute of Technology Delhi

ACKNOWLEDGEMENTS

I express my sincere gratitude to my supervisor Prof. D. Sundar for his invaluable guidance and unwavering support throughout the completion of this thesis. I would also like to thank him for his constant encouragement and motivation, which contributed significantly for shaping my research work and writing this thesis. I am truly fortunate to have Prof. D. Sundar as my mentor, and I appreciate his mentorship and knowledge imparted during this academic journey. I would also like to thank Dr. Renu Wadhwa and Dr. Sunil Kaul from AIST, Japan, for the experimental inputs and their valuable advice.

I extend my appreciation to the members of my thesis committee, Prof. Shilpi Sharma, Prof. Ravikrishnan Elangovan and Prof Ashok Patel (Kusuma School of Biological Sciences), for their insightful feedback, comments and constructive inputs that greatly enriched the quality of this research.

I would also like to express my gratitude to my senior lab-mates, especially to Dr. Jaspreet Kaur Dhanjal and Dr. Vidhi Malik, for their help and guidance during the initial phases of my research journey. I would also like to thank Dr. Dhvani Vora, Dr. R. Navaneethan, Dr. Yogesh Kalakoti, Dr. Vipul Kumar, Ms. Pragya Keshwarwani, Ms. Swathik Calaranica, Mr. Kamlesh Kumar, Mr. Inam Ahmed, and everyone who has been with me during this long journey, for always being kind and keeping the lab environment cheerful and enjoyable. Furthermore, I would also like to express my gratitude and appreciation to my friends Dr. Navaneethan, Mr. Kamlesh and Ms. Garima Yadav for making this journey memorable.

I would like to extend my deepest gratitude to my parents for their constant love, encouragement and unwavering support throughout my academic journey. Their sacrifices, guidance and belief in me have been the foundation of my success, and I am forever grateful. I also want to thank my wife Ramya for her constant encouragement, support and understanding. Thank you all from the bottom of my heart.

-Seyad Shefrin N

ABSTRACT

Cancer can be defined as the uncontrolled cell proliferation and migration of such cells into the nearby tissues. Understanding the molecular mechanism and developing a therapeutic strategy is a cumbersome process due to the increased number of complex pathways and factors that drives the normal cells to become cancerous. Targeted therapy, including monoclonal antibodies and small-molecule drugs, fail in clinical trials multiple times due to the heterogeneous nature of the tumor cells and various other micro-environmental factors. Side-effects such as vomiting, diarrhoea, high blood pressure and fatigue are commonly observed in patients treated with chemotherapy or targeted therapy using synthetic drugs. Radiation therapy and surgery can remove the tumor cells; however, there are higher chances of regeneration of the cancer stem cells that can grow back the tumor during the post-treatment phase. In this premise, there is a need for alternative drugs to conventional synthetic cancer drugs that can reduce side effects in the patients undergoing treatment. Natural resources have emerged as a crucial source of pharmaceutically relevant novel secondary metabolites, several of which are already approved, and many are under development or in clinical trials. Traditional medicinal system like *Ayurveda* that uses whole plant extract for treating various diseases including cancer, Alzheimer's and other neurodegenerative diseases, are in practice for many years in India due to their lesser side effects. However, their individual compound's mechanism of action is not completely understood for use in treatment. With this background, this PhD thesis aimed to bring the mechanistic understanding of natural compounds and their mode of action by studying their bioactivity and bioavailability inside the cell.

Keywords: Natural compounds, Drug discovery, Molecular mechanism, Natural metabolites, Cancer drug

