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College of Science



Biology Department

Evaluation of *In-silico* and *In-vitro* Anti-Diabetic Efficacy of Phytochemicals of *Phoenix dactylifera*

A Thesis Submitted in Partial Fulfilments of the Requirements for the
Master's Degree of Science in Biological Sciences

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Thesis Discussion Committee Decision

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The Discussion Committee met on 29/6/2025 in S41-0078 upon discussing the student whose details are stated above in public. The discussion committee recommends the following:

The student is awarded the grade Pass in the thesis course BIOLS699.

- ☐ Without making any changes to the thesis.
- ☒ After making the modifications attached with this decision.

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Abstract

Type 2 diabetes mellitus (T2DM) is a widely spread chronic disease threatening individual health and increasing mortality due to complications. *Phoenix dactylifera*, is an indigenous Bahraini plant referred as palm tree, that has phytochemicals with high medicinal effect against different disease including diabetes. Plant-derived antihyperglycemic agents represent a promising direction for novel drug discovery, offering a cost-effective, accessible, and potentially safer alternative to synthetic drugs. The current research aims to analyze the phytochemical compounds of *P. dactylifera*, and assess the inhibition kinetic mechanism of the lead compound against diabetic related enzymes.

The *in silico* network pharmacology and molecular simulation are carried out with multiple bioinformatical software considering **ADMET** profile for a potential therapeutic effect. A total of 17 bioactive compounds retrieved from **IMPPAT** were found with high drug likeness and oral bioavailability extracted from *P. dactylifera*. Exploring compound characteristics by **ADMET** profile eliminates poor pharmacokinetic properties at early development stages. 5-O-caffeoylshikimic acid is a phenolic compound interacts with proteins enrolled in diabetic related pathways according to **Metascape** and **ShinyGo** databases. Molecular docking in **PyRx** revealed strong binding affinities of 5-O-caffeoylshikimic acid to α -amylase (-7.7 kcal/mol) and α -glucosidase (-7.8 kcal/mol), indicating a stable and potent interaction.

In vitro enzyme inhibition kinetics are implemented to affirm the effectiveness of *in silico* findings of the compound against α -amylase and α -glucosidase and determine the required IC₅₀ value. 5-O-caffeoylshikimic acid was found to have a strong inhibition with IC₅₀ of 17.862 μ M against α -amylase and 7.668 μ M against α -glucosidase in contrast to acarbose, the positive control with IC₅₀ of 8.621 μ M and 30.978 μ M. 5-O-caffeoylshikimic acid has a significant inhibitory effect against the main digestive enzymes that triggers T2DM progression as anti-hyperglycaemic compound suggesting a promising compound for diabetic patients' pharmaceutical use.

الملخص

داء السكري من النوع الثاني (T2DM) مرض مزمن منتشر على نطاق واسع، يُهدد صحة الفرد ويزيد من معدل الوفيات بسبب مضاعفاته. فينيكس داكليليفيرا، وهو نبات بحريني أصلي يُعرف باسم شجرة النخيل، يحتوي على مواد كيميائية نباتية ذات تأثير علاجي قوي ضد أمراض مختلفة، بما في ذلك داء السكري. تُمثل العوامل الخافضة لسكر الدم المشتقة من النباتات توجهاً واعداً لاكتشاف أدوية جديدة، حيث تُقدم بديلاً فعالاً من حيث التكلفة، وسهل المنال، وربما أكثر أماناً من الأدوية الاصطناعية. يهدف البحث الحالي إلى تحليل المركبات الكيميائية النباتية لنبات فينيكس داكليليفيرا، وتقييم آلية تثبيط المركب الرئيسي ضد الإنزيمات المرتبطة بداء السكري.

أُجريت محاكاة جزيئية ودوائية حاسوبية باستخدام برامج معلوماتية حيوية متعددة، مع الأخذ في الاعتبار ملف تعريف **ADMET** للحصول على تأثير علاجي محتمل. وُجد أن 17 مركباً حيويًا نشطاً، تم استخلاصها من **IMPAT**، تتمتع بتشابه دوائي عالٍ وتوافر حيوي فموي عالٍ، مستخلصة من *P. dactylifera*. يُسهّم استكشاف خصائص المركبات من خلال ملف تعريف **ADMET** في استبعاد ضعف خصائص الحركية الدوائية في مراحل التطوير المبكرة. حمض **O-caffeoylshikimic-5** هو مركب فينولي يتفاعل مع البروتينات المسجلة في مسارات مرتبطة بمرض السكري، وفقاً لقواعد بيانات **ShinyGo** و **Metascape**. أظهر الالتحام الجزيئي في **PyRx** ألفة ارتباط قوية لحمض **O-caffeoylshikimic-5** مع ألفا-أميلاز (-7.7 كيلو كالوري/مول) وألفا-غلوكوزيداز (-7.8 كيلو كالوري/مول)، مما يشير إلى تفاعل مستقر وفعال.

تم تطبيق حركية تثبيط الإنزيم في المختبر لتأكيد فعالية النتائج الحاسوبية للمركب ضد ألفا أميليز وألفا جلوكوزيداز وتحديد قيمة IC_{50} المطلوبة. وُجد أن حمض **O-caffeoylshikimic-5** له تثبيط قوي مع IC_{50} يبلغ 17.862 ميكرومولار ضد ألفا أميليز و 7.668 ميكرومولار ضد ألفا جلوكوزيداز على عكس الأكاربوز، التحكم الإيجابي مع IC_{50} يبلغ 8.621 ميكرومولار و 30.978 ميكرومولار. يتمتع حمض **O-caffeoylshikimic-5** بتأثير مثبط كبير ضد الإنزيمات الهضمية الرئيسية التي تحفز تقدم مرض السكري من النوع 2 كمركب مضاد لفرط سكر الدم مما يشير إلى مركب واعد للاستخدام الدوائي لمرضى السكري.

Dedication

I dedicate this work to my parents, whose constant support and confidence in my potential has inspired me to start the journey and accomplish this achievement.

Acknowledgement

I am grateful to Allah for granting me with strength and passion to accomplish this research and navigate the challenges of this journey.

I am grateful to my supervisor, Dr. Qamar Abbas, for his patience, supervision, insight, time, and efforts. His expertise has been a cornerstone in carrying out this work.

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Table of Contents

Defense Committee Approval.....	a
Abstract	b
Dedication	d
Acknowledgment	e
Table of Contents	f
List of Tables	h
List of Figures.....	h

Chapter One

Introduction to the Study and Theoretical Background

1.1 Background	2
1.2 Research Problem	4
1.3 Research Questions	4
1.3 Secondary Questions	4
1.4 Research Objectives.....	5
1.5 Research Significance	5
1.6 Research Setting.....	6
1.7 Research Terminology	6

Chapter Two

Theoretical Framework

2.1 Pathophysiology of Diabetes Miletus Type 2	9
2.2 Treatment Approaches	11
2.3 Phytochemicals compounds and T2DM	12
2.4 <i>Phoenix dactylifera</i> and T2DM	17
2.5 Research Gap	20

Chapter Three

Materials and Methods

3.1 Plant selection.....	23
3.2 <i>In silico</i> methods.....	23
3.2.1 Screening of Phoenix dactylifera bioactive phytochemicals	23
3.2.2 Compound target T2DM proteins	25
3.2.3 GO and KEGG pathway analysis.....	26
3.2.4 Protein-Protein Network	27
3.2.5 Molecular docking	28
3.3 <i>In vitro</i> methods.....	29
3.3.1 α-Glucosidase Inhibition Assay	29
3.3.2 α-amylase Inhibition Assay	30

Chapter Four

Research Study's Results, Discussion, and Recommendations

4.1 <i>In Silico</i> Results	33
4.1.1 Active compounds and Gene Targets.....	33
4.1.2 ADMET Profile of 5-O-Caffeoylshikimic acid	39
4.1.3. Protein-Protein Interaction.....	40
4.1.4 GO and KEGG pathway	43
4.1.4. Molecular docking	46
4.2 <i>In vitro</i> Results.....	51
4.2.1. α-Glucosidase and α-Amylase Activity	51
4.3 Discussion	52
4.4 Conclusion	58
4.5 Research Study's Limitation and Recommendations.....	59
References.....	60
Appendices.....	73

List of Tables

Table 1 Potential Bioactive Compounds Found in <i>P.dactylifera</i>	34
Table 2 ADMET Profile of 5-O-caffeoylshikimic acid (C ₁₆ H ₁₆ O ₈)......	39
Table 3 Top Gene Enriched in KEGG Pathways.	43
Table 4 <i>Binding and Interaction of C₁₆H₁₆O₈ and α-Amylase</i>	47
Table 5 Binding and Interaction of C ₁₆ H ₁₆ O ₈ and α-Glucosidase.....	47
Table 6 Affinity of Binding Pockets of C ₁₆ H ₁₆ O ₈ with Target Proteins	48

List of Figures

Figure 1 Insulin Resistance Pathophysiology	10
Figure 2 In silico Workflow for Bioactive Compound Identification	25
Figure 3 Venn Diagram of Potential Targets between T2DM And C ₁₆ H ₁₆ O ₈	41
Figure 4 Protein-Protein Interaction Using STRING Tool.....	41
Figure 5 CytoScape Protein-Protein Interaction.	42
Figure 6 Top 10 Closeness Nodes by CytoHubba.	42
Figure 7 Metascape Enrichment Analysis of Pathways Enriched with Genes Aligned Between T2DM And C ₁₆ H ₁₆ O ₈	44
Figure 8 Go Biological Pathway	44
Figure 9 GO Cellular Pathway	45
Figure 10 GO Molecular Pathway.....	45
Figure 11 KEGG Pathway.	45
Figure 12 Molecular Docking of C ₁₆ H ₁₆ O ₈ with α-Amylase.....	49
Figure 13 Molecular Docking of C ₁₆ H ₁₆ O ₈ with α-Glucosidase.....	50
Figure 14 IC ₅₀ Curves Of 5-O-caffeoylshikimic acid and Acarbose Compound	

Chapter One

Introduction of the Study

1.1 Background

Type 2 diabetes mellitus (T2DM), a worldwide chronic disorder that continues to increase in prevalence, posing serious threats to individual health and contributing significantly to mortality rates. This occurs when glucose levels are abnormal due to insulin resistance resulting elevated circulated blood glucose concentrations. According to the International Diabetes Federation's (IDF) 2021 data, 10.5% of the global population had diabetes, with mortality rates estimated to be 6,700,000. Among the population of Bahrain, IDF statistics have revealed the presence of 119,800 (9%) diabetic patients (International Diabetes Federation, 2021). However, burdens are estimated to increase following the global developments and living style habits. Diabetes mellitus characterized as a multisystemic disease; a previous review study has revealed macrovascular, microvascular, and other systematic emerging complications within diabetic patients (Tomic et al., 2022). Management of dietary, life practices, and synthetic medications are common treatments. Regardless to the effectiveness of the antidiabetic drugs, long term prescription causes adverse side effects overtime (Mousavi et al., 2020). However, some plants possess antihyperglycemic characteristics; therefore, they are alternative sources to conventional pharmaceutical drugs due to reduced side effects (Khursheed et al., 2019).

