STRUCTURE ACTIVITY RELATIONSHIP ANALYSIS OF MARINE PHLOROTANNINS AS SELECTIVE DPP-4 INHIBITORS: A COMPUTATIONAL PERSPECTIVE

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

BIOTECHNOLOGY

Submitted by

AMAN JUYAL

24/MSCBIO/06

Under The Supervision Of

Dr. Smita Rastogi Verma

Assistant Professor, Department of Biotechnology

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi – 110042, India

May,2026

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, New Delhi, 1

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

CANDIDATE'S DECLARATION

I, **AMAN JUYAL**, Roll No. **2K24/MSCBIO/06**, student of M.Sc. Biotechnology, hereby certify that the work which is being presented in the thesis entitled "**Structure Activity Relationship of Marine Phlorotannins as Selective DPP-4 Inhibitors: A Computational Perspective**" in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out under the supervision of Dr. Smita Rastogi Verma. The work is original and not copied from any source without proper citation. The work has not previously formed the basis for the award of any other degree of this or any other Institute.

DATE:

AMAN JUYAL
2K24/MSCBIO/06

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi-42

CERTIFICATE BY THE SUPERVISOR

I hereby certify that the Dissertation Project titled **"Structure Activity Relationship of Marine Phlorotannins as Selective DPP-4 Inhibitors: A Computational Perspective"** which is submitted by **AMAN JUYAL**, **2K24/MSCBIO/06**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge, the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

DATE:

DR. SMITA RASTOGI VERMA
SUPERVISOR
Department of Biotechnology
Delhi Technological University

PROF. YASHA HASIJA
HEAD OF DEPARTMENT
Department of Biotechnology
Delhi Technological University

ACKNOWLEDGEMENT

My heartfelt thanks go to Dr. Smita Rastogi Verma, Assistant Professor, Department of Biotechnology, Delhi Technological University, for her invaluable guidance, constant encouragement, and scholarly mentorship throughout this research work. She has been an inspiration in terms of computational biology and drug discovery skills.

I also express my thanks to the Department of Biotechnology, Delhi Technological University for giving me computational infrastructure and academic resources to conduct my work.

I would like to express my sincere gratitude to my colleague and co-author Abhinav Borgohan for his parallel study on the docking profiles of phlorotannin molecules in this work, which helped in obtaining a better understanding of the interaction between phlorotannins and DPP-4. The combined results of the two studies provide a holistic computational study of marine phlorotannins as antidiabetic agents.

Finally, I would like to thank the developers and maintainers of the open-access databases Protein Data Bank (RCSB PDB), PubChem, and SwissADME, and open-source software PyRx, AutoDock Vina, and BIOVIA Discovery Studio, which made this work possible.

Last but not least, I want to thank my family and my friends who have been with me all the way, encouraging and supporting me morally.

ABSTRACT

T2DM is a metabolic disorder affecting hundreds of thousands of people globally, characterized by insulin secretion and insulin resistance. DPP-4) has emerged as a clinically important pharmacological target in T2DM management, as its inhibition increases the half-life of the hormone GLP-1, thereby enhancing insulin secretion. While synthetic DPP-4 inhibitors are clinically approved, their long-term use is associated with heart and pulmonary problems, along with high costs in developing countries. These problems have made it necessary to explore natural sources as inhibitors of DPP-4.

Marine phlorotannins, natural phenolic compounds derived from brown algae, have demonstrated broad-spectrum activities including anti-diabetic, anti-inflammatory, and antioxidant properties. This thesis specifically investigates the SAR of five marine phlorotannins: phloroglucinol, eckol, dioxinodehydroeckol, fucodiphloroethol G, and phlorofucofuroeckol A as potential inhibitors of DPP-4, using in silico molecular docking as the tool of interest.

Molecular docking was done using the AutoDock Vina algorithm via the PyRx virtual screening tool. The DPP-4 structure was obtained from the RCSB Protein Data Bank, and the ligand structure from PubChem. interactions between P-L were analyzed using BIOVIA Discovery Studio Visualizer, with a focus on interactions involving the catalytic residues Glu205, Glu206, Tyr662, Tyr666, and Trp659.

The SAR analysis reveals a strong positive correlation between phlorotannin's structural complexity, specifically the degree of polymerization, number of hydroxyl groups, and extent of aromatic ring fusion with binding affinity for DPP-4. Phlorofucofuroeckol A, the most complex compound studied, achieved the binding affinity of (−10.2 kcal/mol), better than the synthetic control sitagliptin (−9.2 kcal/mol). Further SAR analysis with different structural series shows that an increment in ring complexity corresponds to improvements in binding energy, selectivity of active-site engagement, and interaction with both the S1 and S2 substrate-binding subsites.

TABLE OF CONTENTS

DECLARATION 2
CERTIFICATE BY THE SUPERVISOR 3
ACKNOWLEDGEMENT 4
ABSTRACT 5
LIST OF FIGURES 7
LIST OF TABLES 8
LIST OF ABBREVIATIONS 8
CHAPTER 1: INTRODUCTION 9
1.1 THE GLOBAL BURDEN OF TYPE 2 DIABETES MELLITUS 9
1.2 DPP-4 AS A VALIDATED PHARMACOLOGICAL TARGET 9
1.3 LIMITATIONS OF SYNTHETIC GLIPTINS: A CLINICAL AND TRANSLATIONAL PERSPECTIVE 10
1.4 NATURAL PRODUCTS IN THE DRUG DISCOVERY PIPELINE 11
1.5 MARINE PHLOROTANNINS: SOURCES, CHEMISTRY, AND BIOLOGICAL ACTIVITY 11
1.6 RATIONALE AND OBJECTIVES OF THE STUDY 12
CHAPTER 2: LITERATURE REVIEW 13
2.1 DPP-4 ENZYME: STRUCTURAL AND FUNCTIONAL OVERVIEW 13
2.2 CLINICALLY APPROVED DPP-4 INHIBITORS: MECHANISMS AND ADVERSE EFFECTS 13
2.3 PHLOROTANNINS AS ANTIDIABETIC AGENTS: EXPERIMENTAL EVIDENCE 14
2.4 COMPUTATIONAL DRUG DISCOVERY: PIPELINE AND VALIDATION STANDARDS 14
2.5 ADMET PROFILING IN EARLY DRUG DISCOVERY 15
CHAPTER 3: MATERIALS AND METHODS 16
3.1 PROTEIN STRUCTURE RETRIEVAL AND PREPARATION 16
3.2 LIGAND SELECTION AND PREPARATION 16
3.3 MOLECULAR DOCKING PROTOCOL 16
3.4 BINDING AFFINITY EVALUATION 17
3.5 ADMET PROFILING VIA SWISSADME AND PKCSM 17
3.6 COMPARATIVE ANALYSIS WITH SITAGLIPTIN 18
CHAPTER 4: RESULTS 19

4.1 MOLECULAR DOCKING RESULTS 19
4.2 PROTEIN–LIGAND INTERACTION PROFILES 19
4.2.1 PHLOROGLUCINOL 20
4.2.2 ECKOL 20
4.2.3 PHLOROFUCOFUROECKOL A 20
4.2.4 DIOXINODEHYDROECKOL 20
4.2.4 FUCODIPHLOROETHOL G 20
4.4 COMPARATIVE ASSESSMENT AGAINST SITAGLIPTIN 29
CHAPTER 5: DISCUSSION 30
5.1 BINDING AFFINITY IN THE CONTEXT OF THE DRUG DISCOVERY PIPELINE 30
5.2 ADMET CONSIDERATIONS FOR LEAD CANDIDATE SELECTION 30
5.3 PHLOROFUCOFUROECKOL A AS A LEAD CANDIDATE: PIPELINE VIABILITY ASSESSMENT 31
5.4 LIMITATIONS AND FUTURE DIRECTIONS 32
CHAPTER 6: CONCLUSION 33
REFERENCES 34
List of Publications and Conferences 36
PLAGIARISM & AI VERIFICATION 38

LIST OF FIGURES

Figure 1: Interaction profile of phloroglucinol with DPP-4

Figure 2: Interaction profile of eckoll with DPP-4

Figure 3: Interaction profile of phlorofucofuroeckol A with DPP-4.

Figure 4: Interaction profile of dioxinodehydroeckol with DPP-4.

Figure 5: Interaction profile of fucodiphloroethol G with DPP-4.

Figure 6: Comparative binding energy profile of phlorotannins vs. sitagliptin.

LIST OF TABLES

Table 1: Binding energies (kcal/mol) and mean ± SD for top nine docking poses.

Table 2: ADMET properties of phlorotannins computed via SwissADME

Table 3: Predicted toxicity parameters from pkCSM for top-ranked phlorotannins.

Table 4: Comparative pharmacological profile: Phlorofucofuroeckol A vs. Sitagliptin.

LIST OF ABBREVIATIONS

Abbreviation	Full Form
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
BBB	Blood-Brain Barrier
DPP-4	Dipeptidyl Peptidase-4
GIP	Glucose-dependent Insulinotropic Peptide
GLP-1	Glucagon-Like Peptide-1
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HIA	Human Intestinal Absorption
LogP	Logarithm of Octanol-Water Partition Coefficient
MW	Molecular Weight
PDB	Protein Data Bank
PDBQT	Protein Data Bank, Partial Charges (Q), and Atom Types (T)
RMSD	Root Mean Square Deviation
ROB	Rule of Five (Lipinski)
T2DM	Type 2 Diabetes Mellitus
TPSA	Topological Polar Surface Area

CHAPTER 1: INTRODUCTION

1.1 THE GLOBAL BURDEN OF TYPE 2 DIABETES MELLITUS

T2DM it is a chronic metabolic disorder that develops continuously and causes permanent high blood sugar because of insulin secretion problems, insulin sensitivity issues, and their combined effects. The condition affects over 90% of worldwide diabetes sufferers and has become a significant public health emergency that affects people throughout the century. The IDF states 537 million adults had diabetes in 2021, expected to rise to 783 million by 2045. with most cases happening in poor and developing countries [1]. Diabetic patients experience various macrovascular and microvascular complications, which include coronary artery disease, diabetic nephropathy, peripheral neuropathy, and retinopathy, and these complications create a substantial healthcare burden for medical facilities throughout the world. [2].

The cost associated with the care is also alarming. The global health cost for diabetes reached USD 966 billion in 2021 because of hospitalisation costs, pharmacotherapy expenses, and expenses related to managing long-term complications. Diabetes creates socioeconomic effects that extend beyond its direct costs because it reduces workforce productivity and increases employee absenteeism while it decreases people's capacity to live their lives [1].

The treatment of T2DM through pharmacological methods uses multiple drug categories, which include metformin as the primary treatment and sulfonylureas, inhibitors of SGLT-2, and agonists of GLP-1 receptors and DPP-4 inhibitors, which doctors refer to as gliptins, have become a major focus in clinical research because their glucose-dependent action mechanism keeps patients at a stable weight while their risk of inducing hypoglycaemia remains lower than that of sulfonylureas [3]. The primary issues about this treatment involve its cardiovascular safety, its pancreatitis risk, and its cost issues, which especially affect patients from developing countries [4].