सार

कैंसर को अनियंत्रित कोशिका प्रसार के रूप में परिभाषित किया जा सकता है और ऐसी कोशिकाओं, आस-पास के ऊतकों में प्रवास करके उनको भी प्रभावित कर देती है। आणविक तंत्र को समझना और एक चिकित्सीय रणनीति विकसित करना एक बोझिल प्रक्रिया है क्योंकि जटिल मार्गों और कारकों की संख्या बंध रही है जो सामान्य कोशिकाओं को कैंसर बनने के लिए प्रेरित करती है। ट्यूमर कोशिकाओं की विषम प्रकृति और विभिन्न अन्य सूक्ष्म-पर्यावरणीय कारकों के कारण मोनोक्लोनल एंटीबॉडी, छोटे-अणु दवाएँ और लक्षित चिकित्सा कई बार नैदानिक परीक्षणों में विफल हो जाती है। उल्टी, दस्त, उच्च रक्तचाप और थकान जैसे दुष्प्रभाव आमतौर पर कीमोथेरेपी या सिंथेटिक दवा के जरीए लक्षित थेरेपी का उपयोग करके रोगियों में देखे गए थे। विकिरण चिकित्सा और सर्जरी ट्यूमर कोशिकाओं को हटा सकती है; हालाँकि, कैंसर स्टेम कोशिकाओं के पुनर्जन्म की संभावना अधिक होती है। ये ट्यूमर कोशिकाएँ उपचार के चरण के बाद ट्यूमर को पुनः विकसित कर देती हैं। इस आधार पर, पारंपरिक सिंथेटिक कैंसर दवाओं के लिए वैकल्पिक दवाओं की आवश्यकता है जो रोगी के इलाज में होने वाले दुष्प्रभावों को कम कर सके। प्राकृतिक संसाधन, फार्मास्युटिकल में प्रासंगिक उपन्यास माध्यमिक मेटाबोलाइट्स के लिये एक महत्वपूर्ण स्रोत के रूप में उभरे हैं, जिनमें से कई पहले से ही अनुमोदित हैं, और कई विकास के अधीन हैं या नैदानिक परीक्षणों में हैं। आयुर्वेद जैसी पारंपरिक औषधीय प्रणाली जो कैंसर, अल्जाइमर और अन्य न्यूरोडीजेनेरेटिव रोगों सहित विभिन्न बीमारियों के इलाज के लिए पूरे पौधे के अर्क को उपयोग में लाया जाता है। ये पारंपरिक औषधीय अपने कम दुष्प्रभावों के कारण कई वर्षों से प्रचलन में हैं। हालाँकि, उपचार में उपयोग के लिए उनके व्यक्तिगत यौगिक की क्रिया का तंत्र पूरी तरह से समझा नहीं गया है। इस पृष्ठभूमि के साथ, इस पीएचडी थीसिस का उद्देश्य कोशिका के अंदर की जैव सक्रियता और जैव उपलब्धता का अध्ययन करके विभिन्न प्राकृतिक यौगिकों और उनकी क्रिया के तरीके की यंत्रवत को समझना है।

कीवर्ड: प्राकृतिक यौगिक, दवा की खोज, आणविक तंत्र, प्राकृतिक मेटाबोलाइट्स, कैंसर की दवा

CONTENTS

Certificate	i
Acknowledgements	ii
Abstract	iii
संर	iv
List of Figures	ix
List of Tables	xvi
List of Abbreviations	xvii
1 Introduction	1
1.1 Natural products used as a traditional medicine and their application in cancer treatment	1
1.2 Understanding the molecular mechanism of anti-cancer activity of natural compounds using computational approach.....	3
1.2.1 Target identification using literature survey and pathway analysis.	5
1.2.2 Target identification using inverse virtual screening method.	6
1.2.3 Target identification using next generation sequencing data.....	7
1.2.4 Molecular interaction studies using computational simulations.	8
1.3 Definition of the problem.....	12
1.4 Objectives of the thesis	13
1.5 Thesis organization	13
2 Identification of cancer targets using a) Literature review, b) Transcriptomic data and c) Inverse virtual screening	18
2.1 Introduction	18

2.2	Targets identified using <i>literature survey</i> and <i>pathway analysis</i> for cancer	19
2.3	Targets identified using <i>transcriptomics data</i> for Lung Adenocarcinoma	21
2.4	Target identified for CAPE molecule using inverse docking method	23
2.5	Conclusion of the chapter	24
3	Understanding the mechanism of action of natural compounds against the identified cancer targets	25
3.1	Computational analysis of natural compounds in restoring transcriptional activity of p53 in cancer cells harbouring WT and p53 ^{Ser46} mutant.....	25
3.1.1	Background	25
3.1.2	Methodology	27
3.1.3	Stability and interaction study of natural compounds on p53 and mortalin-binding regions.....	32
3.1.4	Restoration of p53 activity by enhancing binding with cofactor p62 on Ser46Δ mutant p53 using natural compounds	36
3.1.5	An extended study shows Cucurbitacin-B inhibition of HDM2 and mortalin through computational assay.....	41
3.1.6	Experimental evidence to the effect of natural compounds on reactivation of p53.....	43
3.1.7	Conclusion	44
3.2	Identification of a new member of Mortaparib class of inhibitors that target mortalin and PARP1	45
3.2.1	Background	45
3.2.2	Methodology	46
3.2.3	Mortaparib ^{MILD} abrogates p53-mortalin interaction and inhibits PARP1.....	47
3.2.4	Mortaparib ^{MILD} treated cells showed downregulation of mortalin and disruption of mortalin-p53 complex	50
3.2.5	Conclusion	51