Phoenix dactylifera, commonly known as palm tree, belongs to the *Arecaceae* family. Middle Eastern countries and North Africa have widely known *P. dactylifera* as a crucial agricultural plant (Alkhoori et al., 2022). Hence; it highly distributed in tropical and subtropical areas. It has been a main Bahraini indigenous cultivated plant for centuries. Therefore, *P. dactylifera* is receiving high attention from the agriculture institute along with setting regulations to preserve the national treasure of Bahrain

(Almansoori et al., 2015). *P. dactylifera* especially date fruits, have enriched nutrient value involving carbohydrates, high fiber content, nutritional compounds, and dietary minerals (AlFaris et al., 2021). According to Awad et al. (2020), chemical content of the plant varies between the countries based on the variation in the regional growing conditions. Indeed, soil fertilization quality and cultivation attitudes vary in the nutritional content and profile of date fruits (Al-Alawi et al., 2017). Moreover, date fruits have a significant attractive medicinal effect as it is enriched with pharmaceutical chemical properties against health conditions. Multiple studies have revealed the pharmacological effect of date palm tree residues on the health as reducer to oxidative stress, antimicrobial, controlling inflammation, anticarcinogenic, alleviate diabetes, along with its potential effect on cardiovascular impairment through *in-vivo* and *in vitro* studies (Echegaray et al., 2020). Therefore, *P. dactylifera* phytochemicals may serve as a potential natural origin medication against diabetes minimizing the side effects of current therapies.

P. dactylifera has many phytochemicals that could induce hypoglycaemic properties. A previous study reported the effectiveness of methanolic *P. dactylifera* extract on reducing glucose concentrations in diabetic rabbits which are dexamethasone-induced (Abdullah and Al-bokai., 2022). Date pectin increases glucose adsorption and α -amylase inhibition. Besides that, it reduces starch degradation and glucose intestinal diffusion (Djaoud et al., 2022). Moreover, aqueous suspension of *P. dactylifera* seeds treatment was shown to diminish several diabetic complications as enhancing kidney and liver biochemical parameters and histological investigations (Abdelaziz et al., 2015). Hence, *P.*

dactylifera extract are potential antidiabetic and therapeutic agent made from plant sources.

1.2 Research Problem

Diabetes mellitus is a common persistent metabolic syndrome. According to IDF statistics, diabetic instances and its complications intensity are increasing globally; involving Kingdom of Bahrain. Combating the high incidence of diabetic cases globally, will require additional researches and medications. Hence the availability and high costs of synthetic medications are a global problem. In addition to that, toxicity, and serious complications can be encountered with administration of synthetic medications. Therefore, there are multiple phytochemical compounds that can be obtained from plant sources as a cost-effective and safe therapy for pharmaceutical uses. Some previous studies suggest *P. dactylifera* extracts are effective hypoglycemic agent. However, only limited studies were conducted regarding the effectiveness of *P. dactylifera* extracts against T2DM. Therefore, the lack of comprehensive studies on the bioactive compounds extracted for indigenous plant, and its molecular action limits exploration the native species for pharmaceutical development.

1.3 Research Question

Research Question

- Are *P. dactylifera* bioactive compounds potential inhibitors of the main digestive enzymes?

1.3.1 Secondary Questions

- What are bioactive compounds present in *P. dactylifera*?

- What is the impact of lead compounds on diabetes progression and management?
- How effectively do the lead compounds inhibit α -amylase and α -glucosidase enzymes?

1.4 Research Objectives

- 1- To screen and identify phytochemicals present in *P. dactylifera*.
- 2- To evaluate the drug likeness and antidiabetic potential of the lead compounds.
- 3- To determine the mechanism of inhibition of diabetes using network pharmacology approaches.
- 4- To utilize molecular simulations in the discovery of novel therapeutic compounds.
- 5- To examine the enzyme inhibitory activity of the lead compounds against α -amylase and α -glucosidase.

1.5 Research Significance

The prevalence of diabetic cases is increasing in Bahrain. Traditional treatments are usually unsustainable, expensive, and have associated with adverse side effects. Recently, natural compounds from plant sources seem to be potential agents in therapeutic pharmacology. This assumes a predictive effect of some plants in treating and managing various diseases including diabetes mellitus. *P. dactylifera* is one of the main plants in Bahrain culture that contains multiple active compounds. Previous studies showed a promising treatments of *P. dactylifera* date extracts against different health conditions and diseases (Echegaray et al., 2020). Hence, the exploration of phytochemicals from a widely distributed indigenous plants in the country aids the sustainability of the treatment in reduced prices, considering the safety of these substances in comparison to

synthetic medication. Moreover, a combined approach of *in silico* and *in vitro* experiments encourage timesaving and strong groundwork to be applied *in-vivo* experiments. This research will guide researchers to new mechanisms in treating diseases. Therefore, specifying the phytochemicals, their pathways, and their dose effectiveness would provide insight into new naturally sourced drug developments with less adverse side effects compared to traditional conventional medications.

1.6 The Study Setting

The study was conducted at the College of Science, Department of Biology, University of Bahrain. Experimental studies are conducted in a well-equipped laboratory that provide modern technology, equipment and reagents for research experiments.

1.7 Research Terminology

Term	Definition	Ref.
Insulin	The main pancreatic hormone responsible to maintain circulated glucose concentration as energy sources in the cells.	WHO, 2023
T2DM	Chronic condition caused by impaired use of insulin hormone due to insulin resistance conditions.	CDC, 2023
Hyperglycaemia	Impaired high glucose levels in blood	WHO, 2023
Enzyme inhibition assay	An essential or confirmative laboratory procedure used to analyse the efficiency of ligands against limiting enzyme activity under special concentrations and optimum operation conditions.	Smirnovienė at al., 2021

Network pharmacology	New promising approach in drug discovery that provide the groundwork of upcoming drugs by providing a network of interactions where disease genes are interconnected with drug ligands target genes.	Li et al., 2023
<i>In silico</i>	Virtual experiments based on computer simulation	Asonganyi, 2013
<i>In vivo</i>	Biological experimental studies performed and introduced within living organism	Asonganyi, 2013
<i>In vitro</i>	Biological experiments and procedures that are proceeded outside the living organisms.	Asonganyi, 2013
Gene Ontology	Broad representation of gene functions in living organisms using a computational illustration of biological framework.	Thomas, 2016

Chapter Two

Theoretical Framework

2.1 Pathophysiology of Diabetes Mellitus Type 2

Diabetes mellitus is a long-lasting metabolic syndrome characterized by hyperglycemic levels circulating in blood stream. According to Magliano and her colleagues (2020), the burden is increasing between young-onsets compared to past years which causes an increase the disease onset on future population and offspring. It is estimated to encounter an increase in global diabetic cases to 11.3% by 2030 (IDF, 2021). It is subdivided into two types. Type 1 diabetes mellitus is an insulin-dependent diabetes where the production is misfunctioned due to autoimmune response while Type 2 Diabetes mellitus (T2DM) is a condition of insulin resistance and eventual insulin deficiency due to β -cell dysfunction. World Health Organization (WHO) defined T2DM as insufficient insulin function causing high glycemic concentration in the body causing physiological damage and complications (WHO, 2023). This condition is referred as insulin resistance. Consequently, impaired insulin production occurs as a response to insulin resistance in the target cells leading to T2DM onset cases (Hameed et al., 2015). In addition to impaired insulin function, diabetic related complication as cardiac diseases, diabetic nephropathy, retinopathy, neurological disorders and gangrenes occurs as contribution to glucotoxicity caused by hyperglycemia (Chang et al., 2013) (Figure1). Gastrointestinal enzymes including α -amylase and α -glucosidase are corresponded to hydrolysis starch into glucose; causes postprandial hyperglycemia that are controlled in T2DM through inhibiting their activity in the small intestine to reduce glucose absorption (Chakraborty et al., 2016). Moreover, human islet amyloid polypeptide is found to be responsible factor toward to progression of various diseases including T2DM through unusual and excess amyloid aggregation causing cytotoxicity (Chaari et al., 2020).

Ajiboye, Oloyede, and Salawu (2020) stated the effect of oxidative stress in increasing glucose levels especially in elderly people; which induces inflammations that are contributed to diabetic related complications. Several inflammatory markers are associated with diabetes. The occurrence of T2DM is an indicative of an inflammatory response with high releasing of several inflammation markers as tumor necrosis factor- α , interleukin-1 β , and interleukin-6, that cause diabetic related complications as coronary artery diseases; beside Monocyte chemoattractant protein-1 which increases with obesity (Gothai et al., 2016). Therefore, it is well understood that diabetic related complications are induced due to the effect of glucose on the antioxidant system. However, glycosylated haemoglobin A1c (HbA1C) is significant in monitoring glucose on long term basis (Al-Abachi, et al., 2022).