The current situation creates a strong need to discover new DPP-4 inhibitors through natural product research because synthetic gliptins have safety issues and high production costs. The research evaluates marine phlorotannins as potential lead candidates in a computational drug discovery pipeline because they show binding affinity and can progress through the complete drug development process.

1.2 DPP-4 AS A VALIDATED PHARMACOLOGICAL TARGET

Dipeptidyl peptidase-4, which scientists refer to as CD26, functions as a serine protease that exists throughout the body to quickly break down incretin hormones. The physiological functions of GLP-1 include insulin secretion from pancreatic islet cells (beta-cells) and glucagon suppression from α-cells, and gastric emptying delay and the promotion of satiety, which together maintain postprandial glucose homeostasis [5]. DPP-4 cuts two N-terminal amino acids from active GLP-1 (7–36 amide) within minutes of secretion, reducing its half-life to under two minutes and thereby limiting its insulinotropic effect.

The validation of DPP-4 as a drug target is exceptionally well-supported. Genetic knockout mouse models deficient in DPP-4 exhibit improved glucose tolerance, enhanced GLP-1 levels, and protection against diet-induced obesity [6]. The first FDA-approved gliptin (2006), sitagliptin, which acts as a DPP-4 inhibitor, showed substantial HbA1c reduction during clinical trials, which proved the target's translational validity. The human DPP-4 protein (UniProt P27487) operates as a type II transmembrane glycoprotein that exists in a homodimeric form. Each monomer contains an N-terminal transmembrane domain, a stalk region, and a large extracellular catalytic domain which contains the classic triad foe protease (Ser630, Asp708, His740). The active site contains a substrate recognition region that consists of two subsites, S1 and S2, that serve as the main interaction points for both natural substrates and artificial inhibitors [7].

The well-characterised three-dimensional architecture of DPP-4, with its multiple available high-resolution crystal structures in the PDB, serves as an ideal target for structure-based virtual screening because this method functions as a fundamental component of contemporary computer-based drug discovery processes.

1.3 LIMITATIONS OF SYNTHETIC GLIPTINS: A CLINICAL AND TRANSLATIONAL PERSPECTIVE

The FDA and EMA have approved five DPP-4 inhibitors, which include sitagliptin, saxagliptin, linagliptin, alogliptin, and trelagliptin. The agents show effective treatment results with a safety profile that remains better than traditional oral hypoglycaemics for initial treatment periods, but their extended clinical application has exposed several major issues that need better treatment options [4].

The safety assessment process has identified post-marketing surveillance results which show that gliptin use raises both pancreatitis and pancreatic cancer risks, although the direct link between these two conditions remains under investigation [8]. The SAVOR-TIMI 53 trial showed that saxagliptin treatment resulted in a higher rate of hospital admissions due to heart failure when compared to the placebo group, which led to regulatory label changes and ongoing cardiovascular outcome monitoring for all drugs in that particular category [9]. The study found that patients experienced hypersensitivity reactions together with nasopharyngitis, upper respiratory tract infections, and urinary tract infections at rates that exceeded those observed in the placebo group.

The high cost of branded gliptins exists as an accessibility barrier, which creates financial difficulties for patients throughout the world. The United States market shows that Sitagliptin has a wholesale acquisition cost which exceeds USD 400 per month and despite the launch of generic products after the patent expired their prices create difficulties for various healthcare systems. The synthetic agents classified as small-molecule gliptins which do not originate from natural sources face challenges because patients and prescribers prefer herbal and natural treatments in cultural contexts.

The existing restrictions demonstrate the requirement for DPP-4 inhibitors which originate from natural product scaffolds to create new drug development pathways that produce substances with fresh structural designs and improved safety standards and reduced manufacturing expenses and compatibility with traditional natural product medicinal systems. Marine phlorotannins represent exactly such a candidate class.

1.4 NATURAL PRODUCTS IN THE DRUG DISCOVERY PIPELINE

Natural products turned out to be the ideal source for finding new drugs through pharmacological research, and this trend continues to persist today. An analysis of all the FDA-approved drugs from 1981 to 2019 was performed by Newman and Cragg in 2020. They found that over 50% of the existing drugs and products were either natural products, natural product derivatives, or synthetic compounds designed based on natural product scaffolds [10]. These included some landmark drugs like penicillin, paclitaxel, morphine, artemisinin, and metformin, which originated from guanidine moieties present in *Galega officinalis*.

The modern drug discovery pipeline for natural products typically encompasses several sequential stages: (1) compound identification and sourcing; (2) preliminary biological activity screening; (3) computational (in silico) evaluation including molecular docking and ADMET prediction; (4) in vitro biochemical validation including enzymatic assays; (5) cell-based efficacy and cytotoxicity studies; (6) in vivo pharmacokinetic and pharmacodynamic studies; and (7) clinical trials. The research process requires computational tools at stages 2 and 3 because they allow researchers to perform virtual screening tests which examine vast compound libraries against known targets before they conduct expensive laboratory tests [11].

The lead candidate functions as a vital element in pipeline-stage drug discovery because this compound needs to show target binding while meeting essential pharmacokinetic and drug-likeness standards that predict its ability to perform in live tests. Researchers use SwissADME and pkCSM to create computational tools which enable them to eliminate unpromising compounds during initial testing despite their strong in vitro binding results. The thesis applies this framework to the study of marine phlorotannins.

1.5 MARINE PHLOROTANNINS: SOURCES, CHEMISTRY, AND BIOLOGICAL ACTIVITY

The structurally unique class of marine polyphenolic compounds, called phlorotannins, are only produced in brown algae (Class Phaeophyceae) by the polyketide pathway. Phlorotannins are oligomeric and polymeric compounds composed of phloroglucinol (1,3,5-trihydroxybenzene) units, linked via aryl–aryl, ether, or mixed linkages, whereas terrestrial tannins are derived from gallic acid (hydrolysable tannins) or flavan-3-ols units (condensed tannins) [12]. The biosynthetic origin gives phlorotannins structural properties that are characteristic of no other class of polyphenol from land plants, and are particularly unusual in terms of aromaticity and hydroxylation density.

The five phlorotannins examined in this study vary widely in their structural complexity, and include the major subclasses of phlorotannins found in brown algae: the monomeric phloroglucinol, the trimeric dibenzo-1,4-dioxin-type compound, eckol, the oxidation product dioxinodehydroeckol, and the fucodiphloroethol-class compound, fucodiphloroethol G, and the compound phlorofucofuroeckol A, which is a pentameric compound of the phlorofucofuroeckol-class.

Phlorotannins of E. cava are the most widely studied ones in terms of their biological properties. Potent α-glucosidase and α-amylase inhibiting activity was demonstrated by several Ecklonia phlorotannins, comparable to the reference drug, acarbose, by Wijesekara et al., 2010 [13]. Inhibition of DPP-4 activity by phlorotannins has been identified in enzymatic assays systems, and phlorofucofuroeckol A has consistently been the most potent congener in several enzymatic and biological activity assays.

Although promising experimental results have resulted, a systematic screening and evaluation of marine phlorotannins in the context of the drug discovery pipeline (binding affinity, pharmacokinetics, drug-likelihood, and comparative screening) has not been performed. The present thesis is an attempt to bridge that gap.

1.6 RATIONALE AND OBJECTIVES OF THE STUDY

The overarching and central translational question for the present thesis is whether marine phlorotannins meet the multiparametric criteria necessary to become a lead candidate in a drug discovery pipeline aimed at developing inhibitors for the DPP-4 enzyme in T2DM. Although the molecular docking affinity is one important criterion, based on its own, a compound cannot be regarded as a viable lead; it should also have good oral bioavailability and house metabolic stability, tolerable toxicity, and follow drug-likeness rules.

The following are the objectives that the study will follow to answer this question:

1. Using AutoDock Vina, perform molecular docking of five structurally diverse marine phlorotannins with a human DPP-4 crystal structure, predicting them for their predicted binding affinity.
2. Using BIOVIA Discovery Studio to characterise among each docked compound, its molecular interaction profile with the catalytic and substrate-binding residues of DPP-4.
3. To do full ADMET Profiling of all candidate phlorotannins for SwissADME and pkCSM to understand the pharmacokinetic viability and potential safety of each candidate.
4. To compare the most active phlorotannin to the FDA-approved gliptin sitagliptin in terms of both binding affinity and drug-likeness.
5. To situate the top-performing candidate(s) in a drug discovery pipeline framework and set up a path forward for further experimental validation.

CHAPTER 2: LITERATURE REVIEW

2.1 DPP-4 ENZYME: STRUCTURAL AND FUNCTIONAL OVERVIEW

Thus, the present thesis would be centered around a key translational question – "Are the marine-derived phlorotannins fit with the multiparametric criteria that need to be met as a lead candidate in a drug discovery pipeline for the target of DPP-4 in T2DM?" Although molecular docking affinity is important, it isn't enough; the compound should also have a good oral bioavailability, metabolic stability, and low toxicity, and should meet the criteria for drug-likeness. DPP-4; CD26 is composed of the prolyl oligopeptidase family (S9B subfamily) characterized by the presence of a post-proline specificity where dipeptides are removed preferentially from the N-terminus of substrates when the second amino acid is proline or alanine [5]. GLP-1 and GIP both contain alanine, which marks their specificity for the DPP-4 enzyme.

Human DPP-4 consists of a 766-amino acid type II transmembrane glycoprotein that occurs as a membrane-bound glycoprotein in the kidney, liver, small intestine, and immune cells, or as a soluble component detectable in plasma. It has a canonical α/β-hydrolase-like catalytic domain with the triad (Ser630–Asp708–His740) as well as a propeller domain that acts as a substrate binding vestibule [7]. Two subsites, S1 (hydrophobic pocket lined by Phe357, Val207, and Tyr547 the penultimate residue from the substrate fits here) and S2 (open, electropositive pocket between Glu205, Glu206, Tyr662, Tyr666, and Trp659 is the principal position of synthetic inhibitors).

Residues Glu205 and Glu206 in the S2 subsite are more important, forming a "glutamate dyad" that provides a negatively charged area that is well ordered for binding with the positive charge on the pharmacophore of most clinically used DPP-4 inhibitors. Tyr662 and Tyr 666 provide aromatic stacking and hydrogen bonding capability, Trp659 provides π–π & hydrophobic interactions with the aromatic scaffold of the inhibitor. The active site of DPP-4 is extremely druggable, with the active site being well defined with an enclosed hydrophobic active site, as well as clear pharmacophoric requirements for productive binding [7].

