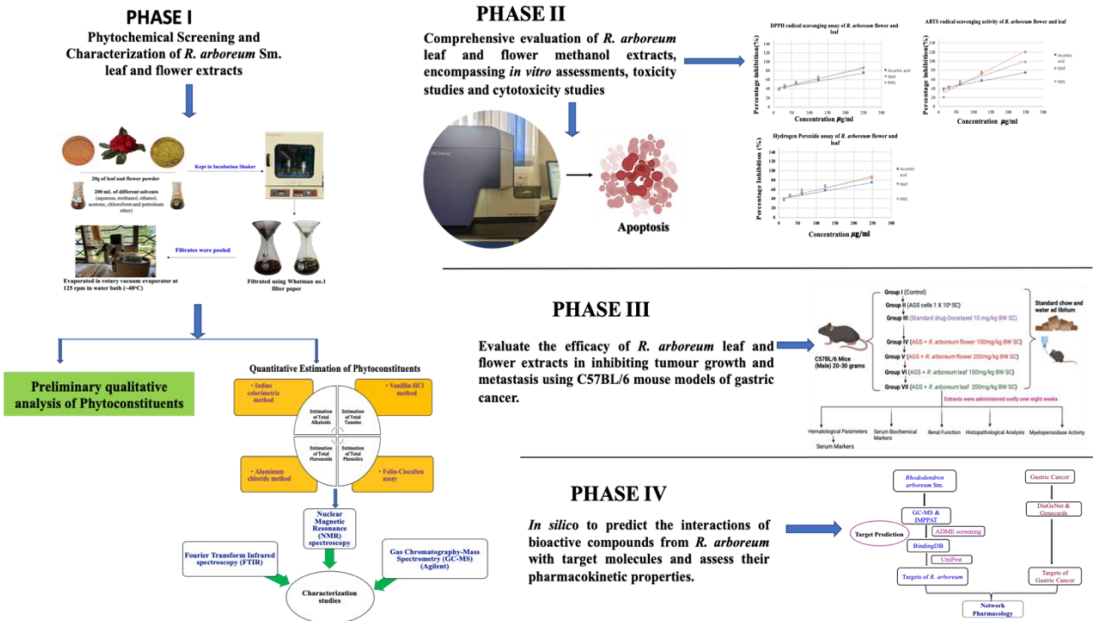
5. Summary and Conclusion



Rhododendron arboreum Sm. a plant known for its significant traditional medicinal uses, has garnered attention for its phytochemical properties and the potential therapeutic value of its extract, especially in anti-cancer activities. This study focused on the qualitative and quantitative phytochemical analyses of *R. arboreum* leaves and flowers, revealing the presence of numerous bioactive compounds in different solvent extracts. The qualitative analyses showed that there was the presence of alkaloids, flavonoids, phytosterols, phenols, tannins, diterpenes, proteins, amino acids, carbohydrates, saponins, quinones, coumarins, acidic compounds, catechins, volatile oils, resins, starch, carotenoids, oxalates and vitamin C. The quantitative analysis revealed that flavonoids present in high concentrations, with total amounts of 117.1 RE/g in the leaves as well as 139.6 RE/g in the flowers. Additionally other important secondary metabolites like alkaloids, tannins and phenols were found in significant quantities, which supports the medicinal properties of the plant.

The study also contributes to the investigation of the phytochemical profile of *R. arboreum* employing standardized protocols for extraction and analysis to ensure reproducibility and reliability. Information on the correlation between the identified and quantified phytochemicals and their biological effects could help bridge existing knowledge gaps and provide insights into how these compounds may contribute to the overall bioactivities of *R. arboreum*

extracts. The free radical scavenging activity was evaluated using various assays, including DPPH, ABTS, H_2O_2, LPO and FRAP. The results showed that the leaf extract possess strong antioxidant activity than the flower extract, except the FRAP assay shows a higher antioxidant activity in flower when compared to leaf extract. These findings suggest a strong correlation between phytochemical content and antioxidant activity, likely attributing the observed antioxidant effects to the high concentration of flavonoids.