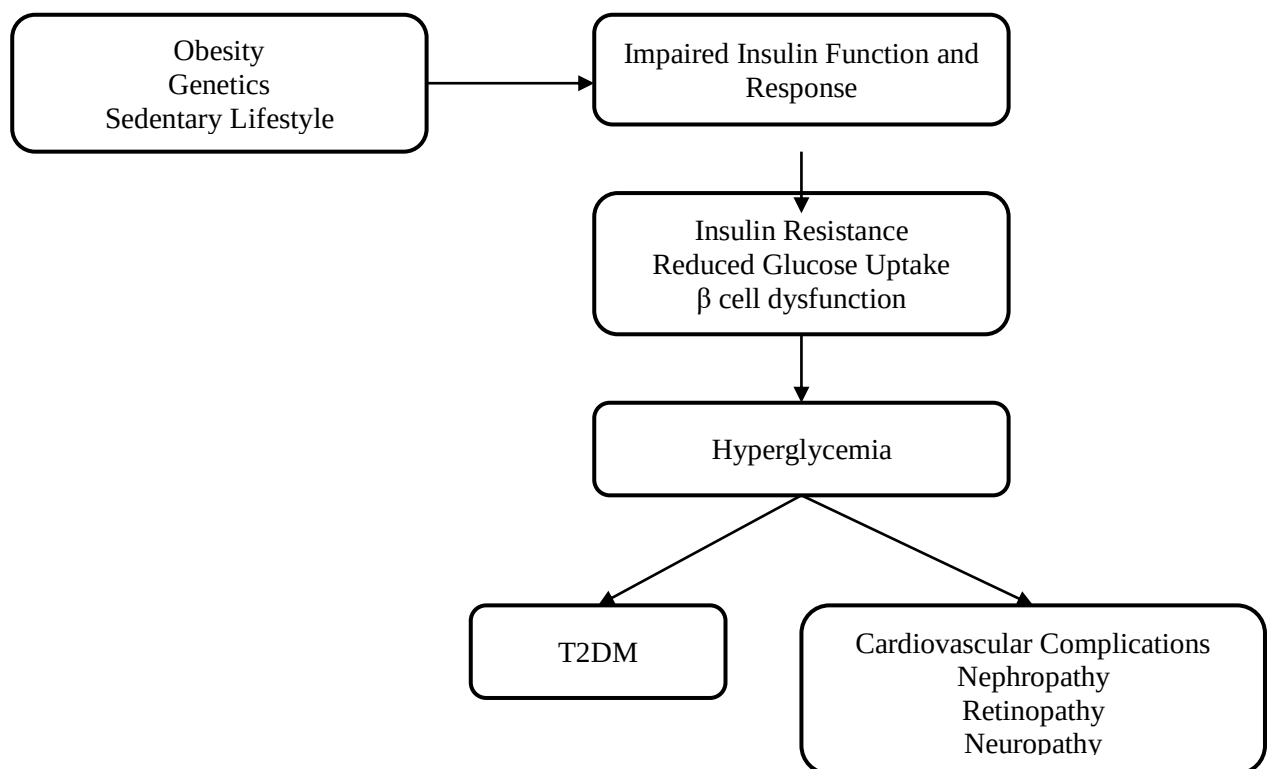


Figure 1 Insulin Resistance Pathophysiology

2.2 Treatment Approaches

Diabetes treatments can be prescribed as monotherapy or conjugated therapy to target the causative pathways of T2DM. With increasing prevalence of T2DM, current onset of the disease is controlled with various pharmacological medications and life quality controlling strategies in order to maintain blood sugar and minimize diabetic adverse effects. As Chang et al., (2013) stated, targeting multiple pathways through insulin doses, secretion inducers and sensitizing agents provides better long term controlling of Hba1c; therefore, severity and disease onset are decreased. Therapeutic plans are effective under competency and adherence to prescribed drugs and life changes (García-Pérez et al., 2013). In a study conducted by Kahn, Cooper and Del Prato (2014), present treatments are mainly targeting gastrointestinal tract to decrease the degradation of complex carbohydrates, antagonism of Sodium-Glucose Transporter-2 to stimulate glucose urinary excretion by kidney, targeting the central nervous system and modified insulin prescription therapy. Main synthetic drugs prescribed for T2DM are such as metformin, acarbose, voglibose and miglitol; are suppressing carbohydrates digestion enzymes to slow starch conversion (Senthila et al, 2019) However, it has been stated that these pharmaceutical drugs as acarbose and miglitol are inducing unlikeable side effects as diarrhea and flatulence to diabetes patients besides their probability to cause cancers as kidney cancers (Chakroun et al., 2016). According to a review article by Khaliq, Sarfraz, and Ashraf (2016), synthetic drugs from different groups have secondary side effects as skin rash, liver and kidney disorders in response to the administration of sulfonylureas, while thiazolidinediones were known to cause obesity, edema and cancers, metformin including α -glucosidase cause

gastrointestinal distress and dizziness with metallic taste. Hence, although available treatments are effective, they suppress the health burden by interfering with other organs. These proposed secondary side effects often require a combined treatment approaches of having multiple antidiabetic drugs along with the standard conventional drug in order to expand the potential intensity of their therapeutic activity (Abbas et al., 2017). Ajiboye, Oloyede, and Salawu (2020) suggested a promising dietary approach including dates from *P. dactylifera* as an alternative medication approach to lower the unfavorable effects and the affordability specially to low income individuals and societies.

2.3 Phytochemicals compounds and T2DM

Plants have generous source of phytochemicals that can be a natural source of therapeutic agent to treat different diseases, which needs an extended exploring on their mechanism of action. These compounds were integrated into medications or used as supplemental drugs to regulate blood glucose levels as galegine, phenols and pycnogenol (Ríos, Francini, & Schinella, 2015). Moreover, under less availability and afford of the conventional therapeutic agents in the developed countries, plant based phytochemical compounds are suggested to be an alternative treatment source for T2DM. According to Gothai et al., (2016), implying extracts with multiple mechanism of actions minimizes drug loads. They declared the use of phytochemical compounds as saponin, myrcelin, flavonoids, pectin, and glucosides in the pharmaceutical drug as they lower blood glucose despite their difference in mechanism of action. These phytochemicals aid in binding with different target proteins of T2DM as the molecular mechanisms for blood postprandial hyper glucose inhibition to resemble antidiabetic agent (Molehin, 2019). Indeed, plants sourced phytochemical constituents were identified to have different modes of

actions regarding suppressing glucose in plasma by reducing glucose absorption, stimulating insulin secretion, or decreasing glucose synthesis and neutralizing the free radical formation that cause diabetic related complications due to their effect on glycoproteins formation (Khaliq, Sarfraz, & Ashraf, 2016).

Tolmie and his colleagues (2021), has conducted a study including phytochemicals presented in widely distributed herbs and spices. Their results suggested a correlation between *in silico* docking trials and enzyme inhibition kinetics; where apigenin, eriodictyol, and piperine are effective glucosidase inhibitors and apigenin, cinnamic acid, and rosmarinic acid as amylase inhibitors. However, daily dose, cytotoxicity and oral absorption are needed to be considered and correlated to acarbose while using phytochemicals as a drug; as some of the compound were found to have high cytotoxicity and others with high oral absorption making them ineffective inhibitors as compound will be absorbed before reaching to the site of action. The analysis of *Boerhaavia diffusa* aerial parts, the phenolic content especially ethanol extract and bioactive compounds were efficiently effective against diabetes related enzymes taking into concern their ability to reduce glucose absorption and encouraging muscle glucose uptake that facilitates managing T2DM (Oyebode et al., 2018). In their study, they focused on different factors that affect the oxidative stress reactions, they justify that different doses of extracts were potentially causing reduction in Fe^{3+} radicals and increased diphenyl-2-picrylhydrazyl radical scavenging, catalase activity, GSH and superoxide dismutase which exhibit antioxidant properties diminishes the pancreatic oxidative injury that was presented before treatment doses. In this context, the extracts are potential against pancreatic dysfunction of diabetic patients as antioxidative agents that will correspond in reducing the complications of

diabetes via enhancing glucose uptake by muscles to modulate high glucose concentrations. Kumar et al., (2020) has stated probabilities of cellular damage in diabetic patients in a consequence to hydrogen radicals results from superoxide anions oxidative stress reaction. They explained proteins glycation and membranes structure are deteriorated due to the excessive oxidation caused by Haber Weiss reaction. This correlates with the hypothesis of the previous study where oxidative injury and high oxidation related parameters and enzymes are increased causing pancreatic dysfunction. According to Kumar and his colleagues (2020), the *in silico* molecular examinations of rutin has revealed the formation of hydrogen bonds in a pi-pi interaction at the hydrophilic region with amino acids in correspondent proteins of GLUT1, PPAR γ , and DPP-IV. The binding affinity were correlated and confirmed with glibenclamide docking to the suggested proteins. In addition to that, DPP-IV inhibition is expected to maintain the efficiency of GIP that is introduced into multiple blood glucose maintaining mechanisms. This was confirmed in their *in-vivo* studies where diabetic induced rats had a decreased glucose levels along with increasing in the body weight taking in concern the different concentrations of doses of glibenclamide in contrast to control groups with diabetes. They proved the impact of antidiabetic factor through the improvement in plasma insulin, better hepatic parameters, and improvement in the growth of pancreatic cells. Therefore, Ríos, Francini, & Schinella, (2015) stated the causes of reduced insulin sensitivity and pancreatic islets dysfunctions in response to oxidative stress; however, the prolonged administration and suppression may link to diabetic cardiovascular complications.

The bark extract of *Cinnamon cassia* has revealed 20 phytoconstituents that are subdivided into aqueous, ethanolic and

methanolic extracts in the study carried out by Vijayakumar et al (2020). They established the implication of different extracts and specially the ethanolic extract in concentration dependent inhibition to the chief antidiabetic enzymes α -amylase and α -glucosidase. Upon their examination of Wister albino mice, diabetic biomarkers and lipid profile were restored during the administration of the ethanolic bark extracts. Due to insulin secretion, gluconeogenesis and glycogenesis are reduced causing glucose hemostasis. The *in silico* has shown a positive good glide score of interaction between the phytochemicals and PPAR α/γ that affect insulin secretion and lipid digestion pathways which consequently decrease diabetes and diabetic related complications such as cardiovascular diseases. Similarly, sinigrin which is one on the phytochemicals that were studied by Abbas et al (2017), where a potential and significant suppression of α -amylase and α -glucosidase are found through a concentration dependent specially with 100 mg/kg with IC₅₀ value of 0.248 mM and 0.0017 mM respectively in comparison to acarbose; the standard conventional reference drug that established 0.00124 and 0.00170 mM. Indeed, the administration of sinigrin in diabetic induced zebra fishes has lowered glycemic ratio significantly in contrast to the control groups with diabetes. They stated through their study the possibility of sinigrin to intercalate by inhibition the enzyme complex formation, and disturbing the catalysis of the enzyme and their substrate; this expectation is supported through the docking studies where it interacted with the active binding sites. Similar findings were found by Wulan, Utomo, and Mahdi (2015), where they focused in their study on the effect of n-hexane fragment of crude methanol from *Ruellia tuberosa* aerial part on lowering blood glucose levels through α -amylase inhibition in 2 weeks. In their study, they confirmed their results of vitro studies with *in-vivo* experiment to ensure the effective reproducible of enzyme inhibition. In their *in vitro* study, plant

extracts with different concentrations potentially inhibited the enzymatic activity from 7.13% to 93.77%. Following up with *in vivo* inhibition where the glucose parameters are reduced to the normal level as the normal control groups parameters significantly by the end of treatment period. Based on the spectrophotometry analysis and previous literatures, they revealed the presence of lipophilic steroids referred as Betulin that has the ability to pass through the membrane, therefore, they estimate the efficient enzyme inhibition and hypoglycemia results are caused by targeting carbohydrate digestion through α -amylase in a non-competitive mechanism by attaching to enzyme-substrate complex. Furthermore, ligands-protein between the suggested compounds as Betulin, Betulinic acid, Bisdemethoxycurcumin, and Curcumin has established a positive interaction with α -amylase considering the impact of hydrogen bonds as an effective docking interaction of amino acids in the active binding sites. According to Nyathi et al (2023), different extracts retrieved from *Xeroderris stuhlmannii* had successfully inhibited the two main digestive enzymes α -amylase (IC_{50} =10.5-29.5 μ g/mL) and α -glucosidase (IC_{50} =8.8-49.5 μ g/mL). The *in silico* molecular simulation has declared the necessity of hydrogen bonds for the ligand-protein interaction between the phytochemicals and the enzymes in their catalytic residues where acarbose had high number of hydrogen bonds which provides a stability for a potential inhibition through higher binding affinities. The researchers established the presence of hydrogen bonds formed between the correspondent phytochemicals and the enzymatic active site amino acids ASP197, GLU233 and ASP300 of α -amylase. Despite of the previous study, Senthila et al (2019), fucoidan extracted from *Turbinaria conoides* has revealed the presence of fewer hydrogen bonds interacted with carbohydrates digestion enzymes compared to acarbose, however, the majority of interactions are with water molecules rather than hydrogen

bonds. The hydrogen bonds are likely to occur between the hydroxyl groups and amino acids in the compound of interest. Despite this, they predict the importance of bond length and binding energy to be essential monitoring targets which can explain the effective dose dependent inhibition ratio of fucoidan. Another criterion mentioned by the previous researchers is the importance of the molecular weight in the effectiveness of drug absorption and side effects occurrence. Indeed, low molecular weight of the compounds is essential to ensure the absorbance to the target site compared to the commercially available drug that has a higher molecular weight, thus, more inhibitory factors and reduced side effects of diarrhea and abdominal discomfort are estimated for Fucidin compared to acarbose.

