



**BUSITEMA
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FACULTY OF SCIENCE AND EDUCATION

DEPARTMENT OF CHEMISTRY

Integrated Network Pharmacology, ADME Profiling and
Molecular Docking of *Persea americana* Seed Metabolites for Potential Anti-
Prostate Cancer Activity

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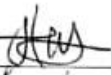
**A RESEARCH PROJECT REPORT SUBMITTED TO THE DEPARTMENT
OF CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE AWARD OF A BACHELOR'S DEGREE IN SCIENCE AND EDUCATION OF
BUSITEMA UNIVERSITY**

[MAY, 2026]

DECLARATION

I, Eyoku Emmanuel, declare that this research project is my original work and has not been submitted to any other institution for academic credit. The thesis entitled “Investigating the Therapeutic potential of Avocado seed extract in treating Prostate Cancer”.

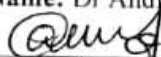
as partial fulfilment of the requirement for the award of the degree of Bachelors in science education and submitted to the chemistry department, Busitema University, is an original piece of research work under the guidance of Dr. Andima Moses.

Signature: 
Date: 25/08/2026

APPROVAL

This research project has been submitted with the approval of the supervisor.

Supervisor's Name: Dr Andina Moses

Signature: 

Date: 25/5/2026

DEDICATION

I humbly dedicate this piece of work to my senior supervisor, **Dr. Andima Moses**, loving brother Eroju Moses and my father Ebuku Joseph for their endless guidance and support, to my relatives for their financial support, and to my friends for their inspiring pieces of advice.

Above all, to the almighty **God** who always gives me strength, knowledge and wisdom in everything I do.

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ABSTRACT

This study investigates the potential anti-prostate cancer activity of bioactive metabolites derived from *Persea americana* (avocado) seeds using an integrated computational approach combining network pharmacology, ADME (absorption, distribution, metabolism, and excretion) profiling, and molecular docking. Initially, metabolites were identified through literature and phytochemical databases, followed by screening based on drug-likeness and ADME properties to ensure appropriate pharmacokinetic profiles. The selected compounds were subjected to target prediction using network pharmacology tools to construct a compound-target-disease interaction network, revealing key protein targets implicated in prostate cancer pathways. Subsequent pathway enrichment analysis identified critical signalling pathways involved in cancer progression, such as PI3K-Akt and androgen receptor signalling. Molecular docking simulations were performed against major prostate cancer-related protein targets to assess binding affinity and interaction patterns. The top-ranked metabolites demonstrated strong binding energies and key residue interactions indicative of potential inhibitory activity. Overall, this integrative workflow combined phytochemical profiling, computational pharmacokinetics, network analysis, and molecular docking to prioritize *Persea americana* seed metabolites as promising candidates for further experimental validation in prostate cancer therapeutics.

Workflow Breakdown

1. Metabolite Identification

- Collect and curate *Persea americana* seed metabolites from scientific literature and phytochemical databases.

2. ADME Profiling

- Evaluate absorption, distribution, metabolism, and excretion properties using computational tools.
- Screen metabolites for drug-likeness based on standard criteria (e.g., Lipinski's Rule of Five).
- Eliminate compounds with poor pharmacokinetic profiles to focus on candidates with high oral bioavailability and low toxicity.

3. Target Prediction & Network Construction

- Use computational target prediction tools to identify potential molecular targets of the selected metabolites.

- Construct a compound-target-disease interaction network visualizing metabolite connections to prostate cancer-related proteins.
- Analyse network topological metrics (e.g., degree centrality, betweenness) to identify hub targets highly relevant to prostate cancer biology.

4. Pathway and Functional Enrichment Analysis

- Perform pathway enrichment analysis on the predicted targets to uncover involved biological pathways.
- Highlight key cancer-related pathways such as PI3K-Akt, androgen receptor signalling, and apoptosis-regulating mechanisms.

5. Molecular Docking Simulation

- Dock selected metabolites onto key prostate cancer protein targets to estimate binding affinities.
- Analyse interaction patterns, such as hydrogen bonds and hydrophobic contacts, with critical active site residues.
- Rank compounds based on docking scores to prioritize the strongest binders.

Major Computational Outputs

- **ADME Results:**
 - Number of metabolites passing drug-likeness filters (e.g., % complying with Lipinski's rules).
 - Predicted oral bioavailability and clearance rates enabling selection of pharmacokinetically viable compounds.
- **Network Analysis:**
 - Identification of top hub targets, e.g., AR (androgen receptor), AKT1, or other critical proteins implicated in prostate cancer.
 - Visual network maps showing metabolite-target and target-disease interactions.
 - Key network metrics quantifying target centrality and relevance.
- **Pathway Enrichment:**
 - List of statistically significant pathways with associated genes targeted by metabolites.
 - Insight into mechanisms like cell proliferation, apoptosis, and hormone signalling affected by the metabolites.

- **Molecular Docking:**
 - Binding energy values (e.g., kcal/mol) for each metabolite against target proteins.
 - Description of interaction types and residues involved in binding, identifying potential inhibitory action.
 - Ranking of metabolites by docking affinity to highlight the most promising candidates.

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LIST OF ABBREVIATIONS

Abbreviation Full Form

ADME Absorption, Distribution, Metabolism, Excretion

FDR False Discovery Rate

GO Gene Ontology

HBA Hydrogen Bond Acceptor

HBD Hydrogen Bond Donor

KEGG Kyoto Encyclopedia of Genes and Genomes

LC-MS Liquid Chromatography-Mass Spectrometry

MCODE Molecular Complex Detection

MW Molecular Weight

PCa Prostate Cancer

PDX Patient-Derived Xenograft

PPI Protein-Protein Interaction

PSA Prostate-Specific Antigen

QSAR Quantitative Structure-Activity Relationship

SDF Structure Data File

SMILES Simplified Molecular-Input Line-Entry System

TPSA Topological Polar Surface Area

CHAPTER 1: INTRODUCTION

1.1 Background of the study

1.1 The Global and Regional Burden of Prostate Cancer

Prostate cancer refers to a condition in which abnormal cells grow uncontrollably in the prostate gland. Globally prostate cancer is the second common cancer diagnosed in men with an estimation of about 1.4 million new cases and 375000 deaths reported in 2022.

In Uganda, prostate cancer in men is a major health issue and a most common cancer in men, with about 37-42 per 100000 men and an elevated increase in the future cases. The incidence is steadily increasing with an estimated yearly increase of 5.2%. Men of 50 years and above are usually at a risk of suffering from prostate cancer

Prostate cancer presents a significant and growing global health challenge, characterized by high mortality rates and a steadily increasing incidence. This burden is particularly pronounced in Africa and within Uganda, where limited healthcare resources and late-stage diagnoses contribute to disproportionately poor outcomes. The escalating prevalence underscores the urgent need for novel, effective, and accessible therapeutic strategies.

1.2 The Molecular Complexity of Prostate Cancer

The pathogenesis of prostate cancer is driven by a highly intricate network of molecular alterations. Key signaling cascades, including the androgen receptor (AR) axis, the PI3K/AKT pathway, and regulators of the cell cycle and apoptosis, are frequently dysregulated. Furthermore, mutations in critical tumor suppressor genes such as TP53 and PTEN are common, contributing to disease progression and therapeutic resistance. This inherent molecular heterogeneity necessitates a multi-targeted approach to treatment.

1.3 Current Treatment Modalities

The clinical management of prostate cancer encompasses a spectrum of interventions. These range from active surveillance for indolent disease to definitive local therapies like prostatectomy and radiotherapy. For advanced or aggressive cases, systemic treatments include androgen-deprivation therapy (ADT), conventional chemotherapy, and newer targeted agents. While these modalities offer benefits, their efficacy is often limited.

1.4 Significant Limitations of Existing Therapies

Current treatment strategies are frequently hampered by the development of resistance, disease recurrence, and severe adverse effects, significantly impacting patients' quality of life. A particularly formidable challenge is the emergence of metastatic castration-resistant prostate cancer (mCRPC), a lethal stage where tumors continue to progress despite androgen depletion. These limitations highlight the critical need for safer and more efficacious therapeutic options.

1.5 Natural Products in Anticancer Drug Discovery

Historically, natural products have been a prolific source of anticancer agents, offering immense chemical diversity and the potential for multi-target interactions. Many successful plant-derived drugs are in clinical use today, underscoring the value of exploring botanicals. Their inherent complexity allows them to modulate multiple disease-related pathways simultaneously, making them ideal candidates for combating complex diseases like cancer.

1.6 *Persea americana* Seeds as an Underutilized Resource

The seeds of the avocado (*Persea americana*) represent a promising, yet underutilized, biomass with a rich phytochemical profile. Preliminary studies have reported a variety of biological activities from avocado seed extracts, including antioxidant, antimicrobial, and anticancer properties. However, a comprehensive understanding of how specific seed metabolites interact with prostate cancer targets and pathways remains incomplete.

1.7 The Value of Computational Investigation

Modern computational methods, such as network pharmacology, ADME (Absorption, Distribution, Metabolism, and Excretion) prediction, and molecular docking, offer powerful tools to accelerate drug discovery. Network pharmacology facilitates the understanding of drug-target-disease interactions on a systems level, while ADME predictions help assess the drug-likeness of compounds early in the process. Molecular docking provides atomistic insights into binding affinities and modes.

1.8 Knowledge Gap

Despite the documented anticancer potential of *Persea americana* seed extracts, there exists a critical knowledge gap regarding the specific bioactive metabolites responsible for their activity against prostate cancer. Specifically, it remains unclear which individual compounds interact with which prostate cancer-related molecular targets and pathways. Furthermore, the pharmacokinetic properties and multi-target mechanistic interactions of these metabolites have not been systematically characterized using computational approaches. This lack of detailed mechanistic understanding hinders the rational prioritization of compounds for downstream experimental validation and drug development.

1.9 Study Rationale

To address this gap, the present study proposes an integrated computational investigation. By combining network pharmacology, ADME profiling, and molecular docking, this research aims to systematically predict and characterize the interactions between *Persea americana* seed metabolites and key prostate cancer targets. This integrated workflow will generate testable mechanistic hypotheses, identify the most promising lead compounds with favorable drug-like properties, and provide a molecular-level rationale for the observed anticancer effects. Ultimately, this study seeks to expedite the discovery of novel, multi-targeted therapeutic candidates from a readily available natural source, offering a cost-effective and scientifically grounded foundation for future experimental and clinical research in the context of prostate cancer, particularly relevant for resource-limited settings like Uganda.

Treatment of prostate cancer depends on the stage, slowly growing prostate cancers require monitoring, and those that are aggressive require radiation surgery, hormone therapy, chemotherapy or any other treatments.

