

CERTIFICATE

I certify that the thesis entitled “Exploring the Anti-Cancer Potential of *Rhododendron arboreum* Sm. in Gastric Cancer using *In vitro* and *In vivo* Models” submitted for the award of Doctor of Philosophy (Ph.D) by Yangchen Dolma Kom is the record of research work carried out by her during the period from January 2020 to April 2025 under my guidance and supervision and that this work has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other Titles in this Institute or any other University or Institution of Higher Learning.


Signature of the HoD


Signature of the Supervisor
with Designation

Dr. R. KARTHIYAYINI
Assistant Professor
Department of Botany
Avinashilingam Institute for Home Science
and Higher Education for Women
Coimbatore - 641 043


Signature of the Dean of Respective School

DECLARATION

I declare that the thesis entitled "**Exploring the Anti-Cancer Potential of *Rhododendron arboreum* Sm. in Gastric Cancer using *In vitro* and *In vivo* Models**" submitted by me for the award of Doctor of Philosophy (Ph.D) is the record of work carried out by me during the period from 2020 to 2025 under the guidance of **Dr. R. Karthiyayini, Assistant Professor (SS), Department of Botany, Avinashilingam Institute of Home Science and Higher Education For Women, Coimbatore** and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this Institute or any other University or other similar Institution of Higher Learning.



Signature of the Research Scholar



Signature of the Supervisor

ACKNOWLEDGEMENT

*First and foremost, I owe my profound gratitude to **God Almighty**, the author of knowledge and wisdom, for showering countless blessings throughout my research work to complete the thesis successfully.*

*I take this opportunity to sincerely thank **Padma Bhusan Dr. T. S. Avinashilingam Ayya Avargal**, Founder and First Chancellor of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and **Padma Shri Dr. Rajammal P. Devadas Amma Avargal** for giving me this opportunity to gain knowledge in this institution.*

*I express my sincere thanks to our Chancellor, **Dr. T. S. K. Meenakshi Sundaram**, Former and Revered Chancellors, **Dr. S. P. Thyagarajan** and late **Dr. P. R Krishna Kumar**, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for all their good wishes and permission to carry out my work in this eminent institution.*

*I express my reverential thanks to **Dr. V. Bharathi Harishankar**, Vice Chancellor, and Former Vice Chancellor, **Dr. Premavathy Vijayan**, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for permitting me to utilize the infrastructure of the University and rendering benevolent support during the tenure of my study.*

*I acknowledge **Dr. H. Indu (i/c)**, Registrar, and **Dr. S. Kowsalya**, Former Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for extending all the possible help towards the completion of this study.*

*My heartfelt thanks to the Controller of Examinations, **Dr. K. Sampath Rani**, Professor, and former Controller of Examinations **Dr. K. Manimozhi**, Professor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her valuable suggestions and the help given for the successful completion of the study.*

*I am thankful to **Dr. P. Lalitha**, Professor, Director of Research and Development Cell, and my special thanks to **Dr. G.P. Jeyanthi**, Professor, Former Director R&D, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for all the prudent suggestions and for the immense help rendered.*

*I am indebted to **Dr. Anitha Subash**, Professor, and Dean, School of Biosciences, and **Dr. A. Vijayalakshmi**, Dean Academics, Professor and Head, Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for their constant encouragement, timely help and tremendous support rendered in carrying out the research work.*

*I extend my profound and earnest gratitude to my Supervisor, **Dr. R. Karthiyayini**, Assistant Professor (SS), Department of Botany, for her meticulous guidance and effective management in the execution of each part of my research. Her unwavering support, insightful feedback, and dedication to pushing the boundaries of knowledge have not only enriched my research but have also inspired me to strive for excellence in everything I do. Her encouragement, moral support and constant rational counseling during every step of the research work and working under her mentorship have been a privilege and a transformative experience that I will always cherish.*

*I am thankful to **Dr. Parimelazhagan Thangaraj**, Professor and Head, Department of Botany, Bharathiar University, the Subject Expert for my Doctoral Committee, for his constructive criticisms and valuable suggestions given for the study.*

*My profound thanks to all the **Staff members** of the Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, who have created a congenial atmosphere to work in throughout my thesis.*

*I am grateful to thank **Dr. Jubie Selvaraj** Associate Professor, Department of Pharmaceutical Chemistry & **Dr. Raman Rajeshkumar** Assistant Professor, Department of Pharmaceutical Biotechnology. JSS College of Pharmacy Ooty. **Dr. G. Ariharasivakumar** Professor, Department of Pharmacology. KMCH College of Pharmacy Coimbatore, for all the possible help rendered and valuable*

01747) for funding my Ph.D. research work.