2.4 *Phoenix dactylifera* and T2DM

P. dactylifera is a rich source of many bioactive compounds that has an influence on human health including flavonoids, polyphenols, sterols, carotenoids and phytoestrogens. These compounds are found on different plant parts and extracts are being examined nowadays to be used as a natural therapeutic agent to compensate the use of synthetic drugs and their adverse secondary side effects and complications. T2DM is one of the attracted diseases to be studied by scientists to examine the therapeutic index of the plant extracts against the disease. Significant findings are found which needs in deep exploring of their mechanism of action.

As *P. dactylifera* is widely distributed indigenous plant in the Arabian gulf including Bahrain; exploring the benefits of its phytochemicals are beneficial to natural drug discovery, ensure the availability of the compounds, and the costs. Bioactive compounds are found on different plant parts including stem, flower, fruit, root, leaf, and

seed. According to Ajiboye, Oloyede, and Salawu (2020), the enzyme inhibition assays and antioxidant parameters had revealed a significant connection between *P. dactylifera* dietary plan and glucose levels. Their study suggests a potential inhibition with IC_{50} of 7.8 $\mu\text{g/mL}$ for α -amylase and 10.7 $\mu\text{g/mL}$ of α -glucosidase from the fruit-based diet which was found to be compatible with acarbose the main medication. Moreover, the inhibition of antioxidant profile through nitric oxide and hydroxyl radicals estimates their probability in reducing severity and disease complications. Therefore, having the plant extract was found to be potential effect on glucose absorption and starch degradation. The findings of Chakroun et al. (2016), had shown an efficient suppression in α -glucosidase than α -amylase compared to a conventional synthetic drug called acarbose. The IC_{50} value of hydroalcoholic extract of *P. dactylifera* leaves was $20\pm 1\text{mg/mL}$ compared to $125\pm 0.3\text{mg/mL}$ of acarbose. Therefore, they estimate the cause of gastrointestinal distress is most probably linked with excess amylase inhibition as their results finds out $30\pm 0.8\text{mg/mL}$ of hydroalcoholic extract is needed compared to $17\pm 0.5\text{mg/mL}$ of synthetic drug.

Regarding amyloid formation in the diabetic patients, a previous study was carried out by Chaari et al (2020) has focused on the contribution of compounds extracted from *P. dactylifera* against human islets amyloid polypeptide to investigate their effect on amyloid aggregation. Their findings established an efficient inhibition regardless to the different concentrations used in the study. Among all the compounds gallic acid ($IC_{50}=1.2\text{ }\mu\text{M}$) and quercetin ($IC_{50}=1.7\text{ }\mu\text{M}$) have the highest inhibition reached to 88.7% and 97%. Moreover, the permeability of flavonoids exhibits a diminished interaction between membrane vesicles and the aggregation. This was clarified though molecular docking where the

inhibition of the aggregations is estimated to be attributed due the presence on hydroxy groups and aromatic moiety; thus, maintain and protect the normal structure of the peptide.

As a consequence, to the noticeable adverse complications of the designed pharmaceutical drugs, the world is shifting toward more green based treatment approaches and decrease the probability of having extensive side effects. The standard drugs used for treating T2DM were known to cause gastrointestinal distress, dizziness, kidney and liver disorders. Therefore, the standard conventional treatments are not favorable as it suppresses the health statues beside lowering the blood glucose levels. Several previous studies focused on using plant-based compounds in treating the diseases including T2DM. Indeed, plants are rich sources of compounds that can be extracted from different plant parts to be used as therapeutically agents to ensure the safety, lowering side effects and ensure the availability source the compounds through the life. They had different procedural methods in investigating the effect of phytochemical compounds as a pharmaceutical drug that can compensate the needs of synthetic based chemicals. Researchers usually command on using plant extracts in general to be applied on either enzyme inhibition assays or administered to animals as zebrafish, albino mice and rabbits. Which gave a promising result the conclude the presence of effective compounds in the extracts that targets the disease genes. The studies use the plant parts to make a grinded extract from the plants to be used in the experiments. However, the composition of the extracts is usually estimated by spectrophotometry and in some studies, they are subdivided based on their composition as aqueous, methanolic, lipid extracts. In general, studies are aimed to investigate the antidiabetic and antioxidants enzyme inhibition kinetics and *in-vivo* biochemical parameters that examine the efficiency of

compounds in lowering blood glucose, diabetic related complications and organ disorders. Few studies have used the *in silico* approaches to build a groundwork base before in-vivo and in vitro examinations. Some studies were based on enzyme assays and others used the extracts on animals directly. Focusing on *in silico* helps to avoid the toxicity of some compounds and ensure the oral bioavailability to reach target side with the proper doses. Hence, having a combined study with are experimental procedures will ensure the validity, reliability, lowering time and expense of the experimental trials.

2.5 Research gap

Limited sources of the plants phytochemicals need a comprehensive exploring in different multiple databases to encounter all plant bioactive compounds. Previous studies usually used general plant extracts in their experiments. Few studies had included the *in silico* molecular mechanisms of the compounds, however, the main targeted pathways are not identified and studied. The *in silico* and *in vitro* examinations are promising, they give cornerstone to new drug discovery approach. Certain phytochemicals extracted from herbal plants and spices were effectively regulators of α -amylase and α -glucosidase, however, daily dose management requires additional *in vivo* examinations for validation (Tolmie, Bester, & Apostolides, 2021). In addition to that, studies are mainly focused on plants extracts for drug used, an extended exploration through studying the responsible anti-diabetic phytochemical is fundamental to clarify the main target phytochemical and their main of mechanism of action. As the study of Abdullah and Al-bokai (2022), suggest an isolation of the fractioned seed extracts to identify the main targeted phytochemical with antihyperglycemic effect and their molecular action. Few studies are conducted regarding *P. dactylifera* phytochemical extracts and their effect

of T2DM. in addition to that, *P. dactylifera* extracts are needed to be cleared from unlikable compounds and high sugar content for more efficient product. Studies are needed to be conducted regarding the effect of different doses on disease progression and complications. a comprehensive study including multiple *in vitro* studies is needed to clarify all the potential aspects needed for a new pharmaceutical bioactive compound followed by *in-vivo* studies confirm the consistency and stability of study results on the living organisms.

Chapter Three

Materials and Methods

3.1 Plant Selection

P. dactylifera is a cultivated species indigenous to tropical and subtropical regions including North Africa and the Middle east. It is classified under the family of Arecaceae and belongs to the genus Phoenix. The date fruit is an abundant and affordable nutrients provider such as minerals, vitamins, antioxidants and fibers, along with secondary bioactive metabolites as phenolics, flavonoids, carotenoids, procyanidins, tocopherol and tocotrienols and sterols which enhance human well-being (Fernández-López et al., 2022). In addition, the availability of indigenous plants is the primary reason for selecting *P. dactylifera* as a sustainable medicinal resource for managing T2DM.

3.2 *In silico* methods

3. 2.1 Screening of Phoenix dactylifera bioactive phytochemicals

Bioinformatical tools are taking the considerations of the researchers into new drug discovery approach that aids in the identification of the main drug target molecules using different *in silico* methods (Neelawala, et al., 2019). The initial stage for the application of network pharmacology is the identification of the main active compounds extracted from various plant parts to be correlated with the disease potential genes. Therefore, large databases are presented to provide a reliable source of phytochemicals presented in the plants along with providing references sources as Indian Medicinal Plants, Phytochemistry, and Therapeutics Database (**IMPPAT**). **IMPPAT** is a large chemical pharmaceutical database that involves different plants with a comprehensive detailed phytochemical, their structure, properties and common names in one web-interface database

(Mohanraj et al., 2018). Following the acquisition of pharmacological active constituents, their Canonical Simplified Molecular Input Line-Entry System (SMILES) is identified beside the 2D structure of the compounds to be incorporated into the bioinformatical tools for a precise network pharmacology. The National Institutes of Health (NIH) developed a database called PubChem, one of the large chemistry databases that includes the chemical compounds, their formula, chemical identifiers (Glüge, McNeill, & Scheringer, 2023).

For an optimal pharmaceutical drug use, several characteristics are needed to be examined to ensure efficiency probabilities of the drug compound. Therefore, absorption, distribution, metabolism, excretion, and toxicity (**ADMET**) profile is necessary for a proficient drug prediction. Hence, undesirable side effects, low efficiency and toxicity are minimized and prevented at early preclinical stage of drug developing to avoid expenses and costs of failure late stage trials (Wu et al., 2020). The phytochemicals compounds of *P.dactylifera* bioavailability are compared in SwissADME web-interface along with drug-likeness scores (DL) of the correspondent compounds using Molsoft software. Based on the molecular properties and chemical structure, DL score evaluates the probability of the compound to resemble an effective drug. Compound with high bioavailability exceeding 30%, DL of >0.18 and optimum ADMET profile is chosen to proceed in the trials. Other compounds that doesn't meet the characteristics are excluded. (Figure2)