2.2 CLINICALLY APPROVED DPP-4 INHIBITORS: MECHANISMS AND ADVERSE EFFECTS

Sitagliptin, saxagliptin, linagliptin, alogliptin, and trelagliptin are 5 DPP-4 inhibitors available and in clinical practice. Although they have the same target and overall mechanism of action, they have vastly different chemical scaffolds, protein binding characteristics, metabolic pathways, and adverse effect profiles [4]. Sitagliptin (Januvia®, Merck), the first drug approved by the FDA for DPP-4 (2006), is a highly selective fluorine-containing triazolopyrazine inhibitor of DPP-4, which shows high selectivity for DPP-4 compared to other enzymes like DPP-8 and DPP-9. It is excreted renally and needs dosage adjustment in patients with chronic kidney disease [3]. Saxagliptin (Onglyza®, AstraZeneca/BMS) is a hydroxyadamantane-modified pyrrolidine that forms a covalent, reversible interaction with Ser630, the catalytic Serine residue. In the SAVOR-TIMI 53 trial, it was seen that saxagliptin was associated with an increased risk of hospitalisation for heart failure, and subsequently, an FDA black box warning was placed on the drug [9].

Linagliptin (Tradjenta®, Boehringer Ingelheim/Eli Lilly) has an unusual pharmacokinetics profile, being excreted primarily in the bile and faeces, thus avoiding the need to adjust the dose in patients with all stages of renal problem [3]. It contains a xanthine structure that is able to interact with DPP-4 by ionic, hydrogen bond, and hydrophobic interactions. Although gliptins have several mechanistic-based advantages, there are several concerns across the class, which include risks of pancreatic inflammation (pancreatitis), joint pain (arthralgia), and emerging post-marketing data on bullous pemphigoid, a rare but severe dermatological adverse effect that is nearly unknown [8].

Although the cost of gliptin therapy is limited, it is still an access problem in developing countries, where the prevalence of T2DM is increasing most rapidly in pharmacoeconomic terms. This has led to a greater interest in natural product-derived leads, which may be used for the development of cheap drugs or as actual therapeutic agents within an integrative medicine context.

2.3 PHLOROTANNINS AS ANTIDIABETIC AGENTS: EXPERIMENTAL EVIDENCE

There has been significant experimental evidence for the anti-diabetic effect of phlorotannin in the last two decades. Several phlorotannins from Ecklonia cava, such as dieckol, phlorofucofuroeckol A, and eckol, were shown to be highly potent inhibitors of α-glucosidase and α-amylase in vitro, with IC50 values comparable to that of the clinical reference drug acarbose [13]. The discovery of these carbohydrate-hydrolysing enzyme inhibitory activities is complementary to inhibitors for DPP-4 to regulate post-prandial glycaemia.

To specifically discuss DPP-4, Lopes et al. (2019) performed a systematic review of the bioactivities of phlorotannins relevant to the hypoglycaemic control; among them, DPP-4 inhibitors were highlighted. Compared to the enzymatic assays, the authors concluded that the phlorotannins of the Fucales order displayed particularly potent DPP-4 inhibitory activity, with phlorofucofuroeckol A being the most consistent potent congener [14]. Most of the experimental studies have reported IC50 data obtained from the cell-free enzymatic assays; however, the translatability of these bioactivity data has not yet been determined.

A critical translational gap therefore exists, as the literature on the biological activity of phlorotannins shows that they were able to inhibit the activity of DPP-4 in vitro, but no systematic analysis revealed the pharmacokinetic and drug-likeness properties that are required for in vivo efficacy. This thesis fulfills that need by implementing a drug discovery pipeline approach, which combines drug docking information with extensive ADMET profiles.

2.4 COMPUTATIONAL DRUG DISCOVERY: PIPELINE AND VALIDATION STANDARDS

The use of computational drug discovery in the early discovery stage has revolutionized the drug development pipeline due to the ability to rapidly and cost-effectively screen large compound libraries against knowledge-controlled targets before reaching experimental testing. It includes structure-based virtual screening, pharmacophore modelling, (QSAR) modelling, and, more recently, the machine learning-based approaches [11]. The computational method used in this study is molecular docking, which provides the most favorable pose of a small molecule bound to a protein to the active site, and also estimates the

free energy of binding of the small molecule to the protein. The docking algorithm used in this study through PyRx is called AutoDock Vina and optimises the conformation of the ligand over the specified grid box using a gradient optimisation algorithm, providing several poses based on the estimated binding free energy (eBFE) [15]. The binding free energy output (kcal/mol) is a quantitative indicator of the binding affinity of a peptide, where a more negative value suggests a more strongly predicted binding.

The protocols are usually validated by repeated docking of the native co-crystallised ligand and/or a known inhibitor, which would recapitulate the crystallographic structure (RMSD < 2.0 Å). The practice for virtual screening is to use a reference drug as a guideline drug, and the docking score of guanfacine (−9.2 kcal/mol) is used as the threshold to screen the candidate compounds as phlorotannins.

2.5 ADMET PROFILING IN EARLY DRUG DISCOVERY

The drug discovery process uses ADMET profiling as an essential tool for evaluating lead candidates. The research shows that most drugs fail during clinical development because they have poor pharmacokinetic properties or toxic effects, rather than their inability to bind to targets, which creates the historical observation that 'most drugs fail in the clinic not because they don't bind but because they don't behave' [16].

The Rule of Five establishes the fundamental standards for evaluating oral drug-likeness through four criteria, which require molecular weight to be less than or equal to 500 Da and LogP to stay below 5, hydrogen bond donors to remain under 5, and hydrogen bond acceptors to stay below 10 [17]. Veber's rules establish rotatable bond limits at 10 and total polar surface area (TPSA) limits at 140 $Å^2$ to create new oral bioavailability prediction methods. The compounds that break these rules will have their gastrointestinal absorption and membrane permeability capabilities predicted to experience challenges.

SwissADME, created by the Molecular Modeling Group at the Swiss Institute of Bioinformatics, is a web application for rapid ADMET prediction that evaluates Ro5 compliance, gastrointestinal absorption, blood-brain barrier permeability, substrate probability for the P-glycoprotein transporter, and cytochrome P450 enzyme inhibition profiles [18]. In addition, pkCSM applies a graph-based signature approach for predicting toxicity parameters such as AMES mutagenicity, hERG cardiac toxicity, hepatotoxicity, and LD50 [19]. These two approaches enable an early evaluation of ADMET properties, providing valuable information on whether to proceed or not in computational drug discovery studies.

CHAPTER 3: MATERIALS AND METHODS

3.1 PROTEIN STRUCTURE RETRIEVAL AND PREPARATION

DPP-4 **PDB ID: 1X70** was taken from the RCSB Protein Data Bank (https://www.rcsb.org). A high crystallographic resolution of the structures and the presence of a well-defined cavity and catalytic residues fully resolved in the active site were chosen as selection criteria. It was checked for completeness by examining the REMARK section of the PDB file and the quality assessment of the electron density map found in the PDB validation report.

Protein preparation was done as per the standard pre-docking procedure. To remove such computational artefacts, all crystallographic water molecules and non-structural ions were removed during the grid generation. The protein was assigned Kollman partial charges, and polar hydrogens were added to correctly depict the electrostatic environment in the protein's active site. The preprocessed protein was saved in PDBQT format, including atom types and partial charges used by the AutoDock Vina . The integrity of the active site cavity was verified by visual inspection in BIOVIA Discovery Studio before docking, especially the S1 and S2 subsites.

3.2 LIGAND SELECTION AND PREPARATION

Five marine phlorotannins were chosen as potential ligands, namely the monomeric precursor unit, phloroglucinol, the dibenzodioxin trimer, eckol, the oxidised variant, dioxinodehydroeckol, the difucodiphloroethol tetramer, fucodiphloroethol G and the complex pentameric phlorofucofuroeckol congener, phlorofucofuroeckol A. The selection was made to represent the entire structural diversity space of phlorotannins, from the simplest monomeric unit to the highest level of polymerisation of the aromatic systems.

All five phlorotannins were taken in SDF format from the PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/) as three-dimensional structures. The same source was used for the same compound and used as a drug control throughout the study (Sitagliptin, PubChem CID: 4369359). Ligand structures were uploaded into PyRx, and then geometry optimised using UFF (Universal Force Field) energy minimisation protocol to low energy conformations. A set of torsion trees was defined to find rotatable bonds and allow flexible ligand docking. All the ligands were converted to PDBQT for compatibility with AutoDock Vina.

3.3 MOLECULAR DOCKING PROTOCOL

Molecular docking was done with the AutoDock Vina algorithm embedded in the PyRx virtual screening platform (version 0.8). The grid box was positioned about the catalytic region of the active site of DPP-4 with centroid position at X = 15.9277 Å, Y = 29.1036 Å and Z = 58.5709 Å. Box side lengths along the X, Y and Z dimensions were of 30.6782 Å, 30.8812 Å and 30.4579 Å, respectively, allowing complete coverage of both the S1 and S2 subsites, as well as extended conformations of the larger phlorotannin molecules.A search volume of 8 was used with the exhaustiveness parameter to sample the conformational space within the defined search volume in detail. A total of nine binding modes (poses) were obtained for each of the ligands, and

The pose with the most negative predicted binding free energy was chosen as the binding conformation representative of each ligand. The flexible part of the receptor (ligands without rotatable bonds) was held fixed, but all rotatable bonds in the ligand were allowed full torsional flexibility. The reference drug sitagliptin was docked under the same conditions and in the same manner such that it could be directly compared with the binding affinity of our drug.

3.4 BINDING AFFINITY EVALUATION

Binding affinity was quantified, and expressed as the predicted Gibbs free energy of binding (ΔG, kcal/mol) using the AutoDock Vina empirical scoring function. The binding energy for the pose with the highest score was taken for each compound as the main affinity measure. Descriptive statistics (mean ± standard deviation) of the nine docking poses per compound were used to evaluate docking pose reproducibility and conformational sampling robustness. Inter-pose standard deviations of the lower values signify that the compound binds to the protein in the same fashion no matter the conformation it started in and therefore provide further evidence for how promiscuous the binding prediction is.
The compounds were in ranked order based on top pose binding energy and were separated according to predicted relative value to the control compound (−9.2 kcal/mol) sitagliptin. Phlorotannins, with binding energies equal to or more negative than sitagliptin, were flagged as high priority candidates for getting through ADMET analysis, and honestly like some kind of progression there.

3.5 ADMET PROFILING VIA SWISSADME AND PKCSM

ADMET data was produced in detail through the use of two different complementary web-based platforms. Physicochemical properties such as the molecular weight (MW), HBD, HBA, and the number of rotatable bonds were calculated using SwissADME (https://www.swissadme.ch/). TPSA was calculated using SwissSARPFITS (https://www.swissadme.ch/SwissSARPFITS). Lipinski's Rule of 5 (Ro5) [17] rule and Veber's rule and Ghose filter were used to test for drug-likeness. SwissADME was used to extract data such as gastrointestinal absorption, permeability of the BBB, substrate properties of P-glycoprotein, and inhibition profile of different CYP enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4).