My sincere and special thanks to my fellow Researchers **Surya S, Vasandhlakshmi R and Indumathi B** for their encouragement and timely support.

On a personal note, I thank my beloved Father, Mother, Sister and Brother and my dear friends for their unshakeable support, prayers, care, motivation, sacrifice, trust, cooperation and untiring patience. They were the strong pillars who supported me to complete this work with determination.

I also acknowledge the contributions of all other unseen hands during the study for the help rendered in the successful completion of the study.

Yangchen Dolma Kom

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1.	Preliminary Phytochemical Estimation of Phytoconstituents	70
2.	Experimental Grouping and Treatment Protocol for <i>In vivo</i> Study	78
3.	Qualitative Analysis of Primary and Secondary Metabolites Present in <i>R. arboreum</i> Leaf Extracts	91
4.	Qualitative Analysis of Primary and Secondary Metabolites Present in <i>R. arboreum</i> Flower Extracts	92
5.	Brine Shrimp Lethality Assay	109
6.	Body weight and Organ Weight of C57BL/6 Mice Models	117
7.	Haematological Parameters of C57BL/6 Mice Treatment with Docetaxel and <i>R. arboreum</i> DMSO RFD and RLD Extracts	117
8.	Effects of C57BL/6 Mice Treatment with Docetaxel and <i>R. arboreum</i> DMSO RFD and RLD Extracts	121
9.	Renal Function Parameters of C57BL/6 Mice Treatment with Docetaxel and <i>R. arboreum</i> DMSO RFD and RLD Extracts	123
10.	Determination of Total Protein, SOD, CAT, GPx, GSH and LPO in C57BL/6 Mice Treatment with Docetaxel and <i>R. arboreum</i> DMSO RFD and RLD Extracts	125
11.	Estimation of Myeloperoxidase of C57BL/6 Mice Treatment with Docetaxel and <i>R. arboreum</i> DMSO RDF and RDL Extracts	126
12.	Molecular Properties of Selected Compounds from <i>R. arboreum</i> Leaf and Flower	134
13.	Pharmacokinetic Properties of Selected Compounds from <i>R. arboreum</i> Leaf and Flower	135
14.	Results of Biological Radar for Selected Compounds	136
15.	KEGG Enrichment Analysis (top 20): Functional Insights into Biological Processes	146
16.	The Binding Energies of Top 4 Key Target Proteins with Top 5 Compounds: Molecular Interaction and Stability	149

LIST OF FIGURES

FIGURE	TITLE	PAGE NO.
1.	Borrmann Classification of Gastric Cancer	5
2.	Gastric Cancer Staging	6
3.	Oxidative Stress Intensity	7
4.	EGFR Pathway in Gastric Cancer	12
5.	Habit and Inflorescence of <i>Rhododendron arboreum</i> Sm.	68
6.	Distribution of <i>Rhododendron arboreum</i> Sm. in India	69
7.	Quantitative Estimation of Secondary Metabolites from <i>R. arboreum</i> Leaf Extracts	93
8.	Quantitative Analysis of Secondary Metabolites from <i>R. arboreum</i> Flower Extracts	93
9.	FT-IR of <i>R. arboreum</i> Methanol Extract of Leaf	95
10.	FT-IR of <i>R. arboreum</i> Methanol Extract of Flower	96
11.	NMR Analysis of <i>R. arboreum</i> Methanol Leaf Extract	97
12.	NMR Analysis of <i>R. arboreum</i> Methanol Flower Extract	98
13.	GC-MS Chromatograms of <i>R. arboreum</i> Leaf Extract	100
14.	GC-MS Chromatograms of <i>R. arboreum</i> Flower Extract	102
15.	DPPH Radical Scavenging Activity of <i>R. arboreum</i> Leaf and Flower Extracts	103
16.	ABTS Radical Scavenging Activity of <i>R. arboreum</i> Leaf and Flower Extracts	104
17.	Hydrogen Peroxide Radical Scavenging Activity of <i>R. arboreum</i> Leaf and Flower Extracts	105
18.	Lipid Peroxidation Assay <i>R. arboreum</i> Leaf and Flower Extracts	106
19.	FRAP Radical Scavenging Activity of <i>R. arboreum</i> Leaf and Flower Extracts	107
20.	Cytotoxicity of Leaf <i>R. arboreum</i> on AGS Cell Line	111
21.	Cytotoxicity of Flower <i>R. arboreum</i> on AGS Cell Line	111