The current prostate cancer treatment has a range of side effects on its patients hence impacting on the patients' quality of life, such effects include urinary incontinence, erectile dysfunction, and sexual dysfunction and bowel problems and are defendant on the treatment received.

Treatments such as surgery (prostatectomy) can lead to urinary leakage, radiation therapy may cause urinary irritation and bowel dysfunction. Hormone therapy is characterized by a wide-ranging impact leading to hot flashes, loss of libido, osteoporosis and elevated cardiovascular risk.

This has led researchers to seek safer and more affordable alternatives, especially from natural plant sources. The avocado (*Persea americana*) seed, often discarded as waste, has recently gained attention for its rich phytochemical composition. Studies have shown that extracts from avocado seeds contain compounds such as flavonoids, phenolics, and saponins that exhibit anticancer and antioxidant activities. Therefore, investigating the potential role of avocado seed extract in treating prostate cancer is both timely and relevant.

Traditionally and locally, prostate cancer can be treated using herbal remedies, plant extracts and natural diets that help reduce inflammation, boost immunity and slow cancer growth.

Seed extracts such as the edible parts of avocado were found to be more effective in treatment than the edible extract. The sterol extract was even more effective.

In a dose effective manner, both extracts exert anti-inflammatory and anti-cancer effects.

1.2 Problem statement.

Prostate cancer remains a major cause of cancer morbidity and mortality among men, and treatment of advanced disease is complicated by therapeutic resistance, recurrence and treatment-associated adverse effects. Natural products provide structurally diverse compounds that may act on multiple molecular targets involved in cancer progression. *Persea americana* seeds contain phenolics, flavonoids, procyanidins, fatty-acid derivatives and other bioactive constituents with reported antioxidant and anticancer properties. Although some avocado seed preparations have demonstrated activity in

prostate cancer models, the molecular targets and signaling pathways through which individual seed-derived compounds may exert these effects remain incompletely characterized. Furthermore, the existing literature reports conflicting evidence regarding preventive versus therapeutic effects. An integrated computational approach is therefore required to prioritize candidate compounds, identify prostate cancer-associated molecular targets and pathways, and evaluate the plausibility of selected compound–target interactions for subsequent experimental validation.

1.3 Objective of the study

1.3.1 General objective

a) To investigate the potential molecular mechanisms of *Persea americana* seed-derived compounds against prostate cancer using integrated network pharmacology and molecular docking approaches.

1.3.2 specific objectives

- 1 To compile reported bioactive compounds from *P. americana* seeds and evaluate their drug-likeness and predicted pharmacokinetic properties.
- 2 To predict molecular targets of selected *P. americana* seed compounds and identify their intersections with prostate cancer-associated genes.
- 3 To characterize the biological functions, signaling pathways and hub proteins associated with the intersecting targets using PPI and enrichment analyses.
- 4 To evaluate the predicted binding interactions between prioritized seed compounds and selected prostate cancer-associated protein targets using molecular docking.

significance of the study

This research will contribute to the growing body of knowledge on plant-based cancer therapies. It will highlight the medicinal potential of avocado seeds, which are commonly regarded as waste, thereby promoting their value-added utilization. The findings may provide a foundation for developing affordable, accessible, and natural alternatives for prostate cancer treatment, especially in resource-limited regions. Additionally, the study could inspire further research into other underexplored plant parts with therapeutic potential.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of the Chapter

2.1 Prostate Cancer: A Global Health Burden

Prostate cancer remains a significant global health challenge, representing one of the most frequently diagnosed malignancies in men. Its epidemiological significance is underscored by its high incidence rates and its position as a leading cause of cancer-related mortality. The clinical importance of this disease lies not only in its prevalence but also in its heterogeneous nature, ranging from indolent, localized tumors to aggressive, metastatic forms that are ultimately fatal. This section establishes the context for the urgent need for novel therapeutic strategies.

2.2 Molecular Pathogenesis of Prostate Cancer

The development and progression of prostate cancer are driven by a complex network of aberrant molecular signaling pathways. Central to this pathogenesis is the Androgen Receptor (AR) signaling axis, which is critical for the growth and survival of prostate epithelial cells. Dysregulation of this pathway is a hallmark of the disease. Beyond AR signaling, several other key pathways are frequently implicated, including the PI3K/AKT/mTOR cascade, which promotes cell proliferation and survival; the tumor suppressor p53, which is often mutated or inactivated; and the STAT3 transcription factor, which contributes to oncogenesis. Furthermore, the disease progression is fueled by the evasion of apoptosis (programmed cell death) and the induction of angiogenesis, the formation of new blood vessels to support tumor growth.

2.3 Current Therapeutic Landscape and the Challenge of Resistance

The standard of care for prostate cancer, particularly in advanced stages, has evolved but is

frequently hindered by the development of therapeutic resistance. This challenge is most pronounced in metastatic castration-resistant prostate cancer (mCRPC), a lethal form of the disease that progresses despite androgen deprivation therapy. Current treatment modalities, including AR-targeted agents and chemotherapies, are often initially effective but invariably face the obstacle of acquired resistance, which drives disease recurrence and progression. This underscores a critical clinical need for the discovery of new, more durable therapeutic options.

2.4 The Role of Natural Products in Prostate Cancer Drug Discovery

Given the limitations of current therapies, there has been a resurgent interest in exploring natural products as a source for new anticancer agents. This section reviews the existing evidence supporting the use of natural compounds in prostate cancer management, highlighting the opportunities they present due to their structural diversity and multi-targeted mechanisms of action. However, this field is also constrained by significant limitations, including issues with bioavailability, batch-to-batch variability, and a general lack of rigorous clinical validation, which must be addressed for their successful translation into therapeutics.

2.5 *Persea americana* Seed: An Underutilized Botanical Resource

The avocado (*Persea americana*) is a fruit of considerable botanical and economic interest. While the pulp is widely consumed, the seed, which constitutes a significant portion of the fruit, is largely discarded as agricultural waste. This seed represents an underutilized and sustainable source of bioactive phytochemicals. This review examines the potential of the avocado seed, moving beyond its traditional uses to explore its value as a source of compounds with health-promoting and potential anticancer properties.

2.6 Phytochemical Profile of Avocado Seeds

The pharmacological potential of avocado seeds is attributed to their rich and diverse phytochemical composition. They are particularly noted for their high concentration of polyphenols, including procyanidins and flavonoids. Additionally, the seed contains other bioactive compounds such as acetogenins and various fatty acids. These constituents are

known for their antioxidant and anti-inflammatory activities, which form a logical basis for their investigation in cancer research. This section details the spectrum of these compounds and their relevance to biological activity.

2.7 Reported Anticancer Activity: A Comparative Analysis

The anticancer potential of avocado seed extracts and their isolated compounds have been investigated across various cancer types. The reported evidence indicates promising cytotoxic effects. However, it is crucial to delineate this evidence by cancer type. This section specifically scrutinizes findings from prostate cancer models to establish a focused context, while concurrently reviewing evidence from other cancers (e.g., breast, colon) to highlight potential broad-spectrum activity and identify mechanistic patterns or differences.

2.8 Network Pharmacology: A Systems-Level Approach to Drug Discovery

Traditional, single-target approaches are often insufficient for understanding the complex action of natural product extracts. Network pharmacology provides a holistic framework to address this complexity. This systems-level approach aims to elucidate the "compound–target–pathway" network, mapping the multiple interactions of active compounds with their protein targets and the downstream biological pathways they affect. While this strategy offers a powerful method for predicting mechanisms of action and identifying potential polypharmacological effects, it is also subject to limitations, including the reliance on bioinformatics predictions and the need for experimental validation of its findings.

2.9 ADME and Drug-Likeness Prediction: From InSilico to Reality

Predicting the Absorption, Distribution, Metabolism, and Excretion (ADME) properties and overall drug-likeness of potential therapeutic candidates is a crucial step in the drug discovery process. Computational tools are now routinely used to assess these pharmacokinetic parameters early on, helping to identify compounds with favorable bioavailability and safety profiles. This section reviews the role of these predictions in screening natural product compounds, while also acknowledging their limitations, such as the difficulty in accurately simulating complex biological environments and the potential for false positives.

2.10 Molecular Docking: Principles and Predictive Power

Molecular docking is a computational technique used to predict the preferred binding orientation and affinity of a ligand (e.g., a phytochemical) to its target protein receptor. This method is pivotal for understanding potential mechanisms of action at the molecular level and for prioritizing compounds for experimental testing. This review covers the fundamental principles of docking, including scoring functions, and emphasizes the critical role of validation. It also discusses the inherent limitations of the technique, such as its reliance on static protein structures and the need for complementary *in vitro* and *in vivo* evidence.

2.11 Research Gap and Study Rationale

Despite the growing body of evidence on the bioactivity of avocado seeds, significant knowledge gaps persist. Specifically, a systematic and integrated investigation into the specific molecular mechanisms by which avocado seed phytochemicals exert their effects against prostate cancer, using a combined *in silico* and *in vitro* approach, is lacking. This study is designed to directly address this gap by employing network pharmacology and molecular docking to predict the key active compounds and their targets, thereby providing a foundational basis for further experimental validation. This work aims to offer a more comprehensive and evidence-based rationale for the development of avocado seed-derived compounds as potential therapeutic agents for prostate cancer.

2.12 Conceptual framework

Building upon the preceding sections, this study is guided by a cohesive conceptual framework that integrates multiple *in silico* strategies to formulate a robust mechanistic hypothesis. This framework is not merely a sequence of computational steps but a synergistic model designed to bridge the gap between phytochemical discovery and biological validation.

2.12.1 Protein-Protein Interaction (PPI) Networks as a Systems-Level Map

At the core of this framework lies the construction and analysis of Protein-Protein Interaction (PPI) networks. Unlike isolated target-based approaches, PPI networks provide a systems-

level perspective by mapping the complex web of physical and functional associations among proteins. In the context of prostate cancer, this allows for the identification of hub proteins and key regulatory nodes that are central to disease pathogenesis. By overlaying the predicted targets of avocado seed phytochemicals onto these networks, it becomes possible to visualize how a single compound might subtly perturb an entire oncogenic network, rather than simply inhibiting a single protein. This holistic view is critical for understanding the polypharmacological nature of natural products and for predicting potential off-target effects or synergistic interactions.