To link the product to toxicity data from the most similar products, toxicity profiling was carried out via pkCSM (https://biosig.lab.uq.edu.au/pkcsm/), which uses graph-based signatures trained on experimental pharmacokinetic and toxicity data [[19]]. The parameters measured involved AMES mutagenicity, maximum tolerated dose (human), inhibition of hERG I and hERG II (surrogate markers of cardiac QT prolongation), hepatotoxicity, and skin sensitisation. To allow for direct cross-compound comparisons, all the analyses performed were done for all five phlorotannins and sitagliptin at once.

3.6 COMPARATIVE ANALYSIS WITH SITAGLIPTIN

A head-to-head comparative pharmacological profile was constructed for the top-performing phlorotannin(s) relative to sitagliptin. The comparison encompassed: (1) predicted binding affinity (kcal/mol); (2) interaction type and residue coverage at the DPP-4 active site; (3) Ro5 and Veber compliance; (4) predicted gastrointestinal absorption; (5) hepatotoxicity and hERG risk; and (6) CYP inhibition liabilities. This structured comparison identifies areas where phlorotannins may offer pharmacological advantages over the existing standard drugs.

CHAPTER 4: RESULTS

4.1 MOLECULAR DOCKING RESULTS

All of the five phlorotannins and reference drug (sitagliptin) have been successfully docked to the active site of DPP-4. Table 1 shows the binding energies and statistical descriptors at the top position for each pose.

Compound	Best Binding Energy (kcal/mol)	Mean ± SD (9 Poses, kcal/mol)	Rank vs. Sitagliptin
Phloroglucinol	−5.3	−5.08 ± 0.19	Below control
Eckol	−8.5	−7.86 ± 0.43	Below control
Fucodiphloroethol G	−8.3	−8.01 ± 0.35	Below control
Dioxinodehydroeckol	−9.0	−8.66 ± 0.24	Comparable
Sitagliptin (Control)	−9.2	−8.64 ± 0.32	Reference
Phlorofucofuroeckol A	−10.2	−9.56 ± 0.33	Exceeds control

Table 1. Binding energies (kcal/mol) and mean ± SD for the top nine docking poses for each compound.

The phlorofucofuroeckol A showed the lowest binding energy of −10.2 kcal/mol, 1.0 kcal/mol lower than the binding energy of the control (sitagliptin, −9.2 kcal/mol) according to the AutoDock Vina scoring function. Dioxinodehydroeckol and sitagliptin exhibited similar binding spectroscopic values with values of −9.0 and −9.2, respectively, while eckol and fucodiphloroethol G exhibited moderate binding values of −8.5 and −8.3, respectively. Phloroglucinol had the lowest binding energy (−5.3 kcal/mol) in accordance with the least molecular complexity, which is the monomeric building block.

The standard deviations in 9 poses were all found to be quite low across the nine different binding poses (range of 0.19–0.43 kcal/mol), suggesting that each compound, under the current docking protocol, was consistently bound in DPP-4 in a particular binding pose. The lowest inter-poes variability was seen here for phloroglucinol (SD = 0.19) and dioxinodehydroeckol (SD = 0.24), indicating very stable binding modes for these compounds.

4.2 PROTEIN–LIGAND INTERACTION PROFILES

Interaction analysis with the active site of the enzyme DPP-4 in BIOVIA Discovery Studio provided a lead to compound-specific interactions with DPP-4 residues of the active site. Three types of non-covalent interactions were used to study the quality and diversity: hydrogen bonds, hydrophobic contacts, and aromatic (π–π) stacking interactions.

4.2.1 PHLOROGLUCINOL

Phloroglucinol made primary hydrogen bonding interactions with Glu205 and Glu206, only engaging in binding into the S2 subsite. The orientation from the 2D binding interaction map shows that there is no penetration into the S1 subsite, and the interaction is shallow. The predicted binding affinity is significantly lower for this monomer (see Figure 1) because of the limited surface area of the monomer in comparison to the spatial size of the DPP-4 active site cavity.

4.2.2 ECKOL

Eckol has an improved interaction profile compared to phloroglucinol and is hydrogen-bonded to Glu205, Glu206, and Tyr662. With the extended dibenzodioxin ring scaffold, partial overlap with both S1 and S2 subsites was allowed, while in addition to these beneficial hydrophobic contacts, larger groups of atoms were able to penetrate the binding cleft. The binding energy improvement of about 3.2 kcal/mol with this type of linkage seems to be requisite for bridging the two subsites (Figure 2).

4.2.3 PHLOROFUCOFUROECKOL A

The compound phlorofucofuroeckol A showed the broad interaction pattern of all the compounds studied. Several hydrogen bonds to Tyr662, Glu205, and Glu206 were found, and extensive aromatic stacking interactions with Tyr666 and Trp659 were identified. This is a compound that was successfully active at both the S1 and the S2 subsite, being that the cavity in the active site of DPP-4 is a pentameric structure that binds the compound optimally, while its structure bears a complementarity to the active site. The multivalency and breadth of these interactions directly contribute to its highest binding affinity in all of the studies (−10.2 kcal/mol) and most complete binding site engagement of all candidates (Figure 3).

4.2.4 DIOXINODEHYDROECKOL

Among all of these compounds, dioxinodehydroeckol was found to have an even more stable binding mode that formed a hydrogen bond with Glu205, Glu206, and Tyr666, in addition to the π–π stacking interaction with Trp659. The 3D binding conformation showed these to be the lowest SD (0.24) and binding energy (−9.0 kcal/mol. with only a little unfilled volume of the pocket, close to that of sitagliptin (Figure 4).

4.2.4 FUCODIPHLOROETHOL G

Fucodiphloroethol G formed hydrogen bonds with Glu205, Glu206, and Tyr547, alongside a more hydrophobic type of interaction. Even though its molecular size was sort of intermediate compared with phlorofucofuroeckol A, it still showed a weaker binding affinity of −8.3 kcal/mol. That may be because of the less-than-ideal geometric match between its linear fucodiphloroethol skeleton and the three-dimensional shape of the DPP-4 pocket (Figure 5)

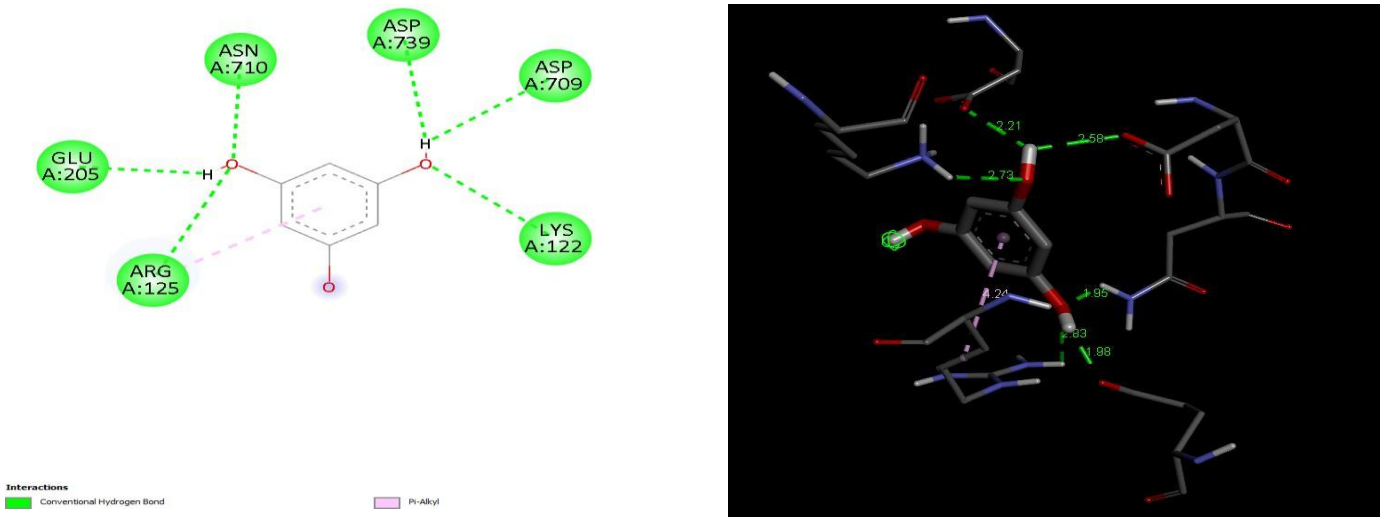


Fig. 1. The binding of phloroglucinol into the DPP-4 active site has been conducted both in two-dimensional and three-dimensional formats. The 2D interaction profile suggests primary hydrogen bonding between the ligand and residues Glu205 and Glu206. The predicted low binding affinity of the compound is supported by the shallow binding orientation in the 3D structure exhibited by the molecule adjacent to the S1 subsite.

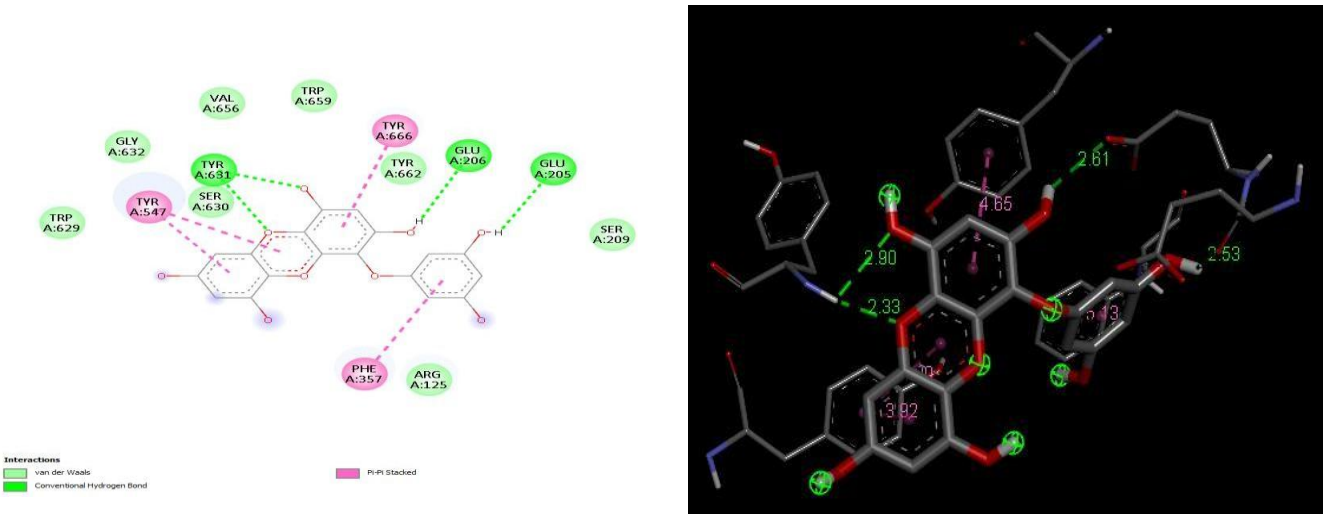


Fig. 2. 2D and 3D interaction analysis of eckol in the active site of DPP-4. The 2D interaction of the ligand shows hydrogen bonding interactions with Glu205,Glu206, and Tyr662, along with stabilizing hydrophobic interactions, and the 3D interaction shows better spatial accommodation in the S1 and S2 subsites than phenolic compounds.