The brine shrimp lethality assay results indicated that the methanolic extracts of *R. arboreum* flower (RFM) and leaf (RLM) exhibited relatively low toxicity compared to the control. Mortality rates for brine shrimp exposed to RFM and RLM were minimal across various concentrations, with only a few shrimps showing mortality even at highest concentration (1500 μg/ mL). The highest mortality rate recorded was 13% for RLM and 10% RFM.

The cytotoxic effects of RFM and RLM were further evaluated using the MTT assay on AGS gastric cancer cell. RLM exhibited the highest cytotoxicity, reducing cell viability from 43.5% to 66.2% at varying concentration, while RFM showed an inhibition ranging from 46.7% to 59.9% the IC_{50} values for RFM and RLM were determined to be 165.6 μl/mL and 270.7

μl/mL respectively, confirming their potentials as anti-cancer agents. Further investigation using flow-cytometry with Annexin V-FITC/PI staining showed that apoptosis was more pronounced in flower-treated cell then in leaves-treated ones, reinforcing the presence of diverse cytotoxic agents in RFM and RLM extracts. These findings helps to understand the potential applications of *Rhododendron* extracts in cancer treatments and highlights the effectiveness of plant derived compounds in inhibiting cancer cell growth.

The investigation of anti-cancer efficacy of methanol extracts from the leaves and flowers of *R. arboreum* using a C57BL/6 mice model of gastric cancer. Following acclimatization, mice were inoculated with AGS gastric cancer cells and tumours were detected on 18th day of post-inoculation. During the treatment period, BW changes were monitored closely, revealing significant variations among the different treatment groups. Specifically, the untreated group experienced a significant weight declined, indicative of tumour burden and inflammations. In contrast, mice treated with the *R. arboreum* extracts showed more stable BW, suggesting a potentials protective effects of

the extracts against the impact of cancer. Additionally, organ weights were assessed, particularly focusing on the stomach and liver. The stomach weights of treated mice were significantly responding to the treatment than those of the untreated group, possibly indicating reduced tumour growth or inflammation, while liver weights reflected potential metabolic changes due the cancer and treatments.

Haematological evaluations were conducted to understand the effects of *R. arboreum* extract on various parameters. The results showed an increase in RBCs counts, particularly in certain treatment groups, suggesting improved erythropoiesis or a reduction in tumour-induced anaemia. Total haemoglobin levels in treated groups were significantly higher compared to other groups indicating improved haemoglobin synthesis or oxygen-carrying capacity. However, WBCs counts remained relatively stable across all groups except for the standard group, indicating the absence of a significant inflammatory response or infection affecting the haematological profiles during the study.

Biochemical analyses revealed important findings regarding liver function and metabolic profile. The treatment with *R. arboreum* extracts and the chemotherapeutic drug docetaxel resulted in decreased total protein levels in the treated mice, which may reflect alteration in protein synthesis or an increase in protein catabolism due to stress from the tumour or treatment. Additionally, elevated liver enzymes levels (such as ALT and AST) were observed in some treated groups, indicating potential liver injury or disruption in metabolic processes as a consequences of the treatments.

In *In-silico* studies, after excluding compounds without known targets, 10 key chemical compounds were identified from the leaves (5) and flowers (5) of *R. arboreum* using GC-MS data and the IMPPAT database. The targets for these compounds were obtained from the BindingDB database. These targets were mapped to human genes by inputting them into the UniProt database, with the species option set to *Homo sapiens*. Additionally, 955 gastric cancer-related targets were identified from the GeneCards database. By comparing the predicted candidate targets of *R. arboreum* with these gastric cancer-related targets, 113 unique targets of *R. arboreum* against gastric cancer were identified. Among them 22 genes were found to be common between the disease and the plant constituents and these were selected for

further Investigation.

The ShinyGO 0.80 identified multiple mechanisms through which *R. arboreum* may act against gastric cancer. This analysis focused on 22 overlapping targets, with a confidence score threshold set at > 0.7. The resulting PPI network comprised 22 nodes and 101 edges, with an average node degree of 9.18 and an average local clustering coefficient of 0.686. The PPI network was analyzed for the top 10 compounds-disease-targets based on betweenness, closeness and degree centrality metrics.

Cytoscape 3.6.1 software was used to visualize the score targets obtained from the STRING database analysis, highlighting the interactions between *R. arboreum* and gastric cancer. The network consisted of 10 nodes and 35 edges, with an average node degree of 7 and an average local clustering coefficient of 0.81. To enhance the clarity and interpretability, the node attributes were customized with adjustments made to both the colour and size of the nodes. This approach effectively depicted the drug-target interactions, highlighting key nodes that represent significant compounds or targets.