22.	Flow Cytometry Analysis of <i>R. arboreum</i> Flower and Leaf Extracts	111
23 a.	Induction of AGS- Gastric Cancer Cell in C57BL/6 Mice Subcutaneously	115
23 b.	Red Arrows Indicate the Subcutaneous Tumour of the Antrum	115
24.	The Microscopic Examination of Stomach Specimens from Different Treatment Groups Provides Valuable Insights into the Cellular Changes Induced by Various Therapeutic Interventions.	128
25.	The Microscopic Observation of Histopathological Examination of C57BL/6 (Stomach) Treated with Flower Extracts	129
26.	The Microscopic Observation of Histopathological Examination of C57BL/6 (Stomach) Treated with Leaf Extracts	130
27.	A Representative Example of Tumour Development Derived from Different Groups of C57BL/6 Mice	132
28.	Venn Diagram Intersecting the Common Targets for Gastric Cancer and <i>R. arboreum</i> (Leaf and Flower)	138
29 a.	Protein-Protein Interaction. 22 Overlapping Targets of Gastric Cancer and <i>R. arboreum</i>	139
29 b.	Top Ten Compound-Disease-Targets	139
30.	Integrated Network of Protein-Drug Interactions for <i>R. arboreum</i> with Gastric Cancer and Associated Diseases	140
31.	GO Enrichment Analysis for Targets across Different Modules	142
32 a.	The Simplified KEGG Pathway for Gastric Cancer (Hsa05226) is shown, with Red-Coloured Nodes Representing the Targets Identified in the Modules	144

32 b.	The Simplified KEGG Pathway for Gastric Cancer (Hsa05226) is shown, with Green-Coloured Nodes Representing the Potential Targets Identified in the Modules	145
33.	2D Structure of PDB ID- 1B3D, 6S9B, 6GQ0 and 1ERR	151
34	3D and 2D Structure of Quercetin (MMP9)	154
34 a.	3D and 2D Structure of Docetaxel	155
35	3D and 2D Structure of Quercetin (EGFR)	155
35 a	3D and 2D Structure of Docetaxel	156
36.	3D and 2D Structure of Quercetin (KDR)	156
36 a.	3D and 2D Structure of Docetaxel	157
37.	3D and 2D Structure of Quercetin (ESR1)	157
37 a.	3D and 2D Structure of Docetaxel	158
38.	Illustration Of RMSD, the Stability of the Protein (Depicted in Blue) and the Ligand (Depicted in Red) within the Complex	160
39.	Illustration of RMSF Plot for Quercetin, with the following PDB Ids: A- MMP9, B- EGFR, C- KDR, D- ESR1	161
40.	Protein-Ligands Contacts- The Bar Graph of Proteins (MMP9, EGFR, KDR and ESR1) with Quercetin	162
41.	The Ligand Properties of Quercetin, encompass the Radius of Gyration (Rgyr), Intramolecular Hydrogen Bonding (Intrahb), Molecular Surface Area (Molsa), Solvent Accessible Surface Area (SASA) and Polar Surface Area (PSA)	164

ABBREVIATIONS

S.No.	Abbreviations	Full Form
1.	1B3D	Crystal Structure of the Human Matrix Metalloproteinase-9
2.	1ERR	Crystal Structure of the Human Estrogen Receptor Alpha (Er α) Ligand-Binding Domain Complexed with an Agonist
3.	¹H NMR	Proton Nuclear Magnetic Resonance
4.	6GQ0	Crystal Structure of the Human Insulin-Like Growth Factor 1 Receptor (IGF-1R) Kinase Domain in Complex with an Inhibitor
5.	6S9B	Crystal Structure of Human Glycogen Synthase Kinase-3 Beta (GSK-3 β) in Complex with an Inhibitor
6.	Å	Ångström
7.	ABTS	2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic Acid
8.	ACP	Acid Phosphatase
9.	ADME	Absorption, Distribution, Metabolism and Excretion
10.	AE/g	Atropine Equivalents Per Gram
11.	AGS	Human Gastric Adenocarcinoma Cells
12.	ALA	Alanine
13.	AlCl₃	Aluminium Chloride
14.	AlCl₃.6H₂O	Aluminum Chloride Hexahydrate
15.	ALP	Alkaline Phosphatase
16.	amu	Atomic Mass Units
17.	ASN	Asparagine
18.	ASP	Aspartic Acid
19.	BBB	Blood Brain Barrier
20.	BCG	Bromocresol Green Solution
21.	BMRB	Biological Magnetic Resonance Data Bank
22.	BSI	Botanical Survey of India
23.	BW	Body Weight