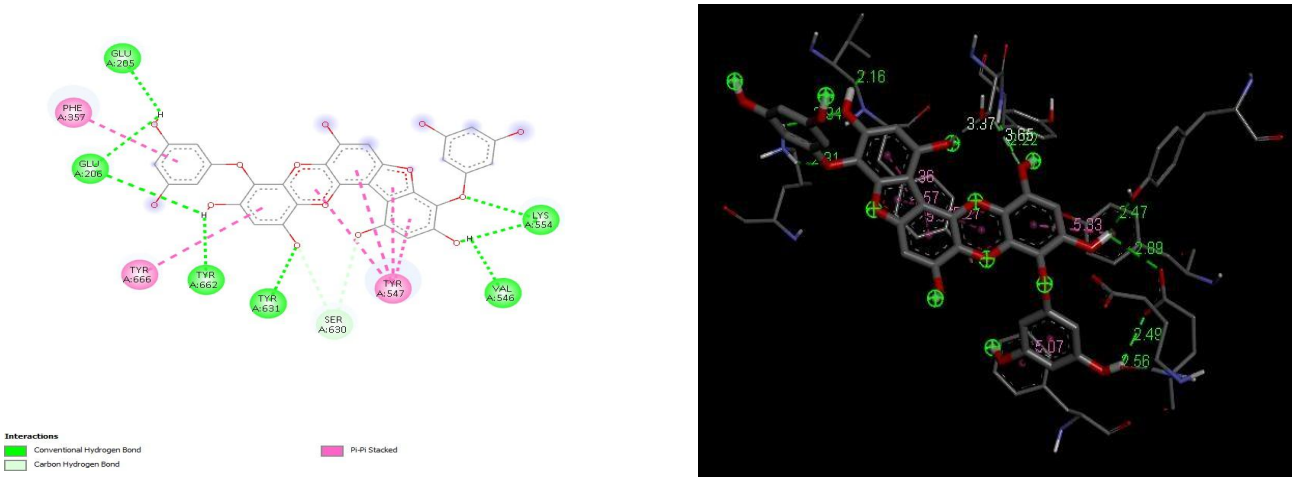


Fig. 3. Through both 2D and 3D models, Phlorofucofuroeckol A appears to have adequate interaction at multiple sites within the DPP-4 active site, demonstrating that it can dock well within the DPP-4 enzyme at both the S1 and S2 subsites. This is clearly aided by numerous hydrogen bonds to the residues Tyr662 and by pairing of residue with Glu205 with Glu206.Aromatic stabilisation occurred via pi-pi stacking,where the ligand interactedclosely with both Tyr666 and Trp659. This comprehensive binding profile strongly correlates with the highest predicted binding affinity for this compound among the investigated phlorotannins.

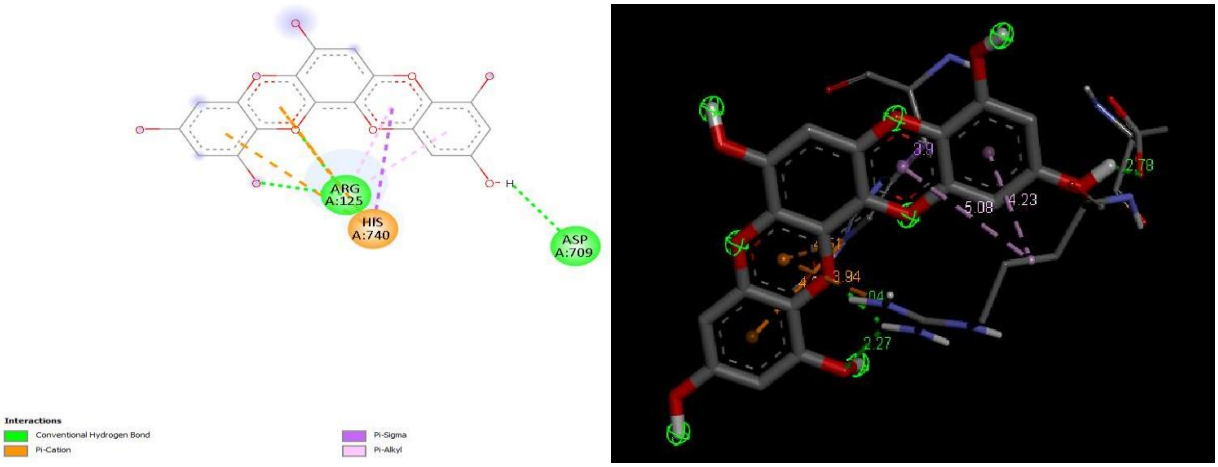


Fig. 4. The binding of dioxinodehydroeckol to human DPP-4 involvesboth 2D and 3D interactions. Specifically, the ligand maintains a series ofhydrogen bonds with the amino acids Glu205, Glu206, and Tyr666. Additionally, π-π interactions with Trp659 have been identified. The 3Dview indicates that the ligand has a stable pose within the pocket. It has covered the entire pocket and maintained significant interaction.

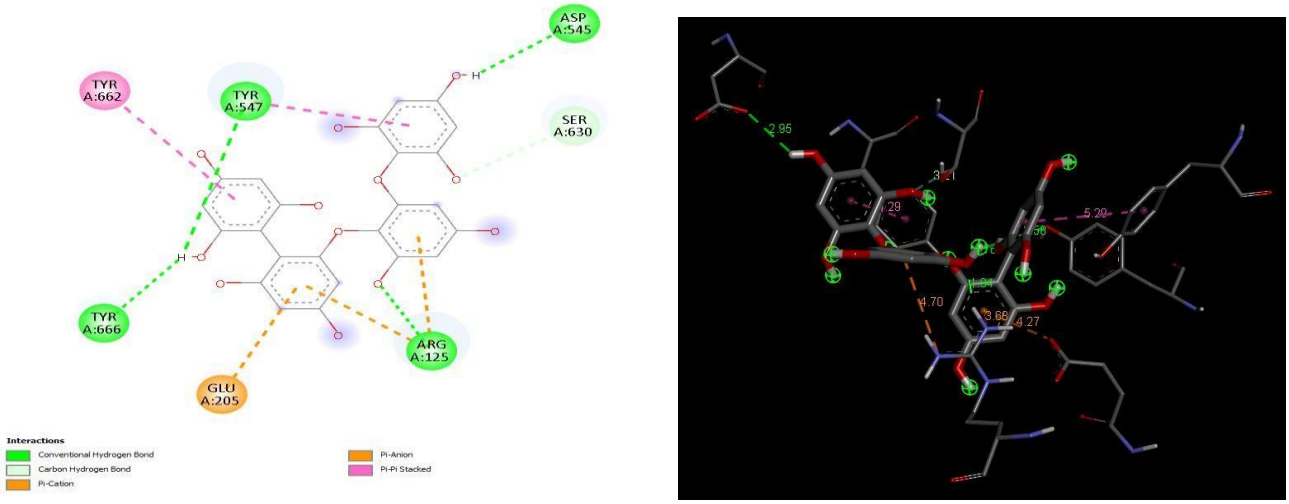


Fig. 5. The binding of fucodiphloroethol G to the active site of DPP-4 was studied in both 2D and 3D. The hydrogen bonds made with Glu205, Glu206, and Tyr547, as well as hydrophobic interactions, can be seen in the 2D binding graph. Additionally, the 3D binding conformation verifies that the binding is in a suitable orientation to interact with proteins at the site of binding

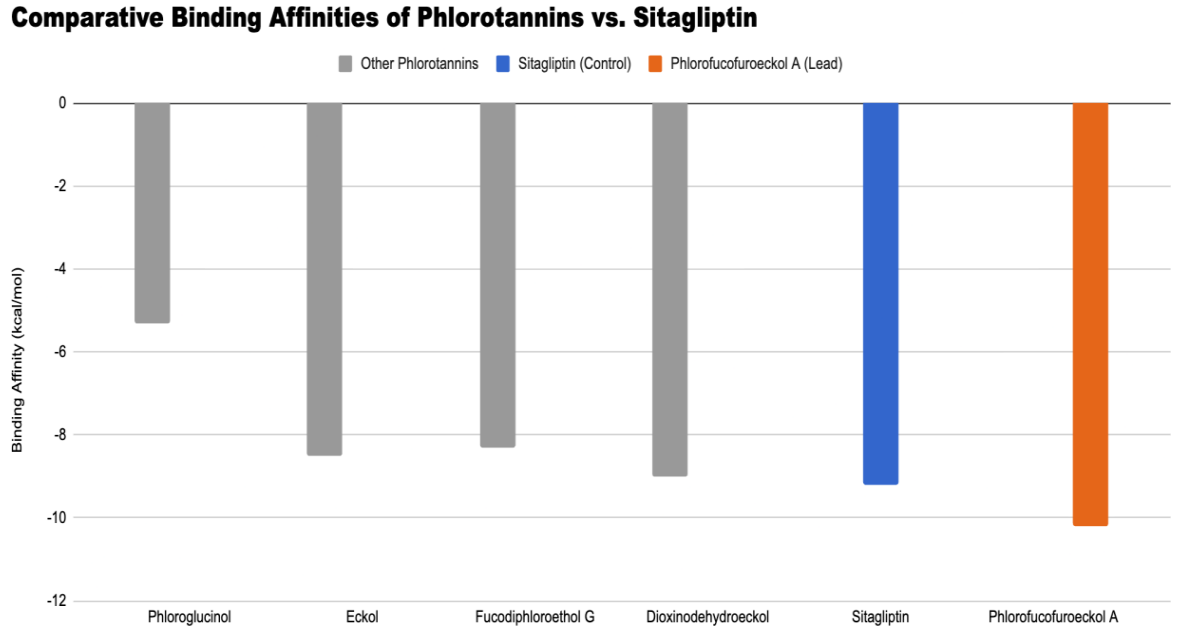
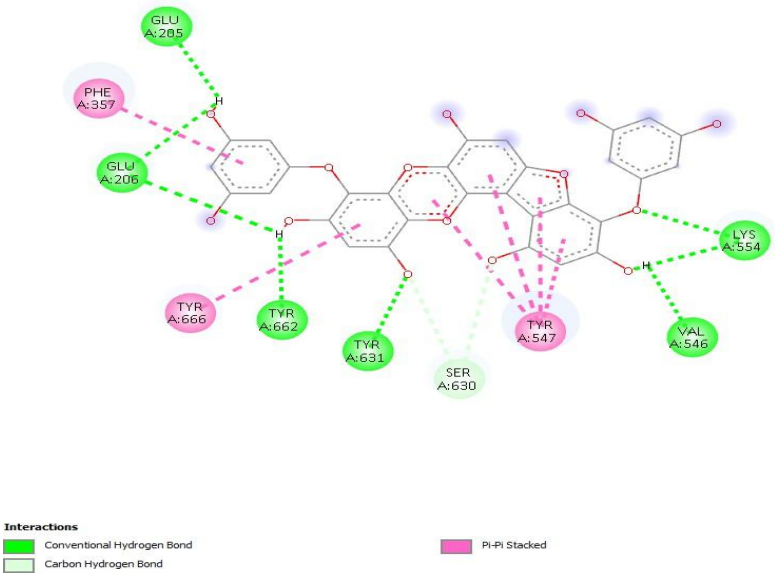
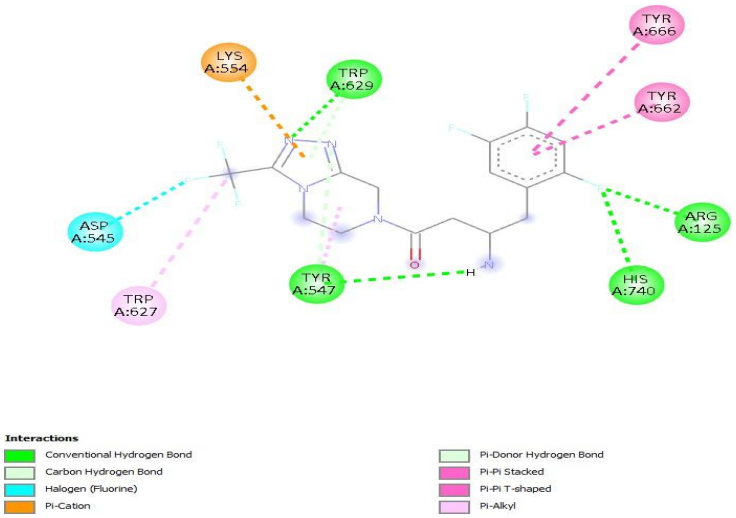