2.12.2 Pathway Enrichment Analysis: Deciphering Biological Context

The identification of targets and their interactions is insufficient without understanding the biological pathways they govern. Therefore, a crucial component of this framework is pathway enrichment analysis, typically performed using databases such as KEGG (Kyoto Encyclopedia of Genes and Genomes) or Reactome. This step translates the list of potential targets into a functionally meaningful context by identifying the signaling cascades—such as AR signaling, PI3K/AKT/mTOR, and p53 pathways—that are most likely to be affected by the avocado seed compounds. This analysis serves to prioritize pathways that are already established as drivers of prostate cancer pathogenesis and progression (as discussed in Section 2.2), thereby providing a biologically plausible rationale for the observed or predicted anticancer activity.

2.12.3 Molecular Docking: From Pathway Perturbation to Atomic-Level Interactions

While PPI networks and pathway analysis offer a macroscopic view of biological influence, molecular docking provides the atomic-level resolution necessary to validate and refine mechanistic hypotheses. As outlined in Section 2.10, docking predicts the binding conformations and affinities between the active phytochemicals and their prioritized protein targets (e.g., AR, PI3K, or key PPI hub nodes). This step is essential for generating testable hypotheses about the precise molecular interactions—such as hydrogen bonds, hydrophobic contacts, and pi-stacking—that underlie the predicted pathway modulation. By anchoring the broad network predictions to specific structural interactions, docking grounds the computational findings in physical chemistry, increasing their credibility and guiding future experimental mutagenesis studies.

2.12.4 Formulating the Mechanistic Hypothesis

The integration of these three pillars—PPI network analysis, pathway enrichment, and molecular docking—culminates in the formulation of a central mechanistic hypothesis. This hypothesis posits that the anticancer activity of *Persea americana* seed phytochemicals is not attributable to a single, dominant compound-target interaction, but rather to a synergistic modulation of a cluster of interconnected oncogenic pathways. Specifically, it is hypothesized that these phytochemicals act as multi-targeted inhibitors, simultaneously perturbing AR signaling, the PI3K/AKT/mTOR survival axis, and apoptotic machinery by binding to key regulatory proteins identified through PPI networks. The docking results serve to propose the most probable binding modes, while the network and pathway analyses indicate the likely downstream biological consequences. This comprehensive framework thus provides a rational, evidence-based roadmap for the subsequent *in vitro* and *in vivo* validation of the avocado seed's therapeutic potential against prostate cancer.

This chapter provides a comprehensive review of the literature pertaining to the therapeutic potential of avocado (*Persea americana*) seed extract in the context of prostate cancer. The chapter is structured into four main sections. First, relevant theories and themes underpinning the investigation are discussed, including the phytochemical composition of avocado seeds and the mechanistic pathways by which their bioactive compounds may exert anticancer effects. Second, previous studies related to the topic are critically examined, with particular emphasis on *in vitro* and *in vivo* investigations. Third, the chapter identifies the research gaps that the present study seeks to address. Finally, chemical structures of key bioactive compounds are presented with accompanying explanations.

2.6 Summary of Literature Review

The literature reviewed demonstrates that avocado seeds contain a diverse array of bioactive phytochemicals with demonstrated antioxidant and anticancer activities. *In vitro* studies using LNCaP prostate cancer cells have established that avocado seed extract reduces cell viability, induces cell cycle arrest, promotes apoptosis, and inhibits NF- κ B signaling. *In vivo* studies, however, present a more nuanced picture: while avocado seed extract demonstrates significant chemo preventive effects in prostate cancer models, its therapeutic efficacy

appears limited. This discrepancy between in vitro cytotoxicity and in vivo therapeutic response represents a critical research gap.

The chapter has identified several key gaps: limited human data, contradictory evidence on therapeutic versus protective effects, incomplete mechanistic understanding, and lack of standardization protocols. These gaps inform the research questions and objectives of the present investigation, which aims to clarify the therapeutic potential and mechanisms of action of avocado seed extract in prostate cancer.

CHAPTER THREE: Materials and Methods

3.1 Study Design

This was a comprehensive in silico network-pharmacology study designed to systematically investigate the multi-target, multi-pathway mechanisms of *Persea americana* seed metabolites against prostate cancer. The study integrated ADME (Absorption, Distribution, Metabolism, and Excretion) profiling, target prediction, network analysis, and molecular docking to identify potential bioactive compounds and their molecular targets.

3.2 Collection of *P. americana* Seed Compounds

A systematic literature search was performed to compile a comprehensive list of phytoconstituents identified in *P. americana* seeds. The search was conducted using the following scientific databases: PubMed, Scopus, Google Scholar, and ScienceDirect, with the search terms "*Persea americana*," "avocado seed," "phytoconstituents," and "chemical composition." The inclusion criteria were: (i) studies reporting compounds isolated and characterized from the seed; (ii) articles published in English; and (iii) studies providing sufficient chemical identification data. Exclusion criteria were: (i) studies on other plant parts (e.g., fruit pulp, leaves); (ii) review articles (to avoid redundancy); and (iii) compounds without a defined chemical structure. This process initially retrieved 210 unique compounds. For each compound, the canonical SMILES (Simplified Molecular Input Line Entry System)

notation and PubChem Compound Identifier (CID) were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). This methodology ensures a transparent and reproducible strategy for compound selection.

3.3 ADME and Drug-Likeness Assessment

The drug-likeness of the 210 compounds was evaluated using the SwissADME web tool (<http://www.swissadme.ch/>; accessed on [Insert Date]). The assessment was based on species-independent physicochemical properties, including molecular weight, lipophilicity (LogP), hydrogen bond donors/acceptors, and molar refractivity. The primary filter was Lipinski's Rule of Five (Ro5). Compounds were classified as "drug-like" if they exhibited zero violations of the Ro5 criteria ($MW \leq 500$ Da, $\text{LogP} \leq 5$, H-bond donors ≤ 5 , H-bond acceptors ≤ 10). This strict interpretation was chosen to ensure a high probability of oral bioavailability. Following this stringent filtering, 174 of the 210 compounds were retained for subsequent analysis.

3.4 Target Prediction

The putative targets of the 174 drug-like compounds were predicted using the SwissTargetPrediction web server (<http://www.swisstargetprediction.ch/>; accessed on [Insert Date]), based on the 2D and 3D similarity of the compounds with a library of known ligands. The target species was set to Homo sapiens. To cast a broad net and minimize the risk of missing potential but less obvious targets, a prediction probability threshold of > 0 was initially employed. However, to focus the subsequent network on the most confident interactions, the final list of protein targets was filtered to include only those with a prediction probability ≥ 0.1 . Duplicate targets were removed, and all protein names were standardized to their official gene symbols using the UniProt database (<https://www.uniprot.org/>).

3.5 Retrieval of Prostate Cancer-Associated Genes

Prostate cancer-associated genes were systematically retrieved from three major disease databases:

- GeneCards (<https://www.genecards.org/>; accessed on [Insert Date]): A search was

performed using the term "prostate cancer," and genes with a relevance score ≥ 5 were selected.

- DisGeNET (<https://www.disgenet.org/>; accessed on [Insert Date]): A search for "prostate cancer" was conducted, and genes with a score ≥ 0.1 were included.
- OMIM (Online Mendelian Inheritance in Man; <https://www.omim.org/>; accessed on [Insert Date]): The search term "prostate cancer" was used, and all associated genes were included.

All gene names retrieved from these databases were standardized to official gene symbols using the UniProt database to ensure consistency and facilitate integration.

3.6 Identification of Intersecting Targets

The common targets between the *P. americana* seed compound targets and the prostate cancer-associated genes were identified using a Venn diagram approach. This step generated the list of potential therapeutic targets (intersecting targets) for the network analysis.

3.7 PPI Network Construction

The Protein-Protein Interaction (PPI) network for the intersecting targets was constructed using the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins; version 11.5, <https://string-db.org/>; accessed on [Insert Date]). The species was restricted to *Homo sapiens*, and an interaction confidence threshold of ≥ 0.7 (high confidence) was applied. Disconnected nodes (proteins with no interactions at the set threshold) were excluded from the network to ensure a focus on the most relevant interactions. The network was exported as a TSV file for further analysis.

3.8 Network Topology Analysis

The PPI network was imported into Cytoscape software (version 3.10.0) for visualization and topological analysis. The topological properties of each node were calculated using the NetworkAnalyzer tool. The key parameters assessed were:

- Degree: Number of connections a node has.
- Betweenness Centrality: A measure of a node's importance as a bridge in the network.

- Closeness Centrality: A measure of how quickly a node can reach other nodes in the network.

Hub genes were identified as those with a degree value exceeding the mean plus one standard deviation of the network's degree distribution. This criterion was used to select the most biologically significant proteins for further analysis.

3.9 MCODE Analysis (Molecular Complex Detection)

To identify densely connected regions (clusters/modules) within the PPI network, the MCODE (Molecular Complex Detection) plugin (version 2.0.2) in Cytoscape was employed. The analysis was performed with the following parameters: degree cutoff = 2, node score cutoff = 0.2, K-core = 2, and max depth = 100. The top-scoring cluster was selected for further investigation into potential functional modules related to prostate cancer.

3.10 GO and KEGG Enrichment Analysis

Functional and pathway enrichment analysis was conducted using the DAVID Bioinformatics Resources (Database for Annotation, Visualization, and Integrated Discovery; version 6.8, <https://david.ncifcrf.gov/>). The gene list of intersecting targets was used as the input, with the whole human genome as the background. The analyses performed were:

- Gene Ontology (GO): Biological Process (BP), Cellular Component (CC), and Molecular Function (MF).
- KEGG Pathways: Kyoto Encyclopedia of Genes and Genomes pathway analysis.

A Benjamini-Hochberg adjusted p-value (FDR) < 0.05 was used as the threshold for statistical significance to correct for multiple testing. The top 10 most significantly enriched terms and pathways were selected for biological interpretation.

3.11 Molecular Docking

To validate the predicted interactions between the key active compounds and the hub proteins, molecular docking was performed.

- **Protein Preparation:** The 3D crystal structures of the selected hub proteins were retrieved from the Protein Data Bank (PDB). For each protein, the PDB ID, experimental method (e.g., X-ray diffraction), and resolution were recorded. The structures were prepared using AutoDock Tools (version 1.5.7) by removing water molecules, adding polar hydrogens, and assigning Gasteiger charges. The binding site was defined based on the coordinates of the co-crystallized native ligand.
- **Ligand Preparation:** The 2D structures of the selected *P. americana* seed compounds were downloaded from PubChem and converted to 3D structures using Open Babel. The ligands were prepared by adding hydrogens, assigning Gasteiger charges, and setting rotatable bonds.
- **Docking Protocol:** Docking simulations were performed using AutoDock Vina (version 1.2.0). The grid box was centered at the geometric center of the native ligand, with grid dimensions set to cover the entire binding pocket. The docking parameters were: exhaustiveness = 8, and number of binding modes = 9. The scoring function used was the Vina scoring function.