24.	C-H	Carbon Hydrogen Bond
25.	C=O	Carbonyl Group
26.	C≡N	Carbon-Nitrogen Triple Bond
27.	CAFs	Cancer-Associated Fibroblasts
28.	CAT	Catalase
29.	CD₃OD	Deuterated Methanol
30.	CH₃OH	Methanol
31.	ChEMBL	Chemical European Molecular Biology Laboratory
32.	Cm⁻¹	Reciprocal Centimeters (wavenumber)
33.	Conc	Concentration
34.	ctDNA	Circulation Tumor DNA
35.	Cu⁺⁺	Copper II Ion
36.	CYP19A1	Cytochrome P450 Family 19 Subfamily A Member 1
37.	D.H₂O	Distilled Water
38.	D₂O	Deuterium Oxide
39.	DAVID	Database for Annotation, Visualization and Integrated Discovery
40.	dL	Deciliter
41.	DMEM	Dulbecco's Modified Eagle Medium
42.	DMSO	Dimethyl Sulfoxide
43.	DNPH	2,4-Dinitrophenylhydrazine
44.	DPPH	2,2-Diphenyl-1-picrylhydrazyl
45.	DTNB	(5,5'-Dithiobis(2-nitrobenzoic acid))
46.	ECM	Extracellular Matrix
47.	EDTA	Ethylenediaminetetraacetic Acid
48.	EGFR	Epidermal Growth Factor Receptor
49.	ELISA	Enzyme-linked Immunosorbent Assay
50.	ESR1	Estrogen Receptor 1
51.	ESR2	Estrogen Receptor 2
52.	FACS	Fluorescence-Activated Cell Sorter
53.	FBS	Fetal Bovine Serum
54.	FDR	False Discovery Rate
55.	Fe³⁺	Ferric Ion

56.	FeCl₃	Ferric Chloride
57.	FeSO₄	Ferrous Sulfate
58.	FITC	Fluorescein Isothiocyanate
59.	FRAP	Ferric Reducing Antioxidant Power Assay
60.	FT-IR	Fourier Transform Infrared Spectroscopy
61.	GAE	Gallic Acid Equivalents
62.	GB/SA	Generalized-Born and Solvent Accessible Surface Area
63.	GC	Gastric Cancer
64.	GC-MS	Gas Chromatography-Mass Spectrometry
65.	GC/TQ	Gas Chromatography/ Triple Quadrupole
66.	GLU	Glutamic Acid
67.	GO	Gene Ontology
68.	GPx	Glutathione Peroxidase
69.	GSH	Reduced Glutathione
70.	GSK3B	Glycogen Synthase Kinase 3 Beta
71.	GSSG	Glutathione Disulphide
72.	H&E	Haematoxylin & Eosin
73.	H₂O	Water
74.	H₂O₂	Hydrogen Peroxide
75.	H₂SO₄	Sulfuric Acid
76.	Hb	Haemoglobin
77.	HCl	Hydrochloric Acid
78.	HDL	High Density Lipoprotein
79.	HER2	Human Epidermal Growth Factor Receptor 2
80.	HIS	Histidine
81.	HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase
82.	Hz	Hertz
83.	IAEC	Institutional Animal Ethics Committee
84.	IC₅₀	Half-maximal Inhibitory Concentration
85.	IGF1R	Insulin-like Growth Factor 1 Receptor
86.	intraHB	Intramolecular Hydrogen Bonds
87.	K₂Cr₂O₇	Potassium Dichromate