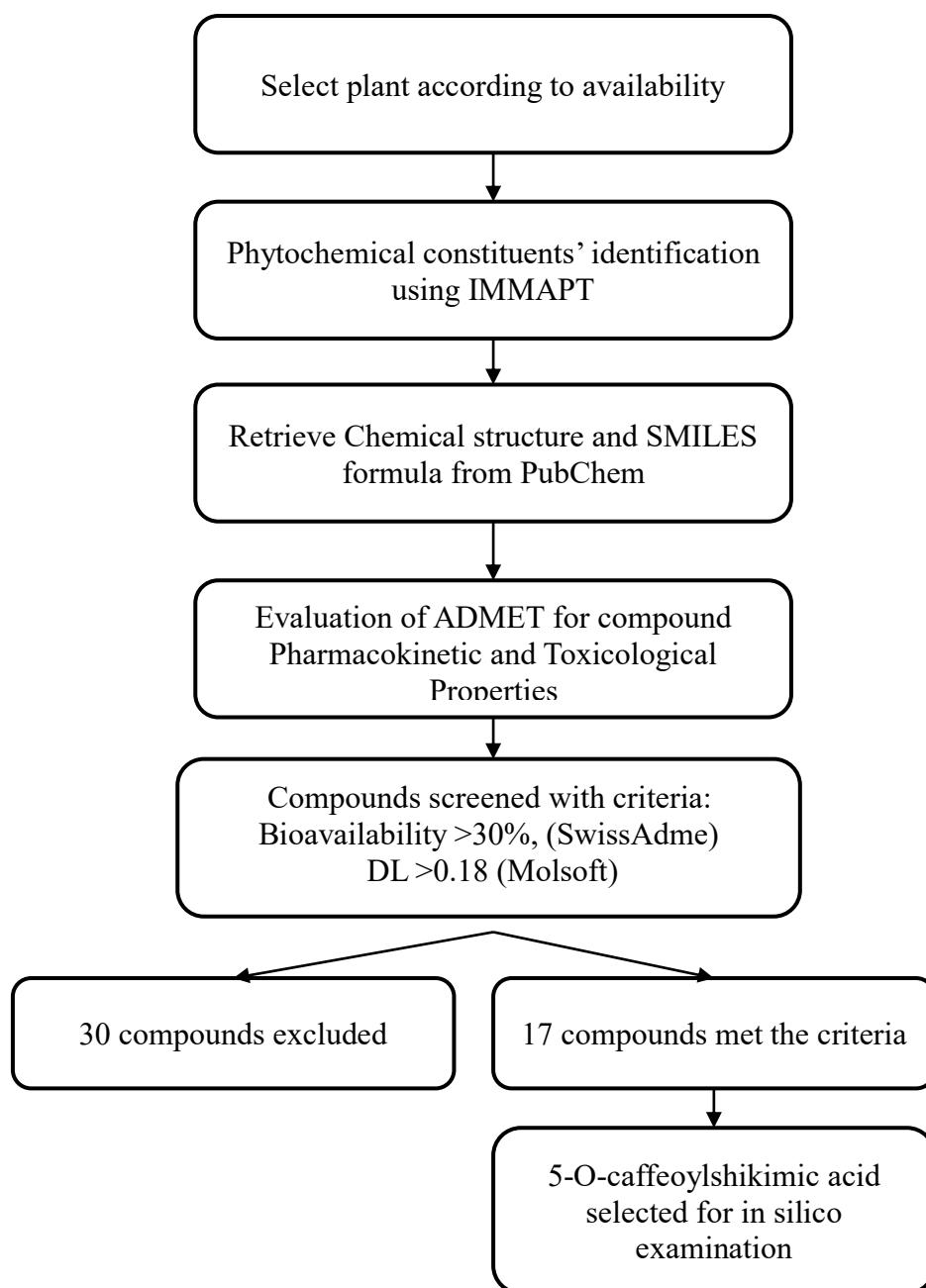


Figure 2 In silico Workflow for Bioactive Compound Identification

3.2.2 Compound target T2DM proteins

Swiss target prediction tool aids in estimating the main 100 genes targeted by the compounds based on chemical similarity of known compounds. Inputting SMILE of bioactive molecule to the software to target genes in *Homo sapiens* species provided scientists with ligand-based

targets with the chemical structure of the compound. The tool with newly launched version contains provides a free access to a large database of experimented chemical compounds with high performance rate of result analysis to be used as drug design bioinformatic tools (Daina, Michielin, & Zoete, 2019). Similarly, **PharmMapper** server requires the 2D structure of the bioactive compound in SDF format obtained from **PubChem** to determine the active target genes of the compound in human targets receptors through a reverse pharmacophore mapping approach. It is an essential step prior molecular docking procedure as it examines best fit scores of the intended dimensional structures properties of the compound molecule to target protein receptors (Ferrari, 2023). After the extraction of the compound target genes, disease target genes are acquired through **GeneCards** server. The web server aids in mining genes in cooperated in the progression of T2DM disease. These target proteins are targeted by drug molecules to induce a therapeutic action against the disease according to multiple web sources. Scientists are able to compare between databases in a graphical picture of the shared genes between the categories. Swiss target prediction genes and **PharmMapper** genes introduced into the category of compound gene targets while **GeneCards** T2DM genes are implied into disease genes. **jVenn** diagram illustrates the aligned genes between ligand target genes and disease progression pathway genes. Thus, common shared genes directed to be examined in the experiments.

3.2.3 GO and KEGG pathway analysis

To demonstrate a graphical chart of Genes enrichment pathways, Metascape analysis tool is used to determine main pathways of *Homo sapiens* species that includes the aligned ligand-gene targets. **Metascape** integrates multiple databases for rapid and comprehensive analysis of gene ontology, while excluding statistically insignificant networks (Zhou et al.,

2019). Therefore, such databases simplify networking interpretations into charts and hairball network structures to visualize the interactions between proteins.

Aligned genes were further analyzed using the **ShinyGo** (0.80) bioinformatical tool to conduct more intensive **gene ontology (GO)** and **Kyoto Encyclopedia of Genes and Genomes (KEGG)** pathways analysis. The tools provide the predominant 10 considerable pathways that supplies with ligand-disease target genes into biological process, cellular component, molecular function and **KEGG** pathways. Search engine is adjusted to human species and $p \leq 0.05$ of significance is chosen. Results are represented into color dot charts that resemble the number of genes, folding of gene over representation and significance.

3.2.4 Protein-Protein Network

Network construction of protein-protein interaction is performed using **STRING** tool (version 12.0). A list of common target genes submitted under the *Homo sapiens* species with a confidence score threshold of >0.9 , ensuring high-reliability interactions. The **STRING** database generates the network based the connection between the proteins physical and functional interactions that are collected from multiple bioinformatical resources, experiments databases, and scientific researches (Szklarczyk et al., 2021). **STRING** network of *P.dactylifera* target genes saved in TSV format is introduced into a **CytoScape** software (version 3.9.1). It is a free computational bioinformatic software used to visualize the biological network interactions of the proteomic data. Data is demonstrating into node structures that resembles protein structures and lines of interaction. **CytoScape** requires data retrieving to the software that is supported with multiples apps with different functions including **clusterMaker**, **PTMOracle** (Doncheva et al., 2018), and **CytoHubba**.

CytoHubba app is free ready to install app in **CytoScape** software that simplify the complex networks in 11 methods based on the researcher requirements of the protein nodes connetions according to the path length such as degree, edge percolated component, maximum neighborhood component, density of maximum neighborhood component, maximal clique centrality, and centralities that includes bottleneck, eccentricity, closeness, radiality, betweenness, and stress (Chin et al., 2014). Therefore, **CytoScape** protein- protein interaction is used in **CytoHubba** tool to retrieve the highest 10 closeness proteins investigated for their enriched pathways of interaction.

3.2.5 Molecular docking

For the intent of predicting the strength of the compound against diabetes mellitus, docking of compound with α -amylase and α -glucosidase are required before *in vitro* examinations. Using **UniProt**, protein structure of the alpha folds structure that contains the highest number of amino acids is retrieved in 3D PDB format. **UniProt** provides protein structures as understanding the structural molecular properties are essential to asses proteins functional actions although, continuous reviewing need to collate scientific literatures and experiments with the protein functional activity (The UniProt Consortium, 2018). Protein structures are prepared for docking by removing solvents and extra non-amino acids structures through **Chimera** software in version 1.16. The tool has a feature refereed as dock prep for preparing the structure for docking by multiple mechanisms such as deleting solvents, extra ions, alternative atoms, return converted amino acids into its original format, adjusting sidechains to be repaired or modified, and adding hydrogens, oxygen and charges (Meng et al., 2023). Indeed, **Chimera** is used to remove all water improve accuracy, decrease complexity of the binding pocket during interaction. In order to

investigate the binding active binding pockets of the receptor proteins, protein modified structure of **Chimera** is introduced to **PrankWeb** website in PDP format. Protein structure further processed to docking in **PyRx** software. Using the binding sites residues predictions of **PrankWeb** tool, the atoms are labelled in the macromolecule in **PyRx** and grid box is adjusted based on the position of the labelled atoms to encounter all active binding site amino acids for an accurate docking interaction. Through **Discovery studio** tool, docking is visualized in the binding pocket with the interacted amino acids between the compounds and proteins along with the bonding types in 2D structure.

3.3 *In vitro methods*

3.3.1 α -Glucosidase Inhibition Assay

The suppressive action of the 5-O-caffeoylshikimic acid toward α -Glucosidase was examined using method designated by Hosseini et al (2024) and Abbas et al (2017). Inhibition assays require the lead compound as the enzyme inhibitor, enzyme solution and substrate solution. A substrate solution composed of 5 mM, p-nitrophenyl- α -D-glucopyranoside, Sigma, USA) is added to 100 mM phosphate buffer (pH 7.0) that contains 0.2 g/L of NaN₃ and 2 g/L bovine serum albumin (BSA). Similarly, Enzyme solution is prepared with α -glucosidase (Sigma, USA) and same buffer mixture. In a 96-well microplate, 10 μ L of lead compound 5-O-caffeoylshikimic acid dissolved 1% of dimethyl sulfoxide. The mixture needs to be incubated 5 min at room temperature with 50 μ L of α -glucosidase. After 5 minutes, 50 μ L substrate solution is added and kept in incubator at 37 °C for 10 minutes. As a stopping solution, 100 μ L of sodium carbonate solution (100 mM) is added to end further enzymatic activity. Same procedure is followed for the control blank, except enzyme

solution. According to results readings, a Thermo Scientific Multiskan GO, USA machine is used for microplate absorbance reader. Absorbance was measured at 405 nm using a Thermo Scientific Multiskan GO microplate reader. Acrabose was used as a standard drug to compare it with 5-O-caffeoylshikimic acid on α -glucosidase. All experiments were performed in triplicate and repeated three times to ensure reproducibility. The equation is used to compute the enzymatic inhibition ratio of corresponding compound,

$$\text{Inhibition (\%)} = [(B - S)/B] \times 100$$

B resembles the absorbance of the blank while S is the absorbance of the test sample. Prism 5.0 software (GraphPad, San Diego, CA, USA) is used to calculate IC₅₀ values of each reaction.