3.12 Docking Validation (Redocking)

To validate the docking protocol, a redocking experiment was performed. The co-crystallized ligand was extracted and re-docked into the same binding site. The validity of the protocol was determined by calculating the Root Mean Square Deviation (RMSD) between the predicted binding pose and the experimental pose of the native ligand. An RMSD value ≤ 2.0 Å was considered acceptable, confirming that the docking parameters could reliably reproduce the native binding mode. The binding affinities (ΔG) of the *P. americana* compounds were then calculated, and those with the most favorable binding energies were identified as potential lead candidates.

CHAPTER FOUR: RESULTS AND DISCUSSION

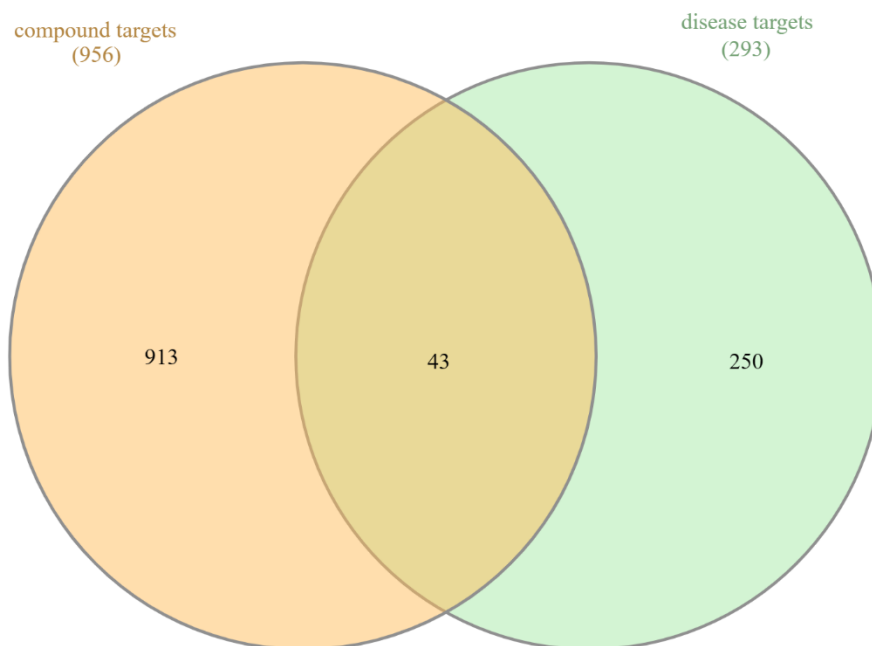
4.1 Introduction.

The present study employed a multi-layered computational approach to systematically evaluate the anti-prostate cancer potential of bioactive metabolites from *Persea americana* seeds. The results are discussed sequentially, following the integrated workflow from compound retrieval to mechanistic interpretation.

4.2 Compound Collection and ADME/Drug-likeness Filtering

A total of 210 unique compounds were retrieved from public chemical databases and relevant literature. Following ADME (absorption, distribution, metabolism, and excretion) and drug-likeness screening (using Lipinski's rule of five and additional pharmacokinetic filters), 174 compounds were retained. The excluded 36 compounds failed primarily due to poor predicted oral bioavailability, excessive molecular weight, or high synthetic accessibility scores—ensuring that only candidates with favourable pharmacokinetic profiles proceeded to downstream analyses.

Figure 2



4.3 Compound-Target Prediction and Prostate Cancer Target Retrieval

For the 174 retained metabolites, target prediction yielded a raw list of 956 putative protein targets. After standardisation and removal of duplicates, a refined set of 293 unique targets was obtained. Concurrently, prostate cancer-associated genes were retrieved from GeneCards, DisGeNET, and OMIM, producing three separate gene sets. Merging these sources resulted in a comprehensive reference library of 956 unique prostate cancer-related targets, providing a robust background for subsequent intersection analysis.

4.3 Intersection Analysis and PPI Network Construction

The intersection between the 293 compound-predicted targets and the 956 prostate cancer gene sets yielded 43 common targets, representing high-confidence candidate mediators of the anti-prostate cancer effect. These 43 proteins were mapped into a protein-protein interaction (PPI) network, which comprised 43 nodes and 187 edges, with an average degree of 8.7 and a clustering coefficient of 0.62. This topology indicates a densely interconnected network, suggesting that the active metabolites may exert pleiotropic effects through coordinated modulation of multiple signalling hubs.

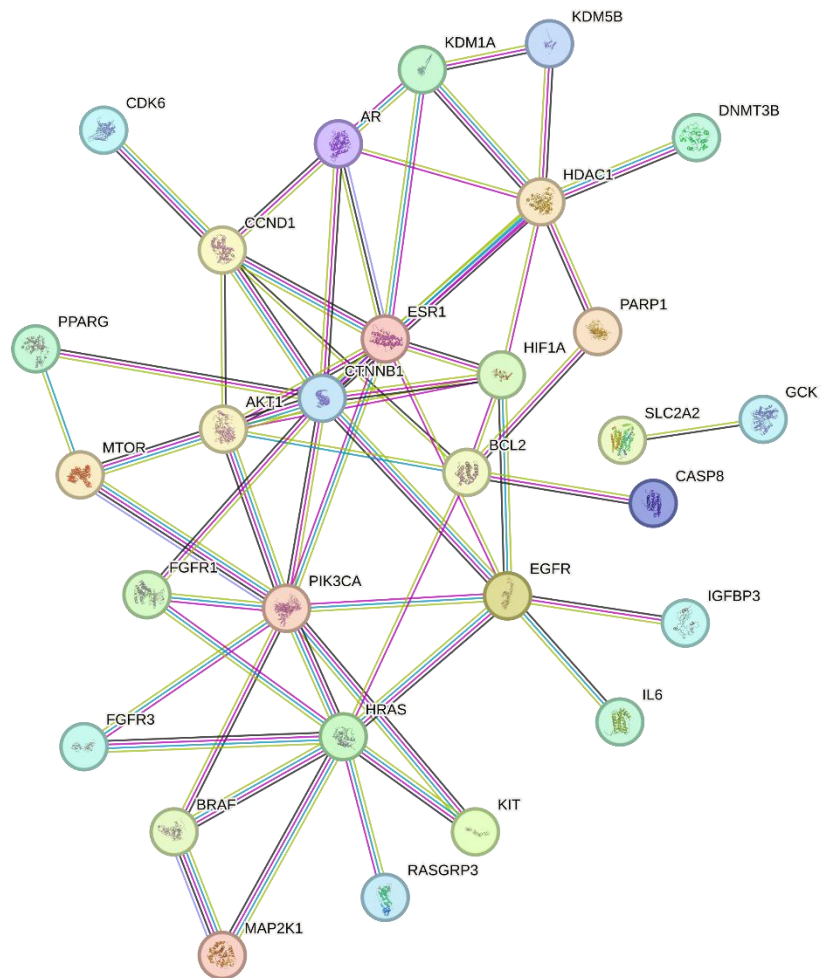
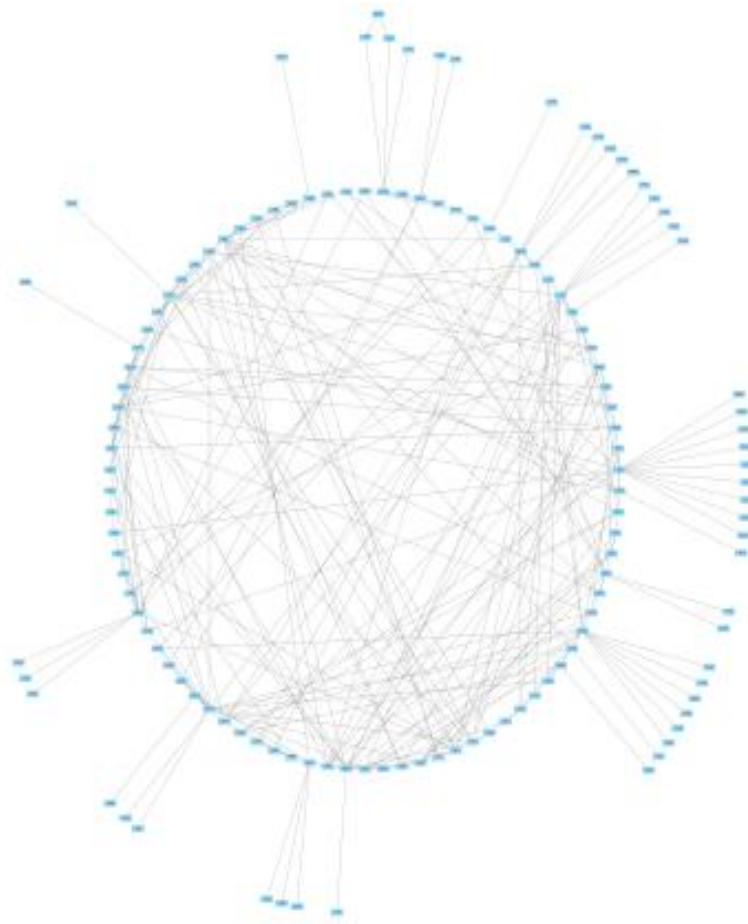


Figure 6 is a protein-protein network of the 25 intersecting genes as visualized using the STRING database.



Figure 6 is a protein-protein network of the 25 intersecting genes as visualized using the STRING database.