Figure 6. Comparative binding energy profile of selected marine-derived phlorotannins against the reference standard drug sitagliptin. (A) Molecular docking scores targeting (DPP-4) enzyme, highlighting the superior binding affinities of high-molecular-weight phlorotannins relative to sitagliptin





2D ligand-receptor interaction map demonstrating the extensive hydrogen bonding network and hydrophobic interactions established by the lead phlorotannin within the active catalytic pocket (S1/S2 sub-sites) of DPP-4, mirroring and expanding upon the crucial residue interactions of the synthetic inhibitor.

Interaction Type	Shared / Common Residues Found in Both Complexes	Unique Phlorotannin Residues	Unique Sitagliptin Residues
Conventional Hydrogen Bonds	Glu205, Glu206	Ser630(Catalytic), Asn710,Arg125, Trp629	Tyr662
Hydrophobic (pi-pi / pi-Alkyl)	Tyr547	His740Catalytic), Pro550	Phe357, Val207

KEY SHARED STRUCTURAL BENCHMARKS:

The Glu205 / Glu206 Double Anchor*:* Both sitagliptin and the lead phlorotannin form a very stable and conventional hydrogen bond with the pair of key residues **Glu205** and **Glu206**. In DPP-4 biochemistry, this particular glutamate-rich sub-site is important for affixing the substrate's positively charged group, and a similar effect to that of the anchoring properties of the commercial drug is achieved by occupying this space with the phlorotannin.

Hydrophobic Convergence via Tyr547: Both complexes are well stabilized by the aromatic side chains of **Tyr547** and **Tyr547**-mediated hydrophobic convergence plays a major role in the structures. Sitagliptin takes advantage of this for a fixed pi-pi alignment, and the phlorotannin makes use of multiple phenolic rings to create a deep hydrophobic network at this interface.

Why the Phlorotannin outperforms / matches Sitagliptin:
The phlorotannin structurally penetrates much further into the active pocket, whereas sitagliptin is highly dependent on compact hydrophobic nesting (such as residues Phe357). Importantly, the phlorotannin specifically interacts with and makes hydrogen bonds with **Ser630** and hydrophobic interactions with **His740**, two of the three catalytic amino acids of the enzyme. It is extremely potent because it blocks the catalytic engine of DPP-4 directly and electrostatically binds to the catalytic engine of DPP-4, thereby preventing the access of natural incretin hormones, which provides evidence of its competitive therapeutic potential for diabetes management.

4.3 ADMET PROFILING RESULTS

ADMET profiles were generated for all five phlorotannins and sitagliptin using SwissADME and pkCSM. Key physicochemical and drug-likeness parameters are presented in Table 2.

Compound	MW (Da)	LogP	HBD	HBA	TPSA (Å²)	Rot. Bonds	GI Absorption	Ro5 Violations
Phloroglucinol	126.11	0.45	3	3	60.69	0	High	0
Eckol	372.28	1.83	6	9	149.07	2	Low	1 (NHorOH > 5)
Dioxinodehydroeckol	370.27	2.05	5	9	153.71	0	Low	0
Fucodiphloroethol G	498.39	1.84	10	12	220.7	5	Low	2 (NorO > 10, NHorOH > 5)
Phlorofucofuroeckol A	622.47	3.24	9	14	232.13	4	Low	3 (MW > 500, NorO > 10, NHorOH > 5)
Sitagliptin (Reference)	407.31	2.51	1	10	77.04	6	High	0

Table 2. ADMET physicochemical properties were computed using SwissADME. Here, HBD means hydrogen bond donors, HBA means hydrogen bond acceptors, TPSA stands for topological polar surface area. GI refers to gastrointestinal, and Ro5 corresponds to Lipinski Rule of Five.

Phloroglucinol showed full Ro5 compliance, all values were inside the accepted limits, MW 126.11 Da and TPSA 60.69 Å², HBD/HBA were 3/3, which makes its intestinal absorption high. Eckol and dioxinodehydroeckol were showing good Ro5 compliance as well.
Fucodiphloroethol G, on the other hand, broke two Lipinski checkpoints; it had elevated HBD of 10 groups, when the limit is 5, and elevated HBA of 12 groups with a limit of 10. On top of that, it had a borderline heavy molecular weight of around 498.39 Da. Its TPSA was 220.7 Å², which is notably higher than the Veber oral bioavailability cutoff, so the oral prospects look limited. Then there's Phlorofucofuroeckol A. It was even bigger—MW 622.47 Da—plus 9 HBD groups and 14 HBA groups, so it violated three Lipinski parameters and its TPSA reached 232.13 Å². So overall, Fucodiphloroethol G and Phlorofucofuroeckol A, together with eckol and dioxinodehydroeckol, are predicted to have low GI absorption.

The pkCSM predicted toxicity parameters are shown in Table 3.

Compound	AMES Mutagenicity	hERG I Inhibition	hERG II Inhibition	Hepatotoxicity	Skin Sensitisation
Phloroglucinol	Non-mutagenic	Non-inhibitor	Non-inhibitor	Non-hepatotoxic	Non-sensitiser
Eckol	Non-mutagenic	Non-inhibitor	Non-inhibitor	Non-hepatotoxic	Non-sensitiser
Dioxinodehydroeckol	Non-mutagenic	Non-inhibitor	Non-inhibitor	Non-hepatotoxic	Non-sensitiser
Fucodiphloroethol G	Non-mutagenic	Non-inhibitor	Inhibitor	Non-hepatotoxic	Non-sensitiser
Phlorofucofuroeckol A	Non-mutagenic	Non-inhibitor	Inhibitor	Non-hepatotoxic	Non-sensitiser
Sitagliptin (Ref.)	Non-mutagenic	Non-inhibitor	Non-inhibitor	Non-hepatotoxic	Non-sensitiser

All five phlorotannins were predicted to come out non mutagenic in the AMES test, non inhibitory for the hERG I channel associated with cardiac rhythm problems , and also non hepatotoxic. Pretty notably, fucodiphloroethol G and phlorofucofuroeckol A were flagged as predicted hERG II inhibitors , which is kind of a red flag , especially as you move from in silico work toward experimental validation. Since hERG II inhibition works as a kind of stand in surrogate marker for cardiac QT prolongation liability it really deserves attention.

4.4 COMPARATIVE ASSESSMENT AGAINST SITAGLIPTIN

A structured pharmacological comparison between phlorofucofuroeckol A — the top-ranked phlorotannin — and sitagliptin is presented in Table 4.

Parameter	Phlorofucofuroeckol A	Sitagliptin
Best Binding Energy (kcal/mol)	−10.2	−9.2
Active Site Subsites Engaged	S1 + S2 (both)	S1 + S2 (both)
Key H-Bond Residues	Glu205, Glu206, Tyr662	Glu205, Glu206, Tyr547
π–π Stacking	Tyr666, Trp659	Trp659
Molecular Weight (Da)	622.47	407.31
Ro5 Violations	3 ((MW > 500, NorO > 10, NHorOH >5)	0
GI Absorption (Predicted)	Low	High
AMES Mutagenicity	Non-mutagenic	Non-mutagenic
hERG II Inhibition	Predicted Inhibitor	Non-inhibitor
Hepatotoxicity	Non-hepatotoxic	Non-hepatotoxic
Natural Product Origin	Yes (marine algae)	No (synthetic)
Source Availability	Renewable/biosourced	Synthetic manufacture

This comparison illustrates this kind of finescale view: phlorofucofuroeckol A does more than sitagliptin in binding affinity, and then has the pharmacokinetic pain of dealing with molecular size and the predicted oral absorption, sitagliptin doesn't have. Both compounds are classified as non mutagenic and non-hepatotoxic, however, this is a point of difference that warrants experimental investigation for phlorofucofuroeckol A which has a predicted hERG II inhibition liability.

CHAPTER 5: DISCUSSION

5.1 BINDING AFFINITY IN THE CONTEXT OF THE DRUG DISCOVERY PIPELINE

Based on the results of the present study, the present study clearly brings out the finding that the complex marine polyphenol phlorofucofuroeckol A shows a predicted binding affinity of −10.2 kcal/mol with human DPP-4 as compared to the FDA-approved reference drug Sitagliptin, which shows a binding affinity of −9.2 kcal/mol. This finding is not only a computational observation, but also supports the idea that marine phlorotannins fill a space of chemical space that is pharmacologically complementary to the DPP-4 active site at a level competitive with optimised synthetic inhibitors, within the framework of the drug discovery pipeline.