3.3.2 α -amylase Inhibition Assay

The α -amylase inhibition assay of the phytochemical compound was examined using the technique described by Shahzad et al. (2019). As per enzyme solution, α -amylase extracted from porcine pancreas (Sigma, USA) was used while the compound serves as the inhibitor solution. In a 1.5 mL Eppendorf tube with buffer of 0.02 M sodium phosphate (pH 6.9) containing 0.006 M sodium chloride, 40 μ L of both inhibitor solution and enzyme solution are added for 10 min of incubation at 15-25°C. Next, starch solution prepared in 1% of dimethyl sulfoxide is added for further 10 minutes incubation at 25°C in the same Eppendorf tube. Then, 100 μ L of coloring reagent prepared from 10 g of sodium potassium tartrate, 1 g of 3,5-dinitrosalicylic acid and 20 mL of 2 N NaOH to a final volume of 100 mL in distilled water. The Eppendorf tube subjected to 5 minutes of hot water bath followed by dilution to 500 μ L with purified water when tube returns to room temperature. The final tube is used for reading the results

at the absorbance 540 nm. Similarly, to α -glucosidase, blank solution includes all the reagents except α -amylase. Same equation and program are used for calculating percentage of inhibition and IC_{50} that were previously described.

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Chapter Four

Research Study's Results, Discussion, and Recommendations

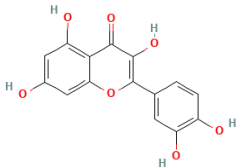
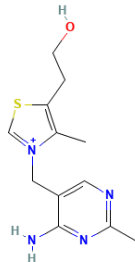
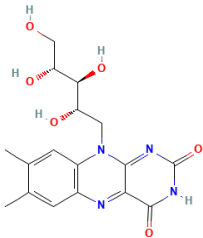
4.1. *In silico* Results

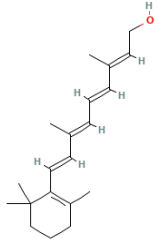
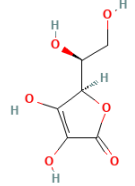
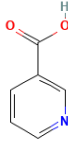
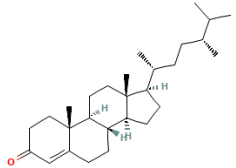
4.1.1 Active compounds and Gene Targets

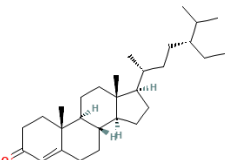
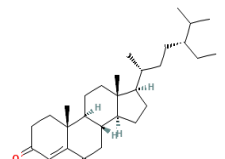
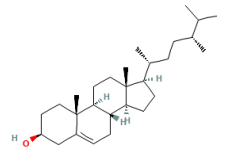
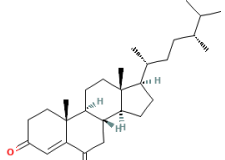
According to the results of **IMPPAT**, 47 bioactive constituents are found in *P.dactylifera* in different plant parts. All the phytochemical compound entries are subjected to be examined for their bioavailability and drug likeness score. Out of 47 entries, 17 compounds were found to meet the chosen criteria of >0.18 DL score and high bioavailability exceeding 30% including Quercetin, Thiamine, Riboflavin, Retinol, Ascorbic acid, Nicotinic acid, Campesterol-4-en-3-one, β -Sitostenone, Stigmast-4-ene-3,6-dione, Campesterol, Campesterol-4-en-3,6-dione, 6 β -Hydroxycampesterol-4-en-3-one, β -Sitosterol, Stigmastanol, 6 β -Hydroxystigmast-4-en-3-one, 5-O-Caffeoylshikimic acid, and Leucocyanidin (Table1). The compound 5-O-caffeoylshikimic acid (C₁₆H₁₆O₈) is chosen to proceed in the experimental study in treating T2DM as it has high drug likeness score reaches up to 0.65 and 56% bioavailability expecting it as a likely proper drug like compound. Based on PubChem chemistry database, the canonical **SMILES** of 5-O-caffeoylshikimic acid is C1C(C(C(C=C1C(=O)O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O.

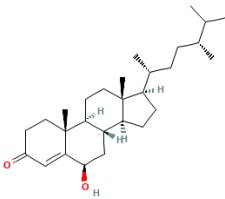
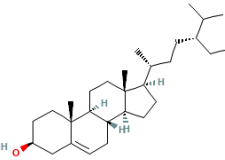
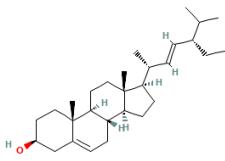
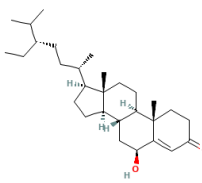
According to Swiss Target Prediction tool, 100 target genes were found to interact with 5-O-Caffeoylshikimic acid. 299 genes were retrieved from PharmMapper that can interact with 5-O-Caffeoylshikimic acid. In total, 397 of genes were targeted by the compound. However, GeneCards revealed a total of 15,407 genes associated with T2DM.

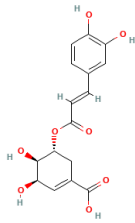
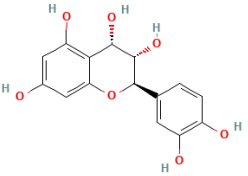
Table 1 Potential Bioactive Compound found in *P. dactylifera*

Compound	Plant part	Molecular weight g/mol	Bioavailability	Drug-likeness	structure	Pubchem ID
Quercetin	Flower Pollen	302.24	0.55	0.52		5280343
Thiamine	Fruit	265.35	0.55	0.87		1130
Riboflavin	Fruit	376.36	0.55	0.62		493570

Retinol	Fruit	286.45	0.55	0.73		445354
Ascorbic acid	Fruit	176.12	0.56	0.74		54670067
Nicotinic acid	Fruit	123.11	0.85	0.30		938
Campest-4-en-3-one	Stem	398.66	0.55	0.73		11988279

β -Sitostenone	Stem	412.69	0.55	0.91		5484202
(8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,7,8,9,11,12,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthrene-3,6-dione	Stem	426.67	0.55	1.09		5490007
Campesterol	stem	400.68	0.55	0.59		173183
Campest-4-en-3,6-dione	stem	412.65	0.55	0.94		15541631

6 β -Hydroxycampest-4-en-3-one	stem	414.66	0.55	1.06		15542141
β -Sitosterol	Stem Pollen	414.71	0.55	0.78		222284
Stigmasterol	Stem Fruit	412.69	0.55	0.62		5280794
6 β -Hydroxystigmast-4-en-3-one	Stem	428.69	0.55	1.22		9823926

5-O-Caffeoylshikimic acid	Fruit	336.29	0.56	0.65		5281762
Leucocianidol		306.27	0.55	0.53		440833

4.1.2 ADMET Profile of 5-O-Caffeoylshikimic acid

Certain properties are considered during drug discovery approach to ensure the effectiveness. **ADMET** profile of 5-O-caffeoylshikimic acid gives a drug like molecule properties considering it to be used as pharmacokinetic molecule (Table 2). Multiple properties are focused on in **ADMET** profile were concerned in the results. Overall, the compound has limited capability to be water soluble and skin permeable, however, has high absorption in the intestine (51.654%), besides being a P-glycoprotein substrate. In addition to that, the ability of the compound pass blood brain barrier (BBB) reduced to avoid the probabilities of having side effects in central nervous system. In contrast to excretion, compound has low clearance and doesn't act as Renal OCT2 substrate to be excreted through urine. Low excretion helps to maintain the drug effect for longer period. Among the main toxicity characteristics as skin sensitization, hepatotoxicity and maximum tolerated zone, the compound assumed to be non-toxic, although, the maximum tolerated was found to be is -0.103 log mg/kg/day. Overall, the **ADMET** analysis suggests that 5-O-caffeoylshikimic acid possesses a favorable safety profile and drug-like characteristics, supporting its further development as a therapeutic candidate for T2DM.

Table 2 ADMET Profile of 5-O-caffeoylshikimic acid (C₁₆H₁₆O₈).

Property	Characteristics	Value
Absorption	Water solubility (log mol/L)	-2.13
	Intestinal absorption (human) (% Absorbed)	51.654
	Skin Permeability (log Kp)	-2.735
	P-glycoprotein substrate	Yes

	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No
Distribution	BBB permeability (log BB)	-1.222
	CNS permeability (log PS)	-3.613
Excretion	Total Clearance (log ml/min/kg)	0.429
Toxicity	AMES toxicity	No
	Max. tolerated dose (human) (log mg/kg/day)	-0.103
	hERG I inhibitor	No
	hERG II inhibitor	No
	Hepatotoxicity	No
	Skin Sensitisation	No

4.1.3. Protein-Protein Interaction

Among 397 of compound target genes, 84 gene targets were found to be compatible and overlapping with T2DM genes according jVenn diagram (Figure 3). On the other hand, 313 gene targets were not intercalated with disease target genes. Certainly, the genes are resembled in a protein-protein interaction in **STRING** tool as nodes structure that are intercalated with each other in lines (Figure 4). After importing PPI network in **CytoScape** software, the network presents 46 nodes and 47 edges (Figure 5). Similarly, to **String** tool, the nodes structure represents the disease target proteins while edges shows the connections between them. Highly connected proteins are shown in clusters with high degree interactions. In total, 4 clusters are found in the PPI, the largest cluster has 17 nodes. Based on **CytoHubba**, the 10

highest closeness node scores are identified as SRC (12.08), MAPK8 (11), CASP3(10.5), PRKCD (10.5), IGF1R (8.8), ESR (8.5), PRKCE (8.5), MMP2 (8.03), CASP8 (7.45) and INSR (7.45). The color is ranked from red to yellow according to degree of closeness (Figure 6).

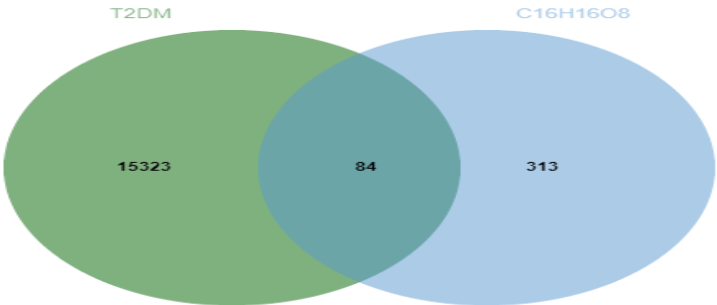


Figure 3 Venn Diagram of Potential Targets Between T2DM

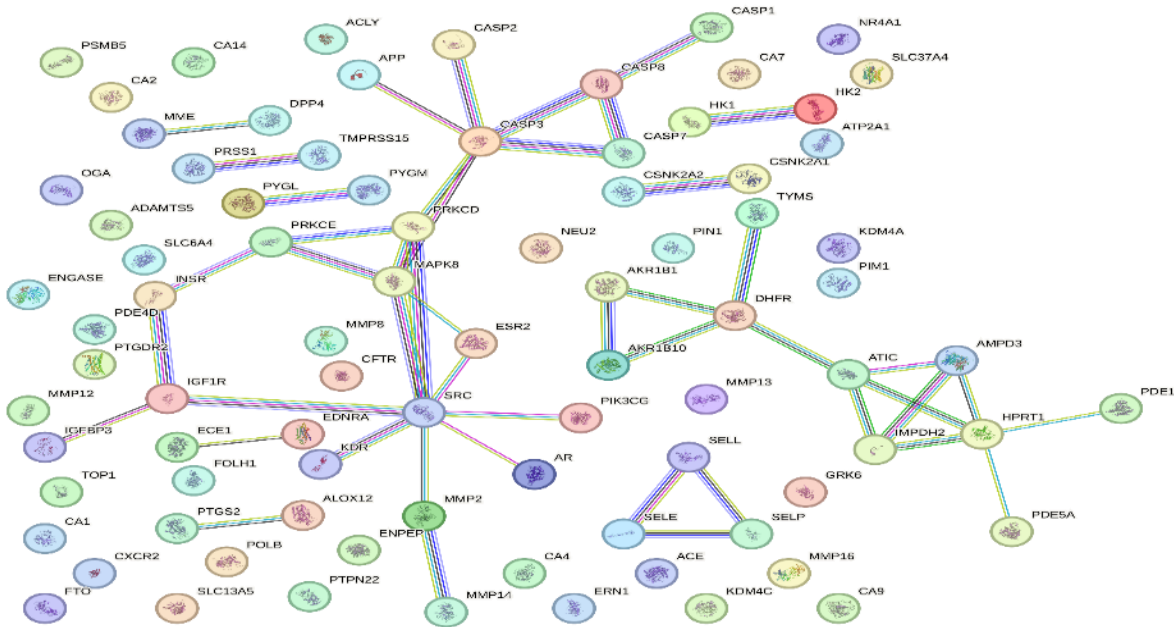


Figure 4 Protein-Protein Interaction using STRING tool.