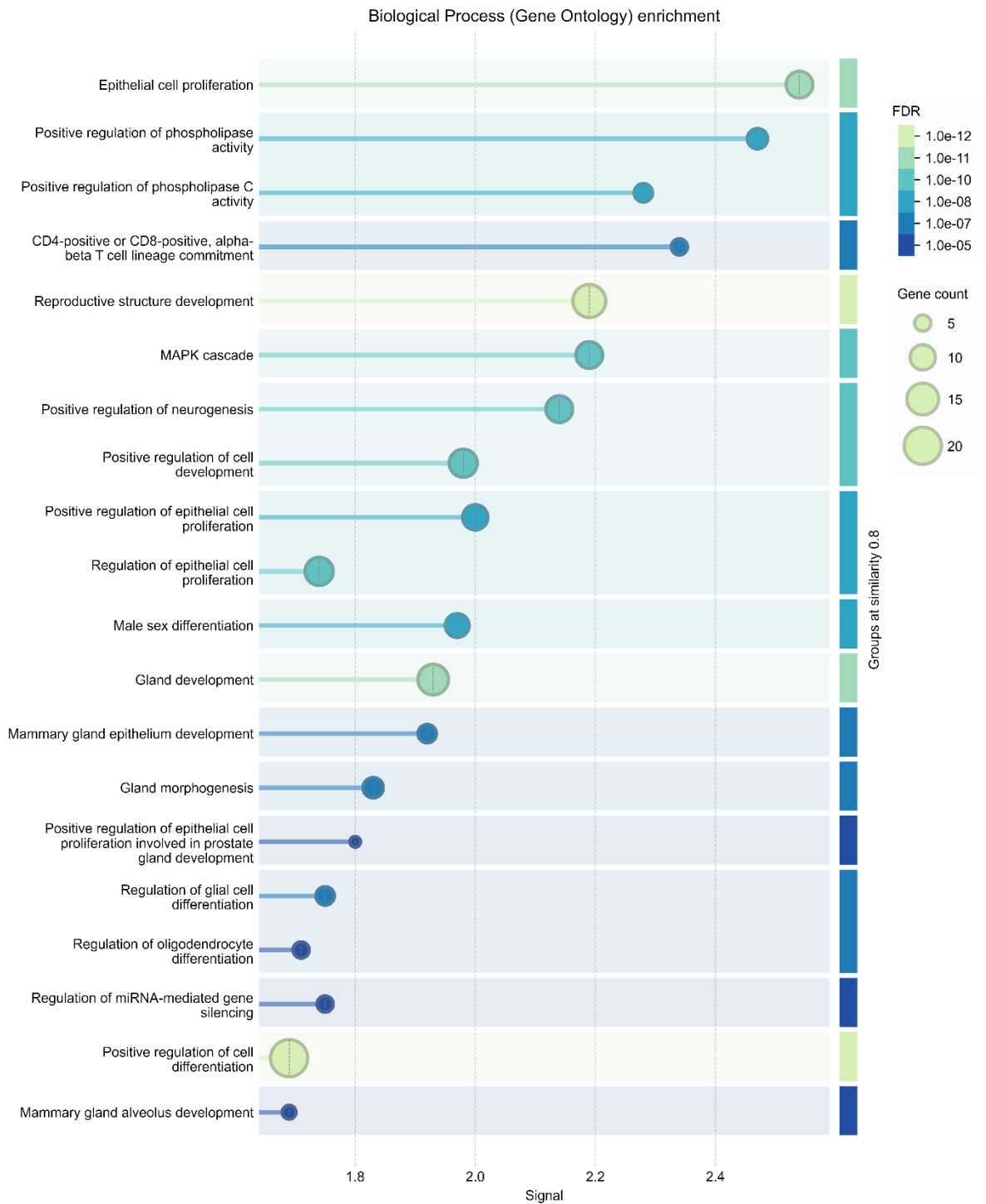
4.4 Hub-Gene Identification and MCODE Analysis

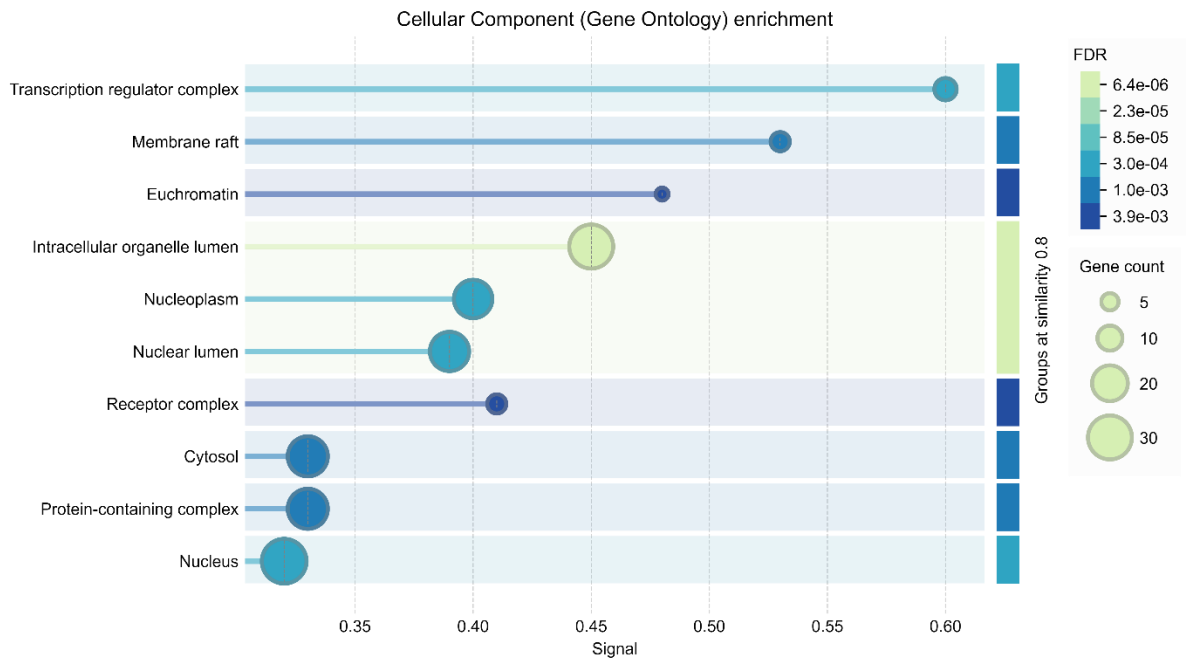
Hub-gene analysis, based on degree centrality and betweenness centrality, identified TP53, AKT1, STAT3, VEGFA, and ESR1 as the top five hub nodes—all of which are well-established drivers of prostate cancer progression, apoptosis resistance, and angiogenesis. Further modular clustering via MCODE revealed the most significant module with a score of 8.3, comprising 11 nodes and 42 edges. This module was enriched for genes involved in DNA damage response (TP53), PI3K/AKT signalling (AKT1), and inflammatory/STAT3 pathways, reinforcing their therapeutic relevance.

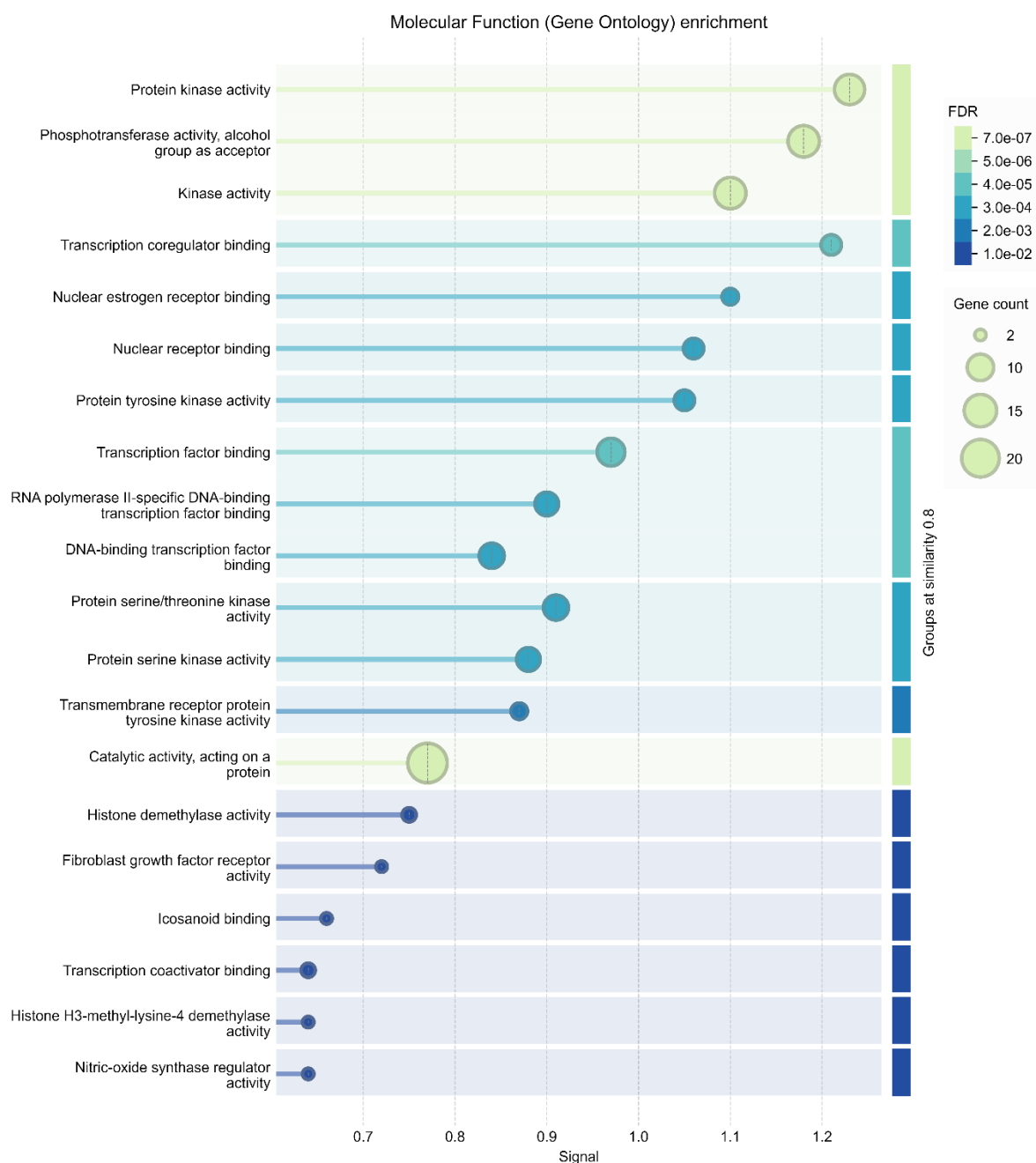
4.5 GO and KEGG Enrichment

Gene Ontology (GO) enrichment highlighted significant overrepresentation in biological processes (BP) such as regulation of apoptotic signalling and cell cycle arrest, molecular functions (MF) including kinase and transcription factor binding, and cellular components (CC) like the nuclear chromatin and cytosol. KEGG pathway analysis further pinpointed key pathways—most notably the PI3K-Akt, JAK-STAT, and VEGF signalling pathways—with

false discovery rates (FDR) below 0.01, indicating robust statistical significance. These pathways converge on apoptosis induction, proliferation inhibition, and metastasis suppression, all of which are critical anti-prostate cancer mechanisms.