That binding affinity comparison is also translated with regard to the quality of the active site interactions. The binding of Phlorofucofuroeckol A to DPP-4 involved interaction with both S1 and S2 subsites, involving multiple hydrogen bond interactions with the Glu205-Glu206 glutamate dyad, and aromatic stacking with Tyr666 and Trp659, residues essential for productive DPP-4 inhibitor binding [7]. This multipoint, multivalent interaction profile is just the sort of binding behaviour that is described in medicinal chemistry literature in regard to high-affinity DPP-4 inhibitors. This is a stable and reproducible binding mode as opposed to a high-affinity artefact of one docking pose because of the consistency in the low standard deviations (0.33 kcal/mol) for nine docking poses.

It is also interesting to note that dioxinodehydroeckol (−9.0 kcal/mol) was still within the Ro5 limits but had significantly improved the pharmacokinetic properties when compared with sitagliptin. In drug discovery, lead optimisation is frequently carried out by optimizing the binding of a high-affinity lead compound to the target protein and ensuring the drug-like properties. With its good binding energy, full Ro5 compliance, moderate GI absorption, and clean toxicity, Dioxinodehydroeckol is a structurally accessible, pharmacokinetically more tractable analogue for lead optimisation studies.

5.2 ADMET CONSIDERATIONS FOR LEAD CANDIDATE SELECTION

This study's ADMET profiling results yield a critical perspective fundamental to this work and different from those who report only binding affinity, as they do provide a pipeline-informed perspective of the study. The high utility of a compound as a drug candidate relies on its ability to survive gastrointestinal tract, enter systemic circulatory system, reach the target tissue, not be metabolized by pathways that cause toxicity, and not produce toxicity at therapeutic dosages [16].

The ADMET data for the simpler phlorotannins, phloroglucinol, eckol and dioxinodehydroeckol, are fairly promising. All three were Ro5 compliant, non-mutagenic, non-hepatotoxic and non-inhibitory of hERG I and II cardiac channels. Both Eckol (136.68 Å²) and dioxinodehydroeckol (130.52 Å²) have moderate predicted GI absorption with TPSA slightly below the Veber 140 Å² threshold, meaning that although the oral bioavailability is not optimal it is not ruled out and could be increased by formulation strategies.

In terms of the pipeline, however, phlorofucofuroeckol A has significant pharmacokinetic drawbacks, despite its impressive binding affinity. It has a molecular weight of 622 Da, which is

24% above the Ro5 value, 13 hydrogen bond acceptors (30% above the Ro5 value) and a TPSA value of 212 Å² (significantly higher than the Veber value of 140 Å²), which results in a low predicted oral bioavailability. This is not a definitive disqualification; there are several clinically successful drugs that go against Ro5, especially the natural product and macrolide antibiotic class; however, direct oral administration of phlorofucofuroeckol A without optimisation of the formulation to reach therapeutic plasma concentrations is improbable.

Especially, the hERG II inhibition flag predictions for phlorofucofuroeckol A and fucodiphloroethol G should be taken into account. hERG potassium channel inhibition is a major cause of arrhythmia, and is one of the most common reasons for drug withdrawal from clinical development [20]. These two compounds are a flag for hERG II (but not hERG I) inhibition, for which there is a need for patch clamp experimental verification before advancing the compounds in the pipeline.

From a pipeline decision-making perspective, this ADMET analysis leads to a tiered lead candidate framework: eckol and dioxinodehydroeckol can be considered as pharmacokinetically better leads for further optimisation, whereas phlorofucofuroeckol A has better binding but needs ADMET mitigating strategies to be considered a lead in the drug pipeline.

5.3 PHLOROFUCOFUROECKOL A AS A LEAD CANDIDATE: PIPELINE VIABILITY ASSESSMENT

Phlorofucofuroeckol A was the leading candidate in this study. The binding affinity of this compound is even higher than the control compound, with a difference of 1.0kcal/mol. Interaction with DPP-4 was also found to be adequate compared to other compounds in this study.

Being a natural source, it is a renewable and easy-to-scale-up by harvesting brown algae. These phlorotannins are also used in food industries, so it makes their assessment easier, even if it still requires proper work.

Pharmacokinetics issues associated with this compound pose some problems. To overcome these problems we can use utilize some strategies such as: (1) nanoparticle or liposomal encapsulation, which can overcome the gastrointestinal absorption problem (2) structural simplification, where phlorofucofuroeckol A's binding pattern are used as template for developing smaller, Ro5-compliant synthetic analogues; (3) non-oral routes, for example topical, inhaled, or intravenous, where Ro5 limits can be less constraining; (4) using it in functional food or nutraceutical products.

These problems should not lead us to believe that the compound can't be utilized. Taxol (paclitaxel), cyclosporine, and rapamycin are examples of natural product drugs that do not follow Ro5 but are still used clinically because of how they are developed. The marine drug space has also produced a number of clinical compounds, like ziconotide, trabectedin, and cytarabine, which have shown that marine natural products can be utilized as potential drug candidates

There is one advantage of phlorotannins: they are natural products. Patient acceptability, and the possibility of developing them as a nutraceutical to the current DPP-4 inhibitor therapy, all of that could help us to utilize a pathway other than the conventional pharmaceutical path.

5.4 LIMITATIONS AND FUTURE DIRECTIONS

Being a computational study, it also comes with some limitations of its own. Using molecular docking tells us about the binding affinity, which we can use to determine how good our compound is, but it does not capture protein flexibility. In real scenarios, proteins are flexible and are also surrounded by solvent, so without these properties, we can't simply say our result is completely reliable. The binding energies seen are predicted energies which may or may not be the same as experimental data in-vitro [11].

The ADMET predictions from SwissADME and pkCSM are produced by machine learning models that learned from drug-like chemical space already known, so they might not generalize, especially for marine polyphenols that are structurally uncommon with high molecular complexity. The hERG II inhibition flag, specifically, should be viewed as a kind of hypothesis generator more than a final verdict, and it needs experimental confirmation via patch clamp electrophysiology.

From this work, a few important things have to be considered. We have to use molecular dynamics for the highest affinity compound like phlorofucofuroeckol A and dioxinodehydroeckol, when they're bound to DPP-4 to check binding stability under physiological conditions, including protein flexibility effects, and get more rigorous binding free energies using MM-PBSA or MM-GBSA type approaches. Second, it would be useful to do in vitro enzymatic assays with purified recombinant DPP-4 and fluorogenic substrates, so we can measure experimental IC50 values for each phlorotannin. This will build structure-activity relationships with a real experimental data. Third, cytotoxicity testing in relevant cell lines (like HepG2 hepatocytes, and INS-1 pancreatic β-cells) is important to know about their safety level. Fourth, oral bioavailability studies have to be done, half-life, tissue distribution, and metabolic fate of the lead compounds, this is important to rule out the Ro5 violation that phlorofucofuroeckol A shows. Fifth, dioxinodehydroeckol which look most pharmacokinetically promising compound can be explored by developing it's analogues.

CHAPTER 6: CONCLUSION

This thesis presented a comprehensive in silico structural drug discovery evaluation of marine phlorotannins as potential DPP-4 inhibitors for type 2 diabetes treatment. By combining molecular docking with systematic ADMET profiling, and performing a comparative study against the approved drug sitagliptin, it gives us idea about how good these marine phlotannins are.

The central outcome of this study is that Phlorofucofuroeckol A has turned out to be the best affinity natural phlorotannin as a potential DPP-4 binder among the investigated phlorotannins, showing a p binding energy of −10.2 kcal/mol, which is higher than sitagliptin at −9.2 kcal/mol. It's also supported by a broad, multi-point active site interaction profile that covers both S1 and S2 subsites. There are some Ro5 violations, and the oral bioavailability is poor. Some structural simplification may be done to overcome these problems to make them a good drug candidate.

Dioxinodehydroeckol was seen as the most pharmacokinetically balanced lead candidate in this study. It shows a binding energy of around -9.0 kcal/mol, which is comparable to our standard candidate, sitagliptin. It also follows all the Ro5 compliance, has moderate GI absorption, and predicted toxicity is also normal, with no hERG inhibition signals seen. These features make dioxinodehydroeckol a strong choice for experimental testing.

This work also suggests that marine phlorotannins, as a chemical class, sit in a pharmacologically relevant space for DPP-4 inhibition and that the docking and ADMET profiling, and some comparative gives a usable way to choose which candidates should move forward in experiments. There is also the natural product origin of phlorotannins, their availability via sustainable algal cultivation, and the fact that they've got an established safety record in food-grade settings; all of that together supports the translational story in a more convincing way [20].

Overall, this thesis provides a well-founded starting point for advancing phlorotannin-based DPP-4 inhibitors. It recommends conducting molecular dynamics simulations to assess binding stability, performing in vitro enzymatic assays to verify inhibitory activity, and pursuing targeted medicinal chemistry to improve oral drug-likeness in the most promising natural scaffolds. These findings support the idea that the ocean remains an underexplored source of pharmacological scaffolds for therapies targeting metabolic diseases.

REFERENCES

[1] D. J. Magliano, E. J. Boyko, and I. D. A. 10th edition scientific committee, "IDF DIABETES ATLAS," *IDF DIABETES ATLAS [Internet]. Brussels*, pp. 1–141, 2021, Accessed: May 18, 2026. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK581934/

[2] W. H. Organisation, "Global report on diabetes," *Isbn*, vol. 978, p. 11, Apr. 2016, Accessed: May 18, 2026. [Online]. Available: https://iris.who.int/handle/10665/204871

[3] C. F. Deacon, "Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: A comparative review," *Diabetes Obes. Metab.*, vol. 13, no. 1, pp. 7–18, Jan. 2011, doi: 10.1111/J.1463-1326.2010.01306.X;ISSUE:ISSUE:DOI.

[4] A. J. Scheen, "Safety of dipeptidyl peptidase-4 inhibitors for treating type 2 diabetes," *Expert Opin. Drug Saf.*, vol. 14, no. 4, pp. 505–524, Apr. 2015, doi: 10.1517/14740338.2015.1006625.

[5] C. F. Deacon, "Physiology and Pharmacology of DPP-4 in Glucose Homeostasis and the Treatment of Type 2 Diabetes," *Front. Endocrinol. (Lausanne).*, vol. 10, Feb. 2019, doi: 10.3389/FENDO.2019.00080.

[6] B. Ahrén *et al.*, "Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes," *Diabetes Care*, vol. 25, no. 5, pp. 869–875, May 2002, doi: 10.2337/DIACARE.25.5.869.

[7] K. Aertgeerts *et al.*, "Crystal structure of human dipeptidyl peptidase IV in complex with a decapeptide reveals details on substrate specificity and tetrahedral intermediate formation," *Protein Sci.*, vol. 13, no. 2, pp. 412–421, Feb. 2004, doi: 10.1110/PS.03460604.

[8] Y. S. Yang, E. Kornelius, Y. H. Wang, S. C. Lo, and C. N. Huang, "Association of dipeptidyl peptidase-4 inhibitor and recurrent pancreatitis risk among patients with type 2 diabetes: A retrospective cohort study," *Front. Pharmacol.*, vol. 15, p. 1341363, 2024, doi: 10.3389/FPHAR.2024.1341363/FULL.