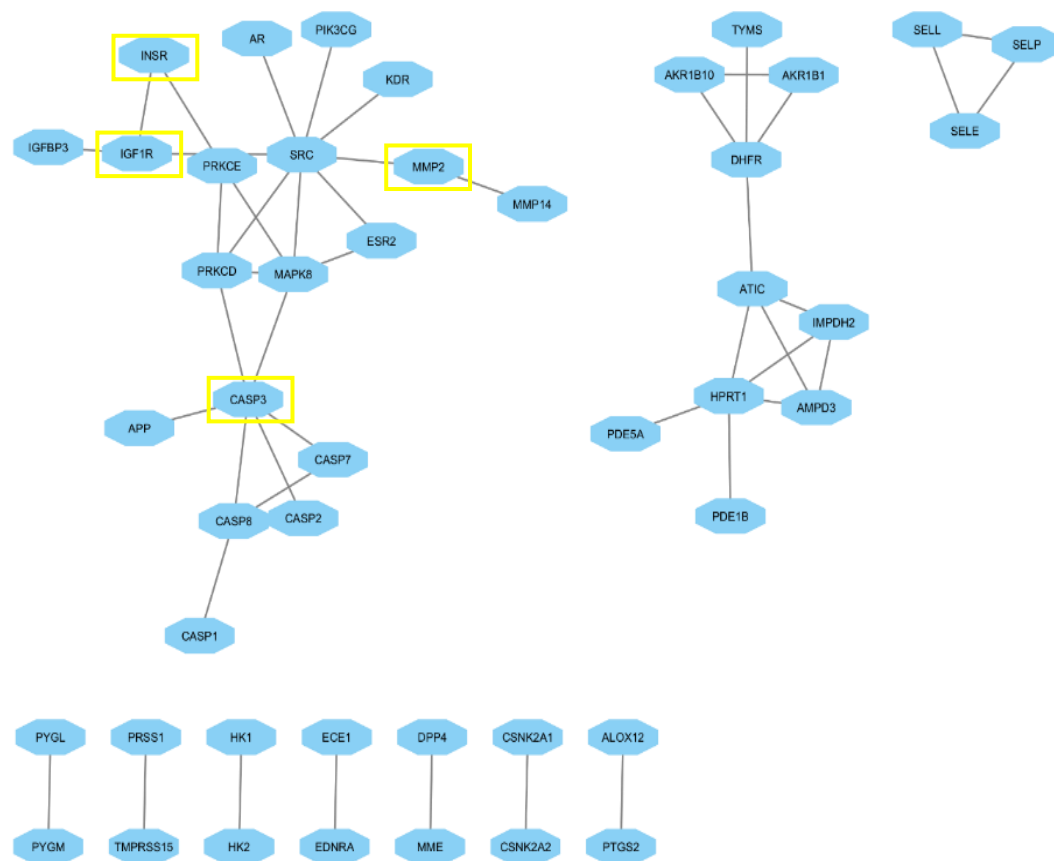


Figure 3 CytoScape Protein-Protein Interaction.

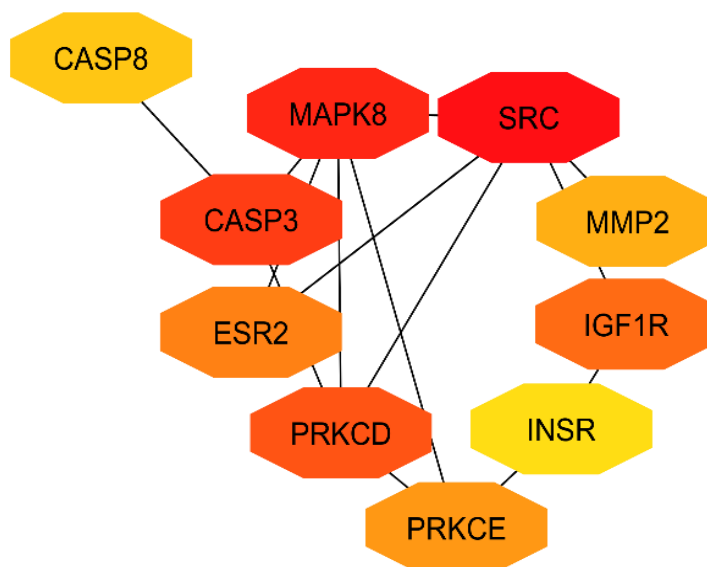


Figure 6 Top 10 Closeness Nodes by CytoHubba.

Table 3 Top Gene enriched in KEGG pathways.

Gene	Name	Score	Pathways
SRC	Proto-Oncogene Tyrosine-Protein Kinase	12.08	Lipid and Atherosclerosis
CASP3	Caspase 3	10.5	Lipid and Atherosclerosis Alzheimer Disease Neurodegeneration Multiple Diseases Age-Race Signaling Pathway in Diabetic Complications TNF Signaling Pathway
MMP2	Matrix Metalloproteinase 2	8.03	Age-Race Signaling Pathway in Diabetic Complications
INSR	Insulin Receptor	7.45	Type II Diabetes Mellitus Insulin Resistance Alzheimer Disease

4.1.4 GO and KEGG pathway

Metascape charts illustrated the presence of target genes in the top 20 pathways enriched with compound target genes that includes both GO and KEGG enrichments analysis (Figure 7). Similarly, the overlapped genes processed to enrichment analysis of GO biological, cellular, molecular and KEGG pathways. According to GO biological pathways, genes were enriched in the mostly in chemical and organic substances pathway, while it is highest significant in the Response to organic substance pathways (Figure 8). In the cellular pathway, membrane raft and membrane microdomain are the most significant pathways although the number of genes is nearly 10 (Figure9). Based on the molecular pathway, catalytic activity acting on protein has the highest significant result, however, the figure resembles carbonate dehydratase with the highest fold enrichments which indicates overrepresentation of the genes in the pathway (Figure 10). In addition to the previous pathways, KEGG pathways indicates Nitrogen metabolism and metabolic pathways as the highest significant pathways, and mostly enriched genes are in the metabolic pathways. Also, Type II diabetes mellitus, AGE-RACE signalling pathway in diabetic complications and Insulin resistance

pathways are presented with less number of of genes compared to nitrogen metabolism pathway (Figure 11). According to CytoHubba 10 closeness degree nodes, Based on KEGG pathway analysis, CASP3 and MMP2 were enriched in Age-Race Signaling Pathway in Diabetic Complications, while INSR was targeting Type II Diabetes Mellitus and Insulin Resistance pathways (Table 3).

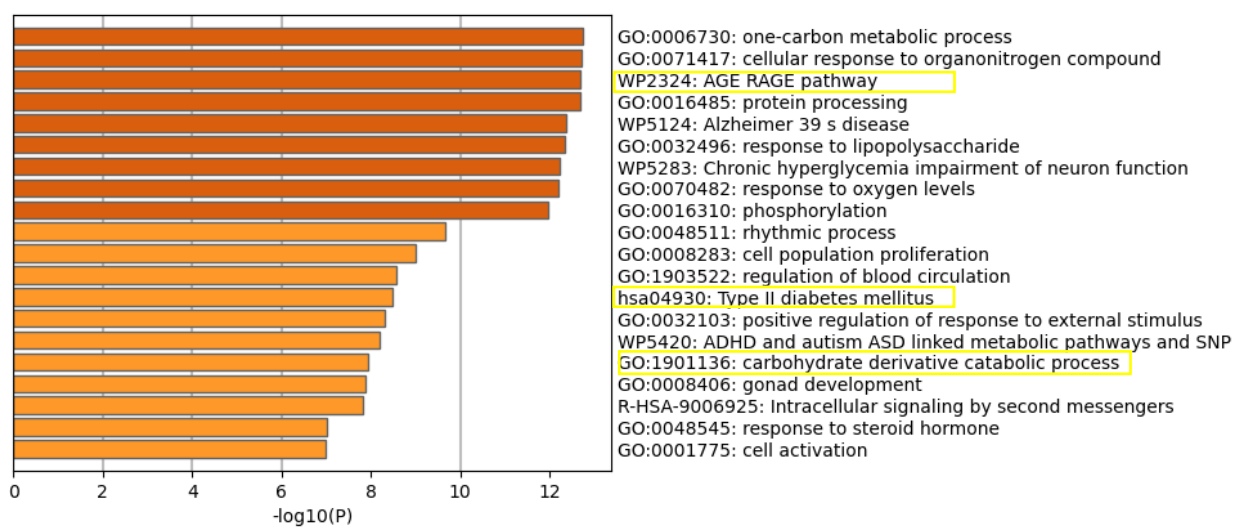


Figure 7 Metascape Enrichment Analysis of pathways enriched with genes aligned between T2DM and C16H16O8