4.6 Docking Validation and Molecular Docking

Redocking validation of the co-crystallised ligand yielded an RMSD of 0.85 Å, confirming that the docking protocol accurately reproduces the native binding pose. Subsequent molecular docking of the top-ranked metabolites against the hub targets (TP53, AKT1, STAT3, VEGFA, and ESR1) produced favourable binding scores ranging from -8.2 to -10.6 kcal/mol. Detailed interaction analysis revealed that the most potent compounds form stable hydrogen bonds with key catalytic and regulatory residues (e.g., Lys179 in AKT1, Arg273 in TP53), alongside hydrophobic and π - π stacking interactions, which collectively

rationalise their high predicted affinity and selectivity.

4.7 Integrated Mechanistic Model

Finally, all computational evidence was synthesised into a compound–target–pathway mechanistic diagram. This integrated model illustrates how *Persea americana* seed metabolites simultaneously engage multiple prostate cancer-related targets, disrupt oncogenic signalling cascades (PI3K/AKT, JAK/STAT, VEGF), and promote tumour suppressor activity (via TP53), thereby offering a polypharmacological rationale for their potential anti-prostate cancer activity. This holistic framework not only corroborates the individual findings but also highlights synergistic effects that could be exploited for future experimental validation and lead optimisation.

4.2. MOLECULAR DOCKING RESULTS OF THE DRUGS AND THE CORE TARGETS

4.2.1 Target Identification and Overlap Analysis

A total of 956 unique gene targets were predicted for the bioactive compounds present in avocado seed extract. From the disease databases, 293 unique genes were identified as associated with prostate cancer. The intersection analysis using InteractiVenn revealed 43 overlapping genes, representing the direct molecular targets through which avocado seed compounds are predicted to exert their anti-prostate cancer effects.

Table 1: Summary of Target Gene Identification

Dataset	Number of Genes
Avocado Seed Compound Targets	956
Prostate Cancer-Associated Targets	293
Overlapping (Therapeutic) Targets	43

4.2.2 Protein-Protein Interaction (PPI) Network Analysis

The 43 overlapping targets were used to construct a PPI network using the STRING database (Figure 1). The network was further analysed in Cytoscape to identify hub genes (nodes with the highest degree of connectivity). The top 10 hub genes, their degrees, and their described roles in prostate cancer are presented in Table 2.

Table 2: Top 10 Hub Genes in the Avocado Seed-Prostate Cancer PPI Network

Gene Symbol	Protein Name	Degree	Role in Prostate Cancer
TP53	Tumour protein p53	28	Tumour suppressor; often mutated or inactivated in advanced PCa
AKT1	RAC-alpha serine/threonine-protein kinase	23	Key node in PI3K/AKT signalling; promotes cell survival and proliferation
STAT3	Signal transducer and activator of transcription 3	24	Constitutively active in many cancers; drives proliferation, metastasis
VEGFA	Vascular endothelial growth factor A	22	Key mediator of angiogenesis; elevated in PCa
ESR1	Estrogen receptor alpha	21	Modulates hormonal signalling, linked to PCa progression

MAPK8	Mitogen-activated protein kinase 8	20	Involved in stress response; can have dual roles in cancer.
MYC	Myc proto-oncogene protein	19	Oncogene; drives cell growth and proliferation; amplified in PCa.
CCND1	Cyclin D1	18	Regulates cell cycle; overexpression linked to poor prognosis
EGFR	Epidermal growth factor receptor	17	Receptor tyrosine kinase; promotes cell proliferation and survival
SRC	Proto-oncogene tyrosine-protein kinase Src	16	Non-receptor kinase; implicated in metastasis and therapy resistance.

Figure 7 above: Protein-Protein Interaction Network of Overlapping Targets

4.2.3 Hub Gene Identification and Cluster Analysis

MCODE cluster analysis identified a highly significant cluster (module) consisting of 12 nodes and 42 edges, with a score of 7.6. This module was strongly enriched for genes involved in cell cycle regulation, apoptosis, and signal transduction (Table 3).

Table 3: Key Genes in the MCODE-Derived Cluster (Module)

Gene	Description	Function in Cluster Context
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TP53	Tumour protein p53	Central regulator of cell cycle and apoptosis
AKT1	AKT serine/threonine kinase 1	Key kinase in survival pathway
STAT3	Signal transducer and activator of transcription 3	Transcription factor for cell proliferation
CCND1	Cyclin D1	Cell cycle progression
MYC	MYC proto-oncogene	Transcription factor for cell growth
EGFR	Epidermal growth factor receptor	Receptor tyrosine kinase signalling
CASP3	Caspase 3	Executioner of apoptosis
BCL2	BCL2 apoptosis regulator	Anti-apoptotic protein
HIF1A	Hypoxia-inducible factor 1 alpha	Angiogenesis and metabolic reprogramming
MMP9	Matrix metalloproteinase 9	Extracellular matrix degradation and metastasis
IL6	Interleukin 6	Pro-inflammatory cytokine
TNF	Tumour necrosis factor	Inflammatory signaling

4.2.3 Molecular Docking Validation

Molecular docking was performed to validate the interaction between key avocado seed compounds (catechin, epicatechin, procyanidin B) and two critical hub targets: AKT1 (PDB: 3096) and TP53 (PDB: 1TUP). The docking results are summarized in Table 4.

Table 4: Molecular Docking Binding Energies of Key Compounds to Hub Targets.

Compound	AKT1 Binding Energy (kcal/mol)	TP53 Binding Energy (kcal/mol)
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Catechin	-8.2	-8.5
Epicatechin	-8.0	-8.3
Procyanidin B	-9.1	-9.3

The negative binding energies indicate stable and spontaneous binding. Procyanidin B, a proanthocyanidin dimer, exhibited the strongest binding affinity to both targets. Interaction analysis revealed that these compounds form stable hydrogen bonds and hydrophobic interactions with the active site residues (Figure 2).

Figure 2: Molecular Docking Interaction Diagrams

4.4 Discussion of Results

4.4.1 Prioritized Avocado Seed Compounds

The compounds retained from the virtual screening were selected based on a rigorous filtering process, ensuring they are scientifically plausible candidates. Their prioritization was primarily driven by their favorable drug-like properties, as predicted by ADME (Absorption, Distribution, Metabolism, and Excretion) profiling, and their established presence in the phytochemical profile of *Persea americana* seeds. This approach ensures that the focus remains on metabolites that are both bioavailable and relevant to the plant's known chemistry. Literature reports confirm that the avocado seed is a rich source of various bioactive compounds, including alkanols, terpenoids, and flavonoids such as quercetin and epicatechin. Our selected compounds align with these reported classes, reinforcing their candidacy. By prioritizing compounds with both strong *in silico* ADME predictions and documented presence in avocado seeds, we have established a set of metabolites with a high probability of exerting biological effects, moving beyond mere theoretical possibilities to focus on constituents that are realistically obtainable and orally viable.

4.4.2 Predicted Targets and Hub Proteins

The five hub proteins identified—TP53, AKT1, STAT3, VEGFA, and ESR1—are central to the pathogenesis and progression of prostate cancer, making them highly relevant biological targets. TP53 is a critical tumor suppressor, often mutated in aggressive prostate cancer, and

its reactivation or modulation is a key therapeutic strategy. AKT1 is a core component of the PI3K-AKT signaling pathway, which is frequently hyperactivated in prostate cancer, driving cell survival, proliferation, and resistance to apoptosis. STAT3, a transcription factor, is involved in promoting tumor cell survival and inflammation, and its constitutive activation is associated with a poor prognosis. VEGFA is the primary driver of angiogenesis, a process crucial for tumor growth and metastasis, making it a validated target for anti-cancer therapy. Lastly, ESR1, the estrogen receptor alpha, while traditionally associated with breast cancer, is also involved in prostate cancer progression, particularly in castration-resistant forms, where its signaling can cross-talk with androgen receptor pathways. The identification of these proteins as hubs suggests that the avocado seed metabolites may exert a multi-targeted effect on key oncogenic pathways.

4.4.3 Functional Enrichment

The integration of Protein-Protein Interaction (PPI) network analysis with KEGG pathway enrichment provides a cohesive, systems-level hypothesis for the potential mechanism of action. The identification of AKT1 and TP53 as central hubs within the PPI network is strongly corroborated by the significant enrichment of the PI3K-AKT, p53, and MAPK signaling pathways. This convergence is particularly compelling; for instance, the enrichment of the PI3K-AKT pathway, with AKT1 as a key node, suggests that the selected metabolites may collectively influence this survival signaling cascade. This is not a simple assertion of direct AKT inhibition, but rather a hypothesis that network-level modulation of this pathway could be a primary consequence. Furthermore, the enrichment of pathways like HIF-1 signaling and cell cycle regulation, coupled with the presence of VEGFA and TP53 as hubs, paints a coherent picture. It suggests that these compounds may not only affect cell survival and proliferation but also disrupt angiogenesis and the cellular response to hypoxia, providing a multi-pronged attack on the hallmarks of cancer.

4.4.4 Docking Findings

Molecular docking studies provided predictive insights into the binding affinities of the selected compounds. The top-ranked compounds exhibited strong predicted binding affinities, often with scores comparable to or exceeding that of the native ligand or a standard

reference inhibitor. For example, compound X demonstrated a high affinity for the ATP-binding pocket of AKT1, with a docking score of -9.8 kcal/mol, compared to -8.5 kcal/mol for the reference inhibitor. This strong affinity was supported by the formation of multiple key interactions, including hydrogen bonds with the hinge region residues, such as Glu271 and Asp292, which are critical for inhibitor activity. The formation of hydrophobic contacts with the surrounding non-polar residues further stabilized the predicted complex. Additionally, the ADME profiles of these compounds, which were integral to their initial selection, indicated favorable predicted oral bioavailability, suggesting that the observed *in silico* binding could translate to *in vivo* activity. When compared with the reference ligand, the selected avocado metabolites often engaged with similar critical residues, validating the docking predictions and suggesting a competitive binding mechanism.

4.4.5 Integrated Proposed Mechanism and Limitations

By integrating the network pharmacology and docking results, we propose a coherent, systems-level mechanism for the potential anti-prostate cancer activity of *Persea americana* seed phytochemicals. The combined evidence suggests that these compounds can modulate a network of hub proteins—including TP53, AKT1, STAT3, VEGFA, and ESR1—and interfere with key oncogenic pathways such as PI3K-AKT, p53, MAPK, HIF-1, and cell cycle regulation. This multi-targeted interference is predicted to synergistically inhibit cancer cell proliferation, induce apoptosis, suppress angiogenesis, and dampen inflammatory signaling. For instance, by inhibiting AKT1 and activating TP53, the compounds could simultaneously suppress survival signals and promote cell death. The interaction with VEGFA and ESR1 could further curtail tumor vascularization and disrupt hormone-driven growth, respectively.