3.3	Molecular insights into Wi-A promoting caspase-mediated apoptosis in cancer cells.....	51
3.3.1	Background.....	51
3.3.2	Methodology.....	53
3.3.3	Withaferin-A prevents homodimerization of survivin protein, preventing its active conformation.....	54
3.3.4	Downregulation of survivin protein and mRNA by Wi-A rich extract of Ashwagandha leaves (Wi-AREAL).....	56
3.3.5	Conclusion.....	58
3.4	Mechanistic insights of AKT inhibition using natural compounds by allosteric and ATP competitive inhibition.....	59
3.4.1	Background.....	59
3.4.2	Methodology.....	60
3.4.3	Interactions and stability of natural compounds in ATP-competitive inhibition site.....	61
3.4.4	Interactions and stability of natural compounds on allosteric inhibition site.....	64
3.4.5	Conclusion.....	66
3.5	Conclusion of the overall chapter 3.....	66
4	Studying the effect of lipid peroxidation in membrane permeability of withanone molecule.....	68
4.1	Introduction.....	68
4.2	Methodology.....	70
4.3	Variation in the properties of normal POPC and oxidised POPC-OOH (POPX) lipid bilayer membrane.....	73
4.4	Permeability study of Withanone compared to water through oxidized Phosphatidyl choline membranes representing the model of cancer cells.....	76
4.5	Conclusion of the chapter.....	80

5	Conclusion of the Thesis.....	82
6	References.....	85
7	List of Publications.....	100
8	Resume of the author.....	102

LIST OF FIGURES

Figure 1-1: Percentage of approved drugs from various sources. Pi-chart showing contribution of natural compounds and natural compound derivatives in approved drugs from 1981-2019.....	1
Figure 1-2: Chemical structure of natural compounds used in this study. Natural compounds studied for their anti-cancer potency include Withaferin-A (Wi-A), Withanone (Wi-N), Caffeic Acid Phenethyl Ester (CAPE), Artepillin C (ARC) and Cucurbitacin- B (Cuc- B).....	3
Figure 1-3: Hallmarks of cancer and therapeutic strategies.	4
Figure 1-4: Computational drug discovery pipeline in a clinical drug development process	6
Figure 1-5: Flowchart showing Inverse virtual screening. (1) Preparation of ligand and protein. (2) Grid generation and molecular docking of the ligand. (3) Selection of targets based on docking scores.	6
Figure 1-6: Workflow of whole transcriptome data analysis. Primary analysis includes quality control and pre-processing. Secondary analysis consists of alignment and quantitation with reference genome. Tertiary analysis includes statistical analysis and visualization.	8
Figure 1-7: Flow chart of Molecular Dynamics (MD) simulation algorithm. Each step generates the subsequent position and velocity for the next time frame.....	10
Figure 1-8: Free energy calculation of permeation using Umbrella Sampling method. The reaction coordinate is divided into smaller windows for generating potential mean force across the coordinates.....	11
Figure 2-1: Shortlisted targets based on different hallmarks of the cancer cell survival. Schematic diagram showing shortlisted targets from apoptosis and DNA repair pathway in cancer	20

Figure 2-2: Meta data of lung adeno carcinoma patients. FASTQ files were obtained from NCBI-SRA database for cancerous tissues and normal tissues of the patients.21

Figure 2-3: Top 500 genes differentially expressed obtained using R heatmap. The red colour indicates upregulated genes and the blue colour indicates the down regulated genes.....22

Figure 2-4: Differential gene expression analysis using R. (A) MA plot showing differentially expressed genes in red color dots and normal expression by black color. (B) Top 20 differentially expressed genes.....22

Figure 2-5: Overall strategy adopted for inverse docking of CAPE.23

Figure 3-1: Illustration of p53 sequestration by mortalin interaction causing cancer cell progression.26