88.	KBr	Potassium Bromide
89.	KDR	Kinase Insert Domain Receptor
90.	KEGG	Kyoto Encyclopaedia of Genes and Genomes
91.	KOH	Potassium Hydroxide
92.	LB	Lower Band
93.	LDL	Low Density Lipoprotein
94.	LEU	Leucine
95.	LPO	Lipid Peroxidation Assay
96.	m/z	Mass-to-Charge Ratio
97.	MAPK	Mitogen-Activated Protein Kinase
98.	MDA	Malondialdehyde
99.	MDS	Molecular Dynamic Simulation
100.	MET	Mesenchymal-Epithelial Transition Factor
101.	MHz	Megahertz
102.	mM	Millimolar
103.	mm³	Cubic Milli Meter
104.	MMP9	Matrix Metalloproteinase 9
105.	MoISA	Molecular Surface Area
106.	MPO	Myeloperoxidase
107.	MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
108.	NaCO₂	Sodium Carbonate
109.	NADPH	Nicotinamide Adenine Dinucleotide Phosphate
110.	NaNO₂	Sodium Nitrite
111.	NaOH	Sodium Hydroxide
112.	NCCS	National Centre for Cell Sciences
113.	NIST	National Institute of Standards and Technology
114.	nm	Nanometre
115.	NMA	N-methyl -Amino Acid
116.	NMR	Nuclear Magnetic Resonance
117.	NPT	Isothermal-Isobaric Ensemble
118.	NRotB	Number of Rotatable Bonds
119.	ns	Nanoseconds

120.	O-H	Hydroxyl Group
121.	O[•]₂	Superoxide Radical
122.	O₂⁻	Superoxide Anion
123.	OH[•]	Hydroxyl Radical
124.	OPLS-3	Optimized Potentials for Liquid Simulations 3
125.	PBS	Phosphate Buffer Solution
126.	PDB	Protein Data Bank
127.	PI	Propidium Iodine
128.	PI3K/AKT	Phosphoinositide 3-Kinase (PI3K) / Protein Kinase B (AKT)
129.	PPI	Protein-Protein Interaction
130.	ppm	Part Per Million
131.	PubChem	Public Database of Chemical Molecules and their Biological Activities
132.	PUFAs	Polyunsaturated Fatty Acids
133.	R[•]	Superoxide Dismutase Activity
134.	RBCs	Red Blood Cells
135.	RCSB	Research Collaboratory for Structural Bioinformatics
136.	RE/g	Rutin Equivalents Per Gram
137.	RFA	<i>Rhododendron</i> Flower Acetone
138.	RFD	<i>Rhododendron</i> Flower DMSO
139.	RFE	<i>Rhododendron</i> Flower Ethanol
140.	RFM	<i>Rhododendron</i> Flower Methanol
141.	rGyr	Radius of Gyration
142.	RLA	<i>Rhododendron</i> Leaf Acetone
143.	RLD	<i>Rhododendron</i> Leaf DMSO
144.	RLE	<i>Rhododendron</i> Leaf Ethanol
145.	RLM	<i>Rhododendron</i> Leaf Methanol
146.	RMSD	Root Mean Square Deviation
147.	RMSF	Root Mean Square Fluctuations
148.	ROS	Reactive Oxygen Species
149.	rpm	Revolutions Per Minute
150.	SAR	Structure-Activity Relationship

151.	SASA	Solvent Accessible Surface Area
152.	SER	Serine
153.	SGOT	Serum Glutamic Oxaloacetic Transaminase
154.	SGPT	Serum Glutamic Pyruvic Transaminase
155.	SOD	Superoxide Dismutase
156.	STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
157.	T1/T2	Relaxation Parameters
158.	TAC	Total Alkaloid Content
159.	TAE/g	Tannic Acid Equivalent Per Gram
160.	TBA	Thiobarbituric Acid
161.	TBARS	Thiobarbituric Acid Reactive Substances
162.	TCA	Trichloroacetic Acid
163.	TFC	Total Flavonoid Content
164.	TIP4P	Transferable Intermolecular Potential with 4 Points
165.	TPC	Total Phenolic Content
166.	TPSA	Topological Polar Surface Area
167.	TPTZ	2,4,6-tris(2-pyridyl)-s-triazine
168.	TSP	3-(trimethylsilyl)-1-propanesulfonic Acid
169.	TWBC	Total White Blood Cells
170.	U/mg	Units Per Milligram
171.	UV	Ultraviolet
172.	v/v	Volume/Volume
173.	VEGF	Vascular Endothelial Growth Factor
174.	VLDL	Very Low Density Lipoprotein
175.	WBCs	White Blood Cells
176.	δ	Chemical Shift
177.	μL	Microliter
178.	μm	Micrometre