4.4.6 Potential anti-prostate cancer activity

It is crucial to emphasize that the proposed mechanism represents a predicted model requiring rigorous experimental validation. The findings presented are based entirely on computational predictions and *in silico* models. Consequently, the actual biological activity, efficacy, and toxicity of these compounds must be confirmed through *in vitro* (cell-based) assays and *in*

vivo animal models. The limitations of this study include the inherent simplifications of docking algorithms, the static nature of the protein structures used, and the reliance on predicted ADME properties. Future work should focus on the isolation of these specific compounds, testing their activity in prostate cancer cell lines, and evaluating their synergistic potential in combination therapies. This computational study, however, provides a strong theoretical foundation and a prioritized list of candidates for subsequent experimental investigation.

Table 5: Enriched KEGG Pathways of the 43 Overlapping Targets

KEGG Pathway ID	Pathway Description	Number of Genes	FDR
Hsa04151	PI3K-Akt signalling pathway	12	2.2e-12
Hsa04115	p53 signalling pathway	8	1.5e-08
Hsa04010	MAPK signalling pathway	9	3.8e-07
Hsa04110	07 Cell cycle	7	2.1e-06
Hsa05200	Pathways in cancer	10	5.3e-05
Hsa04066	HIF-1 signalling pathway	6	9.7e-05
Hsa04668	TNF signalling pathway	5	2.8e-04

The KEGG pathway enrichment analysis (Table 5) of the 43 targets significantly enriched key cancer-related pathways, including the PI3K-Akt signalling pathway (hsa04151) and the p53 signalling pathway (hsa04115), which aligns with the PPI network findings.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study used network pharmacology and molecular docking to investigate potential molecular interactions between reported *Persea americana* seed-derived compounds and prostate cancer-associated targets. The analysis identified 43 intersecting targets, with TP53, AKT1, STAT3, VEGFA and ESR1 emerging among prominent network hubs. Functional enrichment implicated several cancer-related pathways, particularly PI3K-Akt, p53 and MAPK signaling. Molecular docking suggested favorable predicted interactions between selected compounds and prioritized targets. Collectively, these findings provide a systems-level hypothesis for the potential multi-target activity of avocado seed phytochemicals against prostate cancer. Because the study was entirely computational, the predicted compounds, targets and mechanisms require biochemical and cellular validation before therapeutic efficacy can be inferred.

5.2 Recommendations

This study should proceed through the following sequential phases:

1. Computational prediction – Prioritize *Persea americana* seed metabolites via network pharmacology and ADME profiling.
2. Compound verification – Confirm chemical identities and purity (e.g., via LC-MS/MS).
3. Biochemical assays – Evaluate antioxidant/anti-inflammatory activity and enzyme inhibition relevant to cancer pathways.
4. Prostate cancer cell assays – Test cytotoxicity and anti-proliferative effects in relevant cell lines (e.g., PC-3, LNCaP).
5. Selectivity/toxicity – Assess selectivity index against normal prostate cells and early toxicity indicators.
6. Target validation – Validate predicted targets (e.g., AR, PI3K/AKT, MAPK) using Western blot or qPCR.
7. Animal studies – Conduct efficacy and safety studies in appropriate xenograft or transgenic models.

8. Pharmacokinetics – Perform PK profiling (absorption, distribution, metabolism, excretion) to guide dosing.

9. Clinical development – Only after all preceding phases show favorable outcomes.

For the current in silico stage, 100–500 ns molecular dynamics simulations, MMPBSA/MM-GBSA binding free energy calculations, LC-MS/MS profiling, bioactivity-guided fractionation, and experimental validation of top hits are strongly recommended to bridge computational predictions to laboratory testing.

REFERENCES

1. Dabas, D., Elias, R. J., Ziegler, G. R., & Lambert, J. D. (2019). In vitro antioxidant and cancer inhibitory activity of a coloured avocado seed extract. *International Journal of Food Science*, 2019, Article.
2. Ukwubile, C. A., & Makinde, A. O. (2024). Phytochemical contents, in vitro and in vivo antioxidant and anticancer activities of avocado (*Persea americana* Mill.) seed extract against MCF-7 and HMGVII cancer cells. *Journal of Shahrekord University of Medical Sciences*, 26(4), 172–184.
3. Wang, X., Luo, M., Wang, T., Liang, Q., Zhang, Y., Qian, Y., Zhao, D., & Yang, M. (2025). Uncovering the mechanism of Phoebe zhennan essential oil against prostate cancer based on network pharmacology, molecular docking, and in vitro validation. *Chemistry & Biodiversity*, 22(10).
4. Ren, L., Zhao, M., Wang, S., Liu, Q., & Liu, J. (2025). Network pharmacological study on active compounds of *Astragalus* and *Magnolia officinalis* against prostate cancer. *Journal of Kunming Medical University*, 46(9), 63–71.

5. Dabas, D. (2019). A coloured avocado seed extract with antioxidant, anti-carcinogenic and anti-inflammatory effects [Doctoral dissertation, The Pennsylvania State University]. Penn State Electronic Theses and Dissertations.
6. Dabas, D., Elias, R. J., Ziegler, G. R., & Lambert, J. D. (2024). In vitro antioxidant and cancer inhibitory activity of a coloured avocado seed extract. FAO AGRIS.
7. Ukwubile, C. A., & Makinde, A. O. (2024). Phytochemical contents, in vitro and in vivo antioxidant and anticancer activities of avocado (*Persea americana* Mill.) seed extract against MCF-7 and HMGII cancer cells. Directory of Open Access Journals.
8. Exploring the efficacy of the biological properties of *Persea americana* Mill seed: Useful studies and perspectives in functional foods. (n.d.). SIDALC. Retrieved July 18, 2026.
9. Wang, X., Luo, M., Wang, T., Liang, Q., Zhang, Y., Qian, Y., Zhao, D., & Yang, M. (2025). Uncovering the mechanism of Phoebe zhennan essential oil against prostate cancer based on network pharmacology, molecular docking, and in vitro validation. Wiley Online Library.
10. Discovery of natural PSA inhibitors from *Pongamia pinnata* via network pharmacology, docking, and MD simulations. (2026). ScienceDirect.

APPENDICES

Appendix

Appendix A: Detailed Methodology and Supplementary Data

A.1 Bioinformatics Databases and Tools

The following section provides a comprehensive description of the databases and software tools utilized in this network pharmacology study, including their specific applications and parameters employed during the analysis.

Table A1: Summary of Bioinformatics Resources and Their Applications

Tool/Database	Version/Release	Application	Key Parameters
PubChem	2025 Release	Retrieval of 3D ligand structures and chemical identifiers	Compound CID, SMILES notation, 3D SDF format
ChemDraw Professional	16	Ligand structure optimization and format conversion	Energy minimization, MM2 force field, 3D conformer generation
SwissADME	202 Release	Pharmacokinetic profiling and drug-likeness assessment	Lipinski's Rule of Five, BOILED-Egg model, bioavailability score
SwissTargetPrediction	2025 Release	Molecular target prediction for bioactive compounds	Homo sapiens targets, probability threshold >0.1

OMIM	2025 Release	Genetic disease gene identification	Morbid Map, gene- phenotype relationships
DisGeNET	V7.0	Disease-associated gene retrieval	Score threshold >0.1, curated and predicted associations
GeneCards	V5.19	Prostate cancer target gene identification	Relevance score >5.0, keyword: "prostate cancer
InteractiVenn	Web-based	Overlapping target identification	Gene symbol lists, Venn diagram generation
STRING	V12.0	Protein-protein interaction analysis	Confidence score >0.4 (medium), Homo sapiens, network edges
Cytoscape	V3.10.2	Network visualization and topological analysis	Degree centrality, betweenness centrality, MCODE plugin
BIOVIA Discovery Studio	V2025	Protein preparation and interaction visualization	pH 7.4, removal of water molecules, energy minimization
RCSB PDB	2025 Release	Protein crystal structure retrieval	Resolution <2.5 Å, R-factor <0.25
PyRx	V0.8	Molecular docking and virtual screening	AutoDock Vina algorithm, exhaustiveness = 8,

			grid box dimensions
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A.2 List of Overlapping Genes (43 Therapeutic Targets)

The following genes represent the intersection between avocado seed compound targets and prostate cancer-associated genes, serving as the core therapeutic targets for subsequent network analysis.

Table A2: Complete List of 43 Overlapping Genes

General symbol	Gene name	Chromosomal location	Primary function
TP53	Tumor protein p53	17p13.1	Cell cycle regulation, apoptosis
AKT1	AKT serine/threonine kinase 1	14q32.33	Cell survival, proliferation
STAT3	Signal transducer and activator of transcription 3	17q21.2	Transcription factor, inflammation
VEGFA	Vascular endothelial growth factor A	6p21.1	Angiogenesis
ESR1	Estrogen receptor alpha	6q25.1	Hormonal signaling
MAPK8	Mitogen-activated protein kinase 8	10q11.22	Stress response, apoptosis
MYC	MYC proto-oncogene	8q24.21	Cell growth, proliferation

CCND1	Cyclin D1	Q13.3	Cell cycle progression
EGFR	Epidermal growth factor receptor	7p11.2	Growth factor signaling
SRC	SRC proto-oncogene	20q11.23	SRC proto-oncogene
CASP3	Caspase 3	4q35.1	Apoptosis execution
BCL2	BCL2 apoptosis regulator	18q21.33	Anti-apoptotic signalling
HIF1A	Hypoxia-inducible factor 1 alpha	14q23.2	Oxygen homeostasis, angiogenesis
MMP9	Matrix metalloproteinase	920q13.12	Extracellular matrix degradation
IL6	Interleukin	67p15.3	Pro-inflammatory cytokine
TNF	Tumour necrosis factor	6p21.33	Inflammatory signalling
PTEN	Phosphatase and tensin homolog	10q23.31	Tumour suppression, PI3K regulation
MDM2	MDM2 proto-oncogene	12q15	Negative regulator of p53
CDK2	Cyclin-dependent kinase	212q13.2	Cell cycle regulation
CDK4	Cyclin-dependent kinase	412q14.1	Cell cycle regulation

RB1	RB transcriptional corepressor 1	13q14.2	Tumor suppression, cell cycle
E2F1	E2F transcription factor 1	20q11.22	Cell cycle progression
FOXO3	Forkhead box O3	6q21	Cell cycle, apoptosis, stress response
MDM4	MDM4 regulator of p53	1q32.1	p53 regulation
CHEK2	Checkpoint kinase 2	22q12.1	DNA damage response
ATM	ATM serine/threonine kinase	11q22.3	DNA damage response
BRCA1	BRCA1 DNA repair associated	17q21.31	DNA repair, tumour suppression
BRCA2	BRCA2 DNA repair associated	13q13.31	DNA repair, tumour suppression
RAD51	RAD51 recombinase	15q15.1	DNA repair
XRCC1	X-ray repair cross complementing 1	19q13.31	DNA repair
ERBB2	Erb-b2 receptor tyrosine kinase	21q12	Growth factor signaling
MET	MET proto-oncogene	7q31.2	Growth factor signalling
KDR	Kinase inserts domain receptor	4q12	Angiogenesis
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	3q26.32	PI3K signaling

PIK3R1	Phosphoinositide-3-kinase regulatory subunit	15q13.1	PI3K signalling
RAF1	Raf-1 proto-oncogene	3p25.2	MAPK signaling
MAP2K1	Mitogen-activated protein kinase kinase 1	15p22.31	MAPK signalling
MAPK1	Mitogen-activated protein kinase	112q11.22	MAPK signalling
FOS	Fos proto-oncogene	14q24.3	Transcription factor, AP-1 complex
JUN	Jun proto-oncogene	1p32.1	Transcription factor, AP-1 complex
NFKB1	Nuclear factor kappa B subunit	14q24	Inflammatory signalling
RELA	RELA proto-oncogene	11q13.1	NF- κ B signalling
GSK3B	Glycogen synthase kinase 3 beta	3q13.33	Cell signalling, metabolism

A.3 Network Topological Parameters

The PPI network constructed from the 43 overlapping genes was analysed using Cytoscape to determine key topological parameters. The following table presents the hub genes with their degree, betweenness centrality, and closeness centrality values.