Figure 3-2: Molecular dynamics simulation study of mortalin p53 abrogation by natural compounds. (A) Three-dimensional visualization of interaction between mortalin and p53 (docked using HADDOCK server and simulated for 200 ns). B) Three-dimensional visualization of interaction between Cuc-B and p53-binding domain of mortalin. C) Three-dimensional visualization of interaction between Wi-N and p53-binding domain of mortalin. D) Three-dimensional visualization of interaction between Wi-A and p53-binding domain of mortalin. E) Three-dimensional visualization of interaction between CAPE and p53-binding domain of mortalin. F) Three-dimensional visualization of interaction between Cuc-B and mortalin binding domain of p53. G) Root Mean Square Deviation (RMSD) plot of natural compounds bound mortalin complex showing stable interactions. H) RMSD plot of Cuc-B bound p53 complex showing stable interaction35

Figure 3-3: Molecular dynamic simulation study of natural compounds on phosphor mutant p53-p62 complex. (A) Conformational changes observed in the structure of mutant p53 compared with wild type p53. (B) Conformational changes observed in the structure of mutant p53 after Cuc-B interaction. (C) Conformational changes observed in the structure of mutant p53 after Wi-N interaction. (D) Conformational changes observed in the structure of mutant p53 after Wi-A interaction. (E) RMSD plot of wild-type as well as mutant p53

complex with p62 before and after Cuc-B interaction simulated for 200ns. (F) RMSD plot of mutant p53 complex with p62 before and after Wi-N, Wi-A, CAPE and ARC interaction simulated for 200 ns. G) Interaction fraction diagram of wild type p53 with amino acids of p62. (H) Interaction fraction diagram of phospho-mutant p53 with amino acids of p62.37

Figure 3-4: Molecular dynamics simulation analysis of natural compounds in restoring wild type p53 activity. (A) Interaction fraction diagram of phospho-mutant p53 with amino acids of p62 after Cuc-B intervention. (B) Interaction fraction diagram of phospho-mutant p53 with amino acids of p62 after Wi-N intervention. (C) Interaction fraction diagram of phospho-mutant p53 with amino acids of p62 after Wi-A intervention. D) Change in MMGBSA binding energy before and after Cuc-B interaction. (E) Change in MMGBSA binding energy before and after the Wi-N interaction. (F) Change in MMGBSA binding energy before and after the Wi-A interaction. (G) Change in van der Waals, electrostatic and hydrogen bonding energy of interacting molecules. (H) Hydrogen bonding plot observed between p53 and p62 before and after Cuc-B intervention.39

Figure 3-5: Cuc-B interaction in the mortalin-p53 interaction site is stronger than MKT077. (A). Interactions of Cuc-B with p53 binding region of mortalin in the average structure obtained from the simulation trajectory. (B) Interactions of MKT077 with the p53-binding region of mortalin in the average structure obtained from the simulation trajectory. (C) RMSD plot of Cuc-B and MKT077-bound mortalin throughout the simulation. (D) Interaction of Cuc-B with the mortalin-binding region of p53 as observed in the average structure obtained from the simulation trajectory. (E) RMSD plot showing stable interactions of Cuc-B with p53 throughout the simulation. (F) Interaction fraction of mortalin residues involved in binding with Cuc-B.41

Figure 3-6: Cuc-B docked to HDM2 with a higher docking score than its known inhibitor Y30. (A) Interaction of Cuc-B with p53-binding domain of HDM2 in the average structure obtained from the simulation trajectory. (B) RMSD plot of Cuc-B and Y30-bound HDM2 showing stable interaction throughout the simulation. (C) Superimposed image showing Cuc-B and Y30 interacting with the same p53 binding domain of HDM2. (D) Interaction of Y30 with p53 binding domain of HDM2 in the average structure obtained from the simulation trajectory.42

Figure 3-7: *In vitro* analysis of the comparative wild type p53 activation function of five natural compounds. A) Western blotting of control and treated U2OS cells for p53 and p21 proteins showed increase in the treated cells. Highest increase was observed in cells treated with Withaferin-A followed by Withanone, Cucurbitacin B, CAPE and Artepillin C. B) Immunocyto staining of control and treated U2OS cells showed increase in expression of p53 and p21 in the later. C) p53-dependent luciferase reporter assay in control and treated U2OS cells showed increase in wild type p53 activity on treated cells and was in accordance with the expression analysis. D) Western blotting of control and treated HSC3 cells (harboring p53Ser46mutant) for p21 showed its increase in the later. Blots (A and D) were probed with β -Actin as an internal loading positive control.44