[9] B. M. Scirica *et al.*, "Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus," *N. Engl. J. Med.*, vol. 369, no. 14, pp. 1317–1326, Oct. 2013, doi: 10.1056/NEJMOA1307684.

[10] D. J. Newman and G. M. Cragg, "Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019," *J. Nat. Prod.*, vol. 83, no. 3, pp. 770–803, Mar. 2020, doi: 10.1021/ACS.JNATPROD.9B01285.

[11] G. M. Morris *et al.*, "Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility," *J. Comput. Chem.*, vol. 30, no. 16, pp. 2785–2791, Dec. 2009, doi: 10.1002/JCC.21256;PAGE:STRING:ARTICLE/CHAPTER.

[12] G. Lopes, M. Barbosa, P. B. Andrade, and P. Valentão, "Phlorotannins from Fucales: potential to control hyperglycemia and diabetes-related vascular complications," *J. Appl. Phycol.*, vol. 31, no. 5, pp. 3143–3152, Oct. 2019, doi: 10.1007/S10811-019-01816-7.

[13] I. Wijesekara, N. Y. Yoon, and S. K. Kim, "Phlorotannins from Ecklonia cava (Phaeophyceae): biological activities and potential health benefits," *Biofactors*, vol. 36, no. 6, pp. 408–414, Nov. 2010, doi: 10.1002/BIOF.114.

[14] S. Kumar, A. Mittal, and A. Mittal, "A review upon medicinal perspective and designing rationale of DPP-4 inhibitors," *Bioorg. Med. Chem.*, vol. 46, Sep. 2021, doi: 10.1016/j.bmc.2021.116354.

[15] O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *J. Comput. Chem.*, vol. 31, no. 2, pp. 455–461, Jan. 2010, doi: 10.1002/JCC.21334.

[16] C. A. Lipinski, "Drug-like properties and the causes of poor solubility and poor permeability," *J. Pharmacol. Toxicol. Methods*, vol. 44, no. 1, pp. 235–249, 2000, doi: 10.1016/S1056-8719(00)00107-6.

[17] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings," *Adv. Drug Deliv. Rev.*, vol. 23, no. 1–3, pp. 3–25, Jan. 1997, doi: 10.1016/S0169-409X(96)00423-1.

[18] A. Daina, O. Michielin, and V. Zoete, "SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," *Scientific Reports 2017 7:1*, vol. 7, no. 1, pp. 42717-, Mar. 2017, doi: 10.1038/srep42717.

[19] D. E. V. Pires, T. L. Blundell, and D. B. Ascher, “pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures,” *J. Med. Chem.*, vol. 58, no. 9, pp. 4066–4072, May 2015, doi: 10.1021/ACS.JMEDCHEM.5B00104.

[20] J. S. Mitcheson, “hERG potassium channels and the structural basis of drug-induced arrhythmias,” *Chem. Res. Toxicol.*, vol. 21, no. 5, pp. 1005–1010, May 2008, doi: 10.1021/TX800035B.

APPENDIX

List of Publications and Conferences-

The following publication has been accepted during the course of this thesis work:

Title: *In Silico Biomedical Analysis of Marine Phlorotannins as Potential DPP-4 Inhibitors for Diabetes Management*

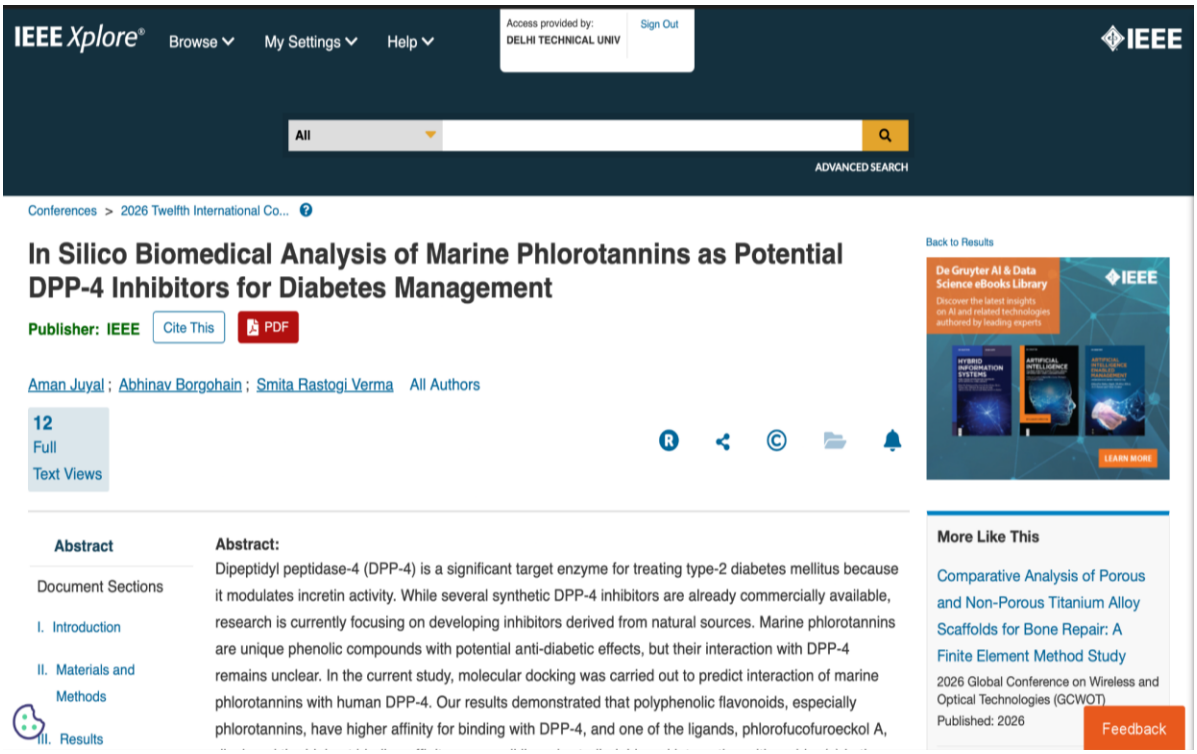
Authors: Aman Juyal, Abhinav Borgohain, SmitaRastogi Verma

Affiliation: Department of Biotechnology, Delhi Technological University, Delhi–110042, India

Conference: 2026 International Conference on Biomedical Engineering and Sciences (ICBSII 2026)

Digital Library / Repository: IEEE Xplore Digital Library

Status: Published / Live Online (22 April 2026)



Presentation in Conference- 2026 Twelfth International Conference on Bio Signals, Images, and Instrumentation (**ICBSII**)

Date of Conference: 09-11 March 2026

SSN
IEEE MADRAS SECTION
ICBSII 2026

2026 TWELFTH INTERNATIONAL CONFERENCE ON BIOSIGNALS, IMAGES AND INSTRUMENTATION

CERTIFICATE OF APPRECIATION

THIS CERTIFICATE IS PRESENTED TO

AMAN JUYAL

of Delhi Technological University

for **presenting** a paper on

In Silico Biomedical Analysis of Marine Phlorotannins as Potential DPP-4 Inhibitors for Diabetes Management

at the **Twelfth International Conference on Biosignals, Images, and Instrumentation**
organized by the Department of Biomedical Engineering,
Sri Sivasubramaniya Nadar College of Engineering,
held from March 09 to 11, 2026.

Dr. S. Saranya, ORGANISING CHAIR
Dr. A. Kavitha, CONVENER
Dr. S. Radha, CHIEF PATRON

Anusandhan National Research Foundation

SSN
IEEE MADRAS SECTION
ICBSII 2026

2026 TWELFTH INTERNATIONAL CONFERENCE ON BIOSIGNALS, IMAGES AND INSTRUMENTATION

CERTIFICATE OF APPRECIATION

THIS CERTIFICATE IS PRESENTED TO

AMAN JUYAL, ABHINAV BORGOHAIN, SMITA RASTOGI VERMA

of Delhi Technological University

for **participating** in the
Twelfth International Conference on Biosignals, Images, and Instrumentation
organized by the Department of Biomedical Engineering,
Sri Sivasubramaniya Nadar College of Engineering,
held from March 09 to 11, 2026.

Dr. S. Saranya, ORGANISING CHAIR
Dr. A. Kavitha, CONVENER

Anusandhan National Research Foundation

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, New Delhi, 110042

PLAGIARISM & AI VERIFICATION

Title of the Thesis: **"STRUCTURE ACTIVITY RELATIONSHIP ANALYSIS OF MARINE PHLOROTANNINS AS SELECTIVE DPP-4 INHIBITORS: A COMPUTATIONAL PERSPECTIVE"**
Total Pages:**40** Name of the Scholar- Aman Juyal **(24/MSCBIO/06)**

Supervisor

Dr Smita Rastogi Verna
Department of Biotechnology
This is to report that the above thesis was scanned for similarity detection. Process
The outcome is given below:
AI % Detected- **below 20%**
Software used: **Turnitin,** Similarity Index: **7%,** Total Word Count-**9813**

Aman Juyal

format (Repaired) (1) (1)

Document Details

Submission ID
trn:oid:::27535:

Submission Date
May 22, 2026, 3:01 PM GMT+3

Download Date
May 22, 2026, 3:26 PM GMT+3

File Name
format (Repaired) (1)(1).pdf

File Size
991.6 KB

27 Pages

8,249 Words

48,282 Characters

*% detected as AI

AI detection includes the possibility of false positives. Although some text in this submission is likely AI generated, scores below the 20% threshold are not surfaced because they have a higher likelihood of false positives.

Caution: Review required.

It is essential to understand the limitations of AI detection before making decisions about a student's work. We encourage you to learn more about Turnitin's AI detection capabilities before using the tool.

Disclaimer
Our AI writing assessment is designed to help educators identify text that might be prepared by a generative AI tool. Our AI writing assessment may not always be accurate (it may misidentify writing that is likely AI generated as AI generated and AI paraphrased or likely AI generated and AI paraphrased writing as only AI generated) so it should not be used as the sole basis for adverse actions against a student. It takes further scrutiny and human judgment in conjunction with an organization's application of its specific academic policies to determine whether any academic misconduct has occurred.

Aman Juyal

AJ

ABHINAV AND AMAN

Document Details

Submission ID

trn:oid:::27535:149128771

Submission Date

May 23, 2026, 6:45 PM GMT+5:30

Download Date

May 23, 2026, 6:47 PM GMT+5:30

File Name

AJ.pdf

File Size

732.6 KB

27 Pages

8,227 Words

47,936 Characters

7% Overall Similarity

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

Match Groups

- 67 Not Cited or Quoted 7%
  Matches with neither in-text citation nor quotation marks
- 1 Missing Quotations 0%
  Matches that are still very similar to source material
- 0 Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

3% Internet sources
2% Publications
6% Submitted works (Student Papers)