The analysis revealed 20 enriched Gene Ontology (GO) terms, each with a statistical significance of $p < 0.05$, indicating a strong association with the biological processes related to gastric cancer. The pathway enrichment analysis also revealed that key genes like EGFR, GSK3B, IGF1R, KDR, MET, ESR1, ESR2 and MMP9 which are significantly involved in multiple cancer-related pathways. For instance, the EGFR tyrosine kinase inhibitor resistance pathway has a fold enrichment of 144.82 and an FDR of 5.42E-09, indicating a strong over-representation. Similarly, the endocrine resistance pathway shows a fold enrichment of 120.43 with an FDR of 9.26E-09. Other significant pathways include gastric cancer with a fold enrichment of 46.38 and an FDR of 0.00018287 and the broadly encompassing pathways in cancer with a fold enrichment of 30.22 and an FDR of 9.81E-09. KEGG pathway enrichment analysis identified the top 20 pathways including EGFR Tyrosine Kinase Inhibitor Resistance. Which highlights the most pertinent regulatory targets, including ESR1, GSK3B, ESR2, CYP19A1, MET, HMGCR, KDR, IGF1R, MMP9 and EGFR.

The Epidermal Growth Factor receptors activates several key intracellular pathways, including the PI3K/AKT pathway, which promotes cell survival and

growth. The MAPK pathway, which regulates cell proliferation, differentiation and migration. These pathways collectively drives essential cellular processes such as growth, proliferation, differentiation, migration and survival. However in many tumours, receptors from the EGF family become dysregulated, leading to excessive signaling and uncontrolled cell growth.

A well-known example is the receptor HER2, which is overexpressed in many cancer cases, making it a significant targets for cancer therapies. The molecular study assessed the binding affinities of 5 compounds such as beta-sitosterol, quercetin, gamma-sitosterol, alpha- amyrin and 2,3,4,6-tetramethoxystyrene against 5 key target proteins. Among them quercetin emerged as the most promising candidate, exhibiting the highest biding affinities among all tested proteins. Significant binding energies included -8.68 kcal/mol for one target protein and lower values for others, indicating a strong interaction of quercetin with critical proteins such as ESR1, KDR, MMP9 and EGFR, identified through KEGG analysis. This highlights quercetin's potential for further exploration due to its interaction with these proteins, crucial for gastric cancer progression.

The molecular docking analysis of docetaxel with key proteins illustrated its binding affinities, comparing its efficacy to the natural compounds from *R. arboreum* extracts. Despite docetaxel's side effects, it remains a fundamental in gastric cancer treatment, and ongoing clinical trials aim to optimize its use.

Molecular Dynamics simulations (MDs) were conducted over approximately 100 ns to evaluate the stability of protein-ligand complexes under various physiological conditions, including changes in pressure, temperature and the presence of water molecules. The Root Mean Square Deviation (RMSD) graph provides insights into the stability and conformational changes of both the protein and the ligand throughout the formation of their complex. Typically, RMSD values indicate the binding energy and interactions between the protein and ligand. In the complex 1B3D, the first fluctuation occurred at 13.10 ns, with the protein RMSD at 2.47 Å and the ligand RMSD at 1.53 Å. For the complex 6S9B, an initial fluctuation was observed at 4.00 ns, with higher RMSD values for both the protein (2.77 Å) and the ligand (4.14 Å). This complex later stabilized at 14.10 ns with increased RMSD values (protein at 3.68 Å, ligand at

6.16 Å). The 6GQ0 complex exhibited initial fluctuation at 4.30 ns, significantly showing a high ligand RMSD of 13.32 Å but stabilized by 40.50 ns, reducing the ligand RMSD to 5.54 Å while the protein RMSD remained relatively stable at 2.05 Å. For the complex 1ERR, fluctuations were first noted at 3.40 ns, with RMSD values of 1.81 Å for the protein and 6.40 Å for the ligand, followed by stabilization at 9.90 ns (protein RMSD: 2.21 Å, ligand RMSD: 6.67 Å) and a subsequent fluctuation at 63.80 ns, where the protein RMSD increased to 3.23 Å and the ligand RMSD decreased to 4.49 Å. These fluctuations may be attributed to changes in the ligand's conformation.