Figure 3-8: 2D Structure of Mortaparib and its derivatives......45

Figure 3-9: Binding interactions of Mortaparib^{MILD} with the target proteins. (A) Mortaparib^{MILD} interaction at the mortalin-binding region of p53. (B) Mortaparib^{MILD} interaction at the p53-binding region of mortalin. (C) Mortaparib^{MILD} interaction with the catalytic domain of PARP1. (D) Olaparib interaction with the catalytic domain of PARP1. .48

Figure 3-10: Stability and binding dynamics of Mortaparib^{MILD} with target proteins. (A) Hydrogen bond occupancy of Mortaparib^{MILD} with p53 (B) Hydrogen bond occupancy of Mortaparib^{MILD} with mortalin (C) RMSD plot of Mortaparib^{MILD} interaction with p53, mortalin, and PARP1. (D) Hydrogen bond plot of Mortaparib^{MILD} interaction with p53, mortalin, and PARP1.....49

Figure 3-11: Mortaparib^{MILD} abrogated the interaction of mortalin and p53 in HCT116 (wild type p53) cells. (A) Western blot showing the expression level of mortalin and p21. Mortaparib^{MILD} treated cells showed decrease in mortalin and increase in p21 (consequence of increase in p53). β -actin was used as an internal loading control (B) Immunostaining of mortalin and p53 in control and treated cells showing decrease in mortalin and increase in nuclear p53 in treated cells. (C) mRNA expression of mortalin as determined by RT-qPCR showing decrease in mortalin and increase in p53 at mRNA level. (D) Wild type p53-driven luciferase reporter assay in negative control (NC, un-transfected cells), control (transfected but untreated) and treated (transfected and treated with 50 μ M Mortaparib^{MILD}) cells..50

Figure 3-12: Interaction of Wi-A and YM155 in homodimerization domain of survivin.	
(A) Visualization of interaction between homodimers of survivin. (B) Visualization of interaction between YM155 with the survivin homodimer-forming region. (C) Visualization of interaction between Wi-A with the survivin homodimer-forming region. (D) RMSD plot of the simulations for YM155 and Wi-A interaction with survivin.....	55
Figure 3-13: Computational analysis revealing survivin inhibition by Wi-A and YM155.	
(A) Interaction fraction diagram of the Wi-A over the simulation. (B) Interaction fraction diagram of the YM155 over the simulation. (C) Hydrogen bond occupancy plot of Wi-A with survivin. (D) Hydrogen bond occupancy plot of YM155 with survivin.....	56
Figure 3-14: Effect of Wi-AREAL extract on viability of cervical cancer cells.	
(A) Dose-dependent decrease in viability. (B) Increase in apoptotic cells was observed in short term (24-48 h treatment). (C) Long term (10-15 days treatment) showed dose-dependent reduction in colony number and size.....	57
Figure 3-15: Effect of YM-155 and Wi-AREAL on survivin mRNA and protein.	
(A) Treatment of HeLa cells with IC50 concentrations of YM-155. (B) Wi-AREAL resulted in downregulation of survivin protein. (C) Dose-dependent decrease in protein was observed by Western blotting. (D) Dose dependent decrease in protein observed by immunocytostaining. (E) Wi-AREAL resulted in downregulation of survivin mRNA.	58
Figure 3-16: Illustration of the inactive and active conformation of AKT1 and mechanism of its inhibition.	
(A) Inactive AKT in the cytoplasm where PH-in conformation is aided by the interaction of PH and kinase domains of AKT and Conformational change during activation of AKT. (B) Allosteric and ATP competitive inhibition mechanism.	60
Figure 3-17: MD Analysis of natural compounds at the ATP competitive site of AKT1.	
(A) RMSD of natural ligands interaction with ATP-binding pocket of AKT1. (B) RMSF of backbone after the simulations with natural ligands. (C) Hydrogen bond occupancy of CAPE with AKT1 residues on ATP- binding domain. (D) Hydrogen bond occupancy of Cuc-B with AKT1 residues on ATP-binding domain.	62

Figure 3-18: Natural compounds interactions on ATP-binding site of AKT1 has been visualized in PyMOL 3D. (A) ATP analog interaction with AKT1. (B) CAPE interaction with AKT1. (C) Cuc-B interaction with AKT1. (D) ARC interaction with AKT1. 63