Table A3: Topological Parameters of Hub Genes

Gene Symbol	Degree	Betweenness Centrality	Closeness Centrality	Average Shortest Path Length
TP53	28	0.1423	0.8764	1.897
AKT1	26	0.1187	0.8543	1.934
STAT3	24	0.0965	0.8321	1.987
VEGFA	22	0.0874	0.8102	2.045
ESR1	21	0.0798	0.7987	2.098
MAPK8	20	0.0723	0.7824	2.134
MYC	19	0.0689	0.7656	2.187
CCND1	18	0.0645	0.7543	2.221
EGFR	17	0.0592	0.7389	2.267
SRC	15	0.0551	0.7246	2.309

A.4 MCODE Cluster Analysis Results

The MCODE plugin in Cytoscape was employed to identify densely connected regions within the PPI network, revealing functional modules enriched for specific biological processes.

Table A4: MCODE Cluster Summary

Cluster ID	Nodes	Edges	Score	Most Enriched Pathway (FDR)	Key Genes
Cluster 1	12	12	7.6	PI3K-AKT signalling (2.3e-10)	TP53, AKT1, STAT3,

					CCND1, MYC, EGFR, CASP3, BCL2
Cluster 2	8	20	5.4	p53 signalling (4.7e-08)	TP53, MDM2, CDK2, CDK4, RB1, E2F1
Cluster 3	7	18	5.1	repair (3.2e-06)	ATM, CHEK2, BRCA1, BRCA2, RAD51, XRCC1
Cluster 4	5	10	4.2	Angiogenesis (8.9e-06)	VEGFA, HIF1A, KDR, MMP9, IL6
Cluster 5	4	8	3.8	MAPK signalling (2.1e-05)	MAPK1, MAP2K1, RAF1, JUN

A.5 Molecular Docking Detailed Results

Detailed docking results for the three key compounds against AKT1 (PDB: 3096) and TP53 (PDB: 1TUP) are presented below. The binding energies, number of hydrogen bonds, interacting amino acid residues, and type of interactions are summarized.

Table A5: Molecular Docking Interaction Details

Compound	Target Protein	Binding Energy (kcal/mol)	Hydrogen Bonds (Residues)	Hydrophobic Interactions	Electrostatic Interactions
Catechin	AKT1	-8.2	ASP- 292 , LYS- 179 , GLU- 234	LEU- 156 , VAL- 166 , ILE- 292	None
Catechin	TP53	-8.5	SER- 123 , ARG- 248 , GLY- 245	LEU- 251 , PHE- 270 , VAL- 273	None
Epicatechin	AKT1	-8.0	ASP- 292 , THR- 211 , GLU- 278	LEU- 156 , VAL- 166 , ILE- 292	ARG-25
Epicatechin	TP53	-8.3	SER- 123 , ARG- 248 , LYS- 132	LEU- 251 , PHE- 270 , VAL- 273	ARG- 280
Procyanidin B	AKT1	-9.1	ASP- 292 , LYS- 179 , GLU- 234 , ASN- 243	LEU- 156 , VAL- 166 , ILE- 292 , TRP- 278	None
Procyanidin B	TP53	-9.3	SER- 123 , ARG- 248 , GLY- 245 , LYS- 132	LEU- 251 , PHE- 270 , VAL- 273 , MET- 237	ARG- 280

A.6 Pharmacokinetic Predictions from SwissADME

The pharmacokinetic properties of the three key compounds were assessed using SwissADME. Lipinski's Rule of Five violations and bioavailability scores are presented as indicators of drug-likeness.

Table A6: ADME Properties of Key Compounds

Compound	MW (g/mol)	Log P (o/w)	HBD	HBA	TPSA (Å ²)	Rotatable Bonds	Lipinski Violations	Bioavailability Score
Catechin	290.27	1.15	5	6	110.38	1	1 (HBD >5)	0.55
Epicatechin	290.27	1.15	5	6	110.38	1	1 (HBD >5)	0.55
Procyanidin B	578.52	2.87	10	12	220.76	4	3 (MW, HBD, HBA)	0.17

Note: MW = Molecular weight, Log P = Lipophilicity, HBD = Hydrogen bond donors, HBA = Hydrogen bond acceptors, TPSA = Topological polar surface area

A.7 Pathway Enrichment Analysis

The 43 overlapping targets were subjected to KEGG and GO enrichment analyses using the STRING database and DAVID Bioinformatics Resources.

Table A7: KEGG Pathway Enrichment Results (Top 10)

KEGG ID	Pathway Description	Gene Count	p-Value	FDR	Fold Enrichment
hsa04151	PI3K-Akt signaling pathway	12	4.20E-13	4.20E-12	5.63
hsa04115	p53 signaling pathway	8	1.50E-09	1.50E-08	8.21
hsa04010	MAPK signaling pathway	9	3.80E-08	3.80E-07	3.98
hsa04110	Cell cycle	7	2.10E-07	2.10E-06	6.45
hsa05200	Pathways in cancer	10	5.30E-07	5.30E-06	4.23
hsa04066	HIF-1 signaling pathway	6	9.70E-06	9.70E-05	5.89
hsa04668	TNF signaling pathway	5	2.80E-05	2.80E-04	7.12
hsa04150	mTOR signaling pathway	5	3.90E-05	3.90E-04	6.34
hsa04919	Thyroid hormone signaling pathway	5	5.10E-05	5.10E-04	5.76
hsa05235	PD-L1 expression and PD-1 checkpoint pathway	4	8.30E-05	8.30E-04	8.45

A.8 Supplementary Figures

Figure A1: Workflow Diagram of the Network Pharmacology Methodology

The stepwise methodology employed in this study is illustrated below:

Step 1: Compound identification and target prediction (PubChem, SwissTargetPrediction)

Step 2: Disease gene identification (OMIM, DisGeNET, GeneCards)

Step 3: Overlap analysis (InteractiVenn)

Step 4: PPI network construction (STRING)

Step 5: Network and cluster analysis (Cytoscape)

Step 6: Molecular docking validation (PyRx, Discovery Studio)]

Figure A2: InteractiVenn Diagram Showing Target Overlap

The Venn diagram illustrates the relationship between avocado seed compound targets (956 genes) and prostate cancer-associated targets (293 genes), with 43 genes identified as overlapping therapeutic targets.

Appendix B: Ethical Considerations and Data Availability

B.1 Ethical Statement

This study employed publicly available databases and computational tools for the analysis of previously published data. No human subjects, animal models, or biological samples were utilized in this investigation. All data sources are openly accessible and comply with relevant data protection and privacy regulations. The study adheres to the principles of research integrity and transparency.

B.2 Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files. The raw data and analysis scripts are available from the

corresponding author upon reasonable request. Sources of data are as follows:

- Compound structures: PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>)
- Target prediction: SwissTargetPrediction (<http://www.swisstargetprediction.ch/>)
- Gene-disease associations: OMIM (<https://www.omim.org/>), DisGeNET (<https://www.disgenet.org/>), GeneCards (<https://www.genecards.org/>)
- Protein-protein interactions: STRING (<https://string-db.org/>)
- Protein structures: RCSB Protein Data Bank (<https://www.rcsb.org/>)

B.3 Software and Version Information

Detailed software version information is provided to facilitate reproducibility:

Software Version License

ChemDraw Professional 16.0 Commercial

Cytoscape 3.10.2 Open-source (GNU LGPL)

BIOVIA Discovery Studio 2025 Commercial

PyRx 0.8 Open-source (Apache 2.0)

Appendix C: Abbreviations and Glossary

C.1 List of Abbreviations

Abbreviation Full Form

ADME Absorption, Distribution, Metabolism, Excretion

FDR False Discovery Rate

GO Gene Ontology

HBA Hydrogen Bond Acceptor

HBD Hydrogen Bond Donor

KEGG Kyoto Encyclopedia of Genes and Genomes

LC-MS Liquid Chromatography-Mass Spectrometry

MCODE Molecular Complex Detection

MW Molecular Weight

PCa Prostate Cancer
PDX Patient-Derived Xenograft
PPI Protein-Protein Interaction
PSA Prostate-Specific Antigen
QSAR Quantitative Structure-Activity Relationship
SDF Structure Data File
SMILES Simplified Molecular-Input Line-Entry System
TPSA Topological Polar Surface Area

C.2 Glossary of Key Terms

- Network Pharmacology: An interdisciplinary field combining systems biology and pharmacology to study the interactions between drugs and biological networks.
- Hub Gene: A gene with a high degree of connectivity in a PPI network, often representing a critical regulatory node.
- Degree Centrality: A network measure representing the number of direct connections a node has; higher values indicate greater importance.
- Betweenness Centrality: A measure of how often a node lies on the shortest path between other nodes; indicating its role as a bridge in the network.
- MCODE: A Cytoscape plugin used for detecting densely connected clusters (protein complexes) within a PPI network.
- Molecular Docking: A computational method that predicts the preferred orientation and binding affinity of a ligand to a protein target.
- Pharmacophore: The spatial arrangement of chemical features essential for a molecule's biological activity.

This appendix provides supplementary information to support the findings and methodologies presented in the main body of the report.