Root Mean Square Fluctuations (RMSF) were analyzed to assess the transient structural changes in both the protein and ligand throughout the simulation. RMSF provides valuable insights into the flexibility of the protein structure during interactions. In one dataset, the C-alpha atoms of Chain A exhibited varying degrees of flexibility, with measurements such as 3.92 Å at residue 137 (GLU) and 1.57 Å at residue 201 (HIS). Chain B showed fluctuations from 2.77 Å at residue 234 (LEU) to 4.19 v at residue 194 (ASN). Another dataset revelaed chain A minimal flexibility, including 0.39 Å at residue 1046 (ASP) and varibilty upto 1.86 Å at residue 459 (NMA). Additionally chain A in a third dataset displayed values ranging from 0.56 Å at residue 445 (ASN) to 3.15 Å at residue 470 (GLU), while chain B presented values such as 1.35 Å at residue 411 (ASP) and the lowest at 0.79 Å for residue 430 (ALA). This comprehensive analysis highlights the dynamic behaviour and variability in the protein’s structural flexibility throughout the simulation.

The properties of the quercetin were assessed, including the radius of gyration (rGyr), RMSD, intramolecular hydrogen bonds (intraHB), molecular surface area (MolSA), solvent accessible surface area (SASA) and polar surface area (PSA). These properties are within optimal ranges and consistent with observations for other substrates and co-crystal ligands, indicating that quercetin is well-suited for drug discovery. During the simulations, Quercetin's molecular surface area (MolSA) and polar surface area (PSA) remained stable up to 100 ns, while other parameters exhibited fluctuations. For the 6S9B protein, only RMSD and SASA showed fluctuations at 10 ns but stabilized for the rest of the simulation. In the case of the 6GQ0 protein, RMSD and SASA

fluctuated at 31 ns, stabilized by 44 ns, then fluctuated again at 88 ns before stabilizing until the end of the simulation. For the 1ERR protein, RMSD and SASA fluctuated at 0.80 ns, stabilized by 6.90 ns, then fluctuated again at 87 ns before achieving stability at 91 ns. All other parameters remained stable throughout the entire simulation.

In conclusion, this study highlights the potential of *R. arboreum* as a rich source of bioactive compounds with significant antioxidant and anticancer properties. Through comprehensive qualitative and quantitative phytochemical analyses, it was confirmed that the leaves and flowers of this plant contain a diverse array of compounds, particularly flavonoids, which were found in high concentrations and are likely key contributors to its therapeutic effects. The antioxidant activity observed in various assays indicates that both the leaf and flower extracts possess substantial free radical scavenging abilities, with leaf extracts exhibiting superior activity. Cytotoxicity assessments using the MTT assay and Brine Shrimp Lethality Assay revealed promising anticancer effects, particularly with the leaf extracts, Which significantly inhibited AGS gastric cancer cell viability. Furthermore, experiments using a C57BL/6 mouse model provided additional evidence of the extract's protective effects against cancer-related weight loss and organ damage, indicating its potential role in managing gastric cancer. Computational study identified key molecular targets and pathways associated with the anticancer mechanisms of *R. arboreum*, paving the way for future pharmacological research. Overall, the findings contribute to the growing body of evidence supporting the therapeutic potential of *R. arboreum* in both traditional and modern medicine, emphasizing its importance as a valuable resource for the development of therapeutic agents against cancer.

This study highlights the antioxidant and anticancer potential of *R. arboreum* leaf and flower extracts, supported by both *in vitro* and *in vivo* findings, including significant tumour suppression and the identification of key bioactive compounds such as quercetin. To further strengthen these outcomes, future research will focus on cellular and molecular-level studies, specifically investigating the underlying mechanisms responsible for these effects. Emphasis will also be placed on exploring the role of *R. arboreum* in gastric ulcer protection and management. These directions aim to enhance the

scientific basis of the current findings and improve translational relevance. Overall, the results offer valuable insights for researchers, clinicians and professionals in cancer therapeutics and natural product-based drug development, aligning the study's significance with practical applications in targeted plant-based treatment strategies.