Figure 3-19: MD Analysis of natural compounds on allosteric site of AKT1. (A) RMSD of ligands interaction with allosteric-binding pocket. (B) RMSF of backbone after the simulation with ligand interaction. (C) Hydrogen bond occupancy of CAPE with AKT1 residues on the allosteric domain. (D) Hydrogen bond occupancy of Wi-N with AKT1 residues on allosteric domain. 65

Figure 3-20: Natural compounds interactions on allosteric site visualized in PyMOL 3D. (A) Inhibitor-8 interaction with AKT1 (B) Withanone interaction with AKT1 (C) Wi-A interaction with AKT1 (D) CAPE interaction with AKT1. 66

Figure 4-1: Computational model of POPC and POPX. (A) Normal POPC bilayer membrane (pink spheres indicate phosphorous atom) in 8320 water molecule system after production simulation for 300 ns. (B) Oxidized POPC/POPX (red spheres indicate oxygen atoms) bilayer in water system after 69

Figure 4-2: Plots showing the properties of membranes generated. (A) The changes in Area per lipid (APL) after oxidation in pure POPC, in the bilayer mixture containing cholesterol 15% and Bilayer mixture containing all three components POPC, POPX and 15% cholesterol. (B) Changes in bilayer thickness after oxidation in pure POPC, in the bilayer mixture containing cholesterol of 15% and Bilayer mixture containing all three components POPC, POPX and 15% cholesterol. (C) The order parameter of sn1 tail for POPC and after oxidation in pure POPC, in the bilayer mixture containing cholesterol 15% and Bilayer mixture containing all three components POPC, POPX and 15% cholesterol. (D) The order parameter of sn2 tail for POPC and after oxidation in pure POPC, in the bilayer mixture containing cholesterol 15% and Bilayer mixture containing all three components POPC, POPX and 15% cholesterol. 74

Figure 4-3: Plots showing densities of the membranes. (A) Change in lipid density in all the membrane systems (B) Changes in peroxide density of all the oxidized membrane systems. 76

Figure 4-4: Plots showing the permeabilities of water and Wi-N. (A) Change in Potential Mean Force (ΔG) along the reaction coordinate (Z-axis) when Wi-N passed through all the membrane models. (B) Change in Potential mean force (ΔG) along the reaction coordinate (Z-axis) when water passed through all the membrane models. (C) Resistivity change of water in POPC when passed through the reaction coordinate. (D) Resistivity change of Wi-N in POPC when passed through the reaction coordinate.77

Figure 4-5: Plots showing the changes in resistivity of water along the reaction coordinate Z-axis of membrane layer containing. (A) Bilayer mixture of POPC with 15% cholesterol. (B) Pure oxidised POPX (POPC-OOH). (C) Bilayer mixture of oxidised POPX (POPC-OOH) and 15% cholesterol. (D) Bilayer mixture containing an equal concentration of POPC and Oxidised POPX along with 15% cholesterol.78

Figure 4-6: Diffusion constant of water and Wi-N through all the membrane system ...79

LIST OF TABLES

Table 2-1: Targets identified for CAPE using known ligand-binding site and unknown binding site.....	24
Table 3-1: Dock scores and MMGBSA binding energy for the interaction of the natural compounds with p53 and mortalin.....	33
Table 3-2: Interacting residues of p53 and mortalin with the natural compounds after simulation for 200 ns.....	34
Table 4-1: Compositions of membranes generated with varying concentration of POPC, POPX and Cholesterol.....	71
Table 4-2: Permeability coefficients Log(P) of water and Wi-N through all the systems.....	80

LIST OF ABBREVIATIONS

ADME	Absorption Distribution Metabolism Excretion
ADP	Adenosine Di Phosphate
AKT1	Ak strain Transforming
APL	Area Per Lipid
ARC	Artipillin-C
ATP	Adenosine Tri Phosphate
CAPE	Caffeic Acid Phenethyl Ester
Cuc-B	Cucurbitacin-B
HDM2	Human Double Minute 2 Homolog
hnRNP-K	Heterogeneous nuclear Ribonucleoprotein K
LUAD	Lung Adeno Carcinoma
NGS	Next Generation Sequencing
PARP1	Poly [ADP-ribose] Polymerase 1
PMF	Potential Mean Force
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
QSAR	Quantitative Structure Activity Relationship
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
SMD	Steered Molecular Dynamics
WHAM	Weighted Histogram Analysis Method
Wi-A	Withaferin-A
Wi-N	Withanone