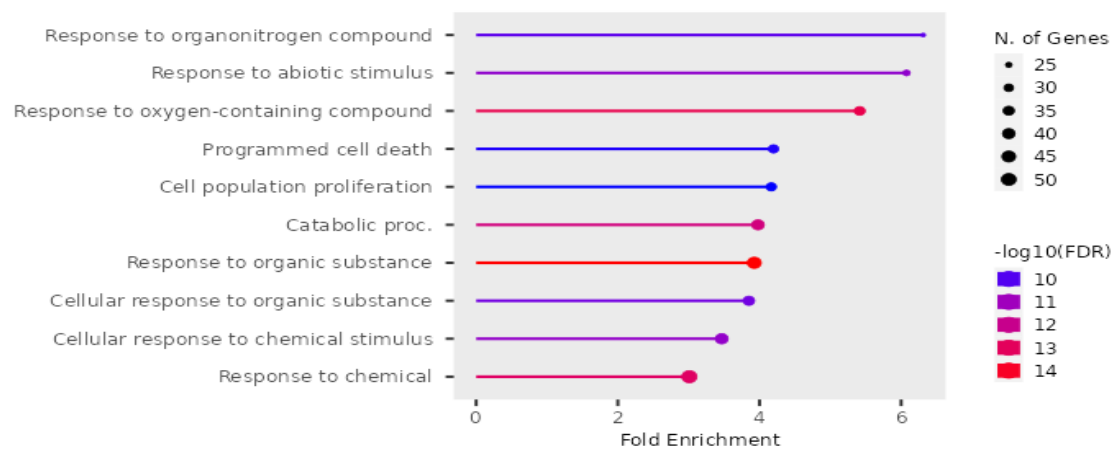


Figure 8 GO Biological Pathway

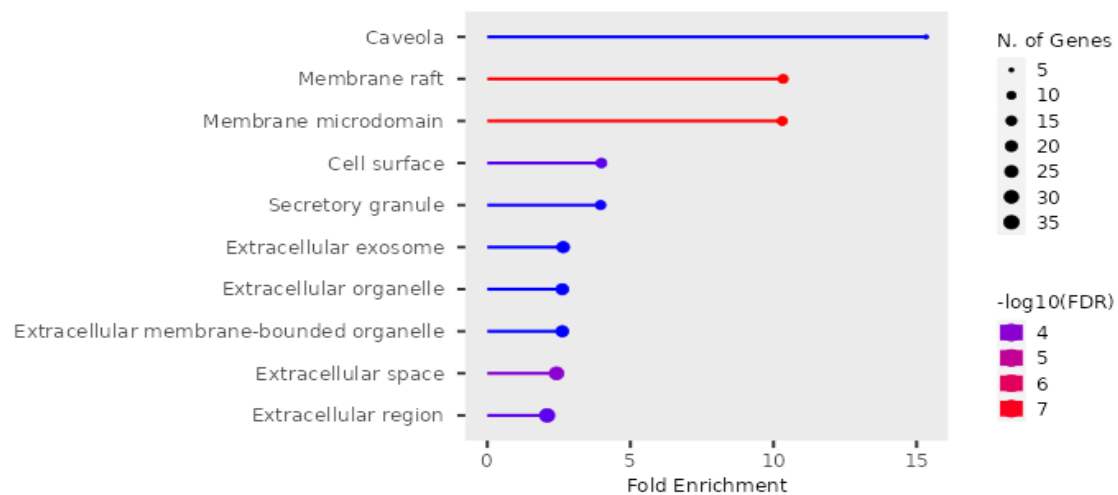


Figure 9 GO cellular Pathway.

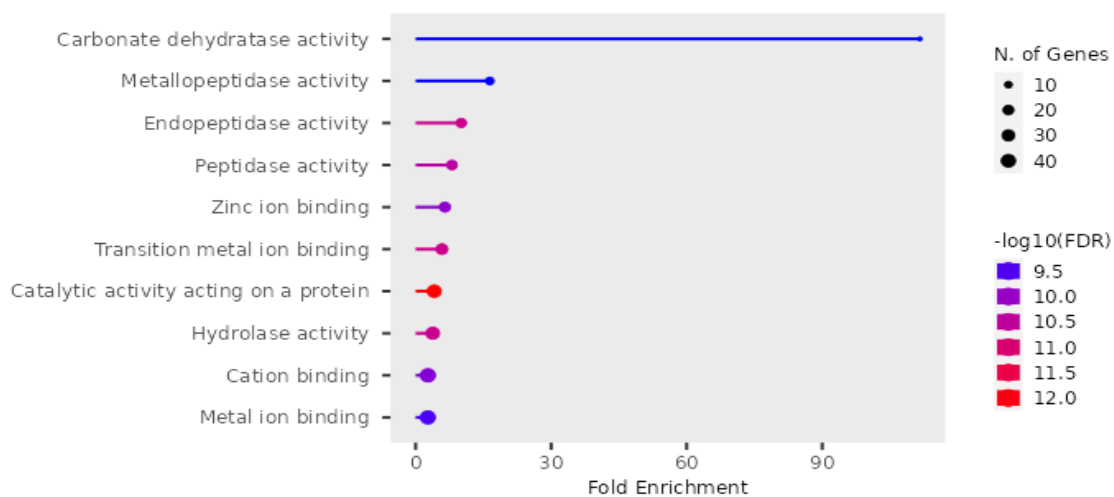


Figure 10 GO molecular Pathway

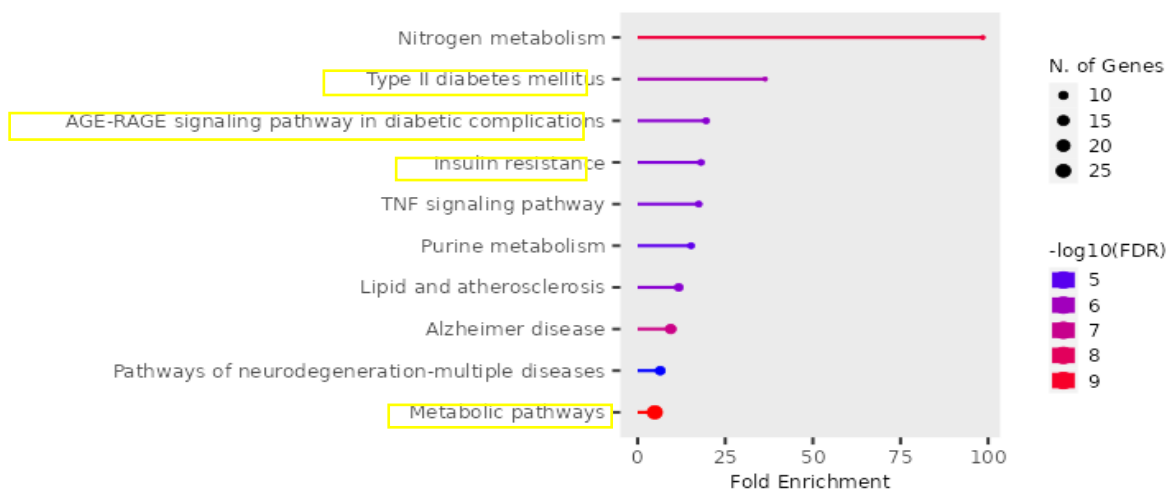


Figure 11 KEGG Pathway.

4.1.4. Molecular docking

5-O-caffeoylshikimic acid undergone docking analysis with the main diabetic enzymes. Therefore, Alpha folds of α -amylase and α -glucosidase predicts maximum amounts of amino acids (1-511, 1-944) presented in the protein structure (UniProt: P0DUB6, Q14697). Based on PrankWeb tool, two active binding pockets are found in α -amylase where ligands can bind. The highest score of the binding pockets is 20.94, hence, it the most effective binding site interacts with the compound structure (Table 4). The amino acids of the binding pocket include HIS_116, TYR_166, LEU_177, SER_178, LEU_180, ASP_212, ALA_213, LYS_215, HIS_216, GLU_248, ILE_250, ASP_315, HIS_320, GLY_321, LYS_367, ASP_371, TRP_372, ASN_68, PRO_69, TRP_73, TRP_74, TYR_77, and GLN_78. The docking is visualized by labelling the binding pockets active sites and positioning the grid to include them in the docking area, therefore, autodocking grid is positioned to X: 13.1819, Y: 10.6967, and Z: -5.6770 and with a dimension of X: 35.4559, Y: 25.0000, and Z: 29.6576. Using the highest model of docking binding affinity (-7.7) (Table 6), discovery studio identified the protein-ligand structure with their binding amino acids (ASP212, GLU248, SER178, GLY78) that are attached by conventional hydrogen bonds (Figure 12). Moreover, compound interacts with TRP74 by Pi-donor hydrogen bonds and Pi-Pi interaction with TYR77. However, only one unfavourable interaction occurs between the ligand and ARG210.

Regarding α -glucosidase, PrankWeb tool has identified three active binding pockets. The best binding pocket shown has scored 11.82 (Table 5). The amino acids of the binding pocket include ASP_283, PHE_285, TRP_401, ASP_429, TRP_503, TRP_540, ASP_542, MET_543, PHE_549, VAL_554, ASP_618, PHE_651, HIS_676, and HIS_678. The autodocking grid is positioned to X: -3.4002, Y: -12.8601, and Z: -12.6263 and with a

diminution of X: 28.3819, Y: 25.0000, and Z: 25.0000. Autodocking results revealed a highest affinity (-7.8) (Table 6). Through discovery studio, ligand has interacted with conventional bonds with ASN37 and ASP618 (Figure 13). Also, Pi-Pi interaction is shown with glucosidase TRP503.

Table 4 Binding and interaction of C₁₆H₁₆O₈ and α -Amylase.

α-Amylase (AF-P0DUB6-F1)				
Name	Rank	Score	Probability	Residues
Pocket 1	1	20.94	0.849	HIS_116, TYR_166, LEU_177, SER_178, LEU_180, ASP_212, ALA_213, LYS_215, HIS_216, GLU_248, ILE_250, ASP_315, HIS_320, GLY_321, LYS_367, ASP_371, TRP_372, ASN_68, PRO_69, TRP_73, TRP_74, TYR_77, GLN_78.
Pocket 2	2	0.89	0.004	ASN_152, LYS_155, ARG_173, ASP_182, LEU_183, GLY_186, HIS_216, MET_217, TRP_218, ASP_221

Table 5 Binding and interaction of C₁₆H₁₆O₈ and α -Glucosidase.

α-Glucosidase (AF-Q14697-F1)				
Name	Rank	Score	Probability	Residues
Pocket 1	1	11.82	0.653	ASP_283, PHE_285, TRP_401, ASP_429, TRP_503, TRP_540, ASP_542, MET_543, PHE_549, VAL_554, ASP_618, PHE_651, HIS_676, HIS_678.
Pocket 2	2	1.81	0.033	ALA_387 LEU_388 ALA_584 ASP_585 ARG_588 ARG_596 PRO_597 ARG_610 PHE_611 HIS_719 PHE_852 TYR_854 GLN_855
Pocket 3	3	1.16	0.011	GLN_703, SER_706, ILE_779, TYR_782, ARG_812, MET_814, PHE_831

Table 6 Affinity of Binding pockets of C16H16O8 with target proteins

α -Amylase/C ₁₆ H ₁₆ O ₈	Models	Binding Affinity	rmsd/ub	rmsd/lb
	Model 1	-7.7	0	0
	Model 2	-7.7	2.637	1.728
	Model 3	-7.4	7.115	2.423
	Model 4	-7.3	6.781	2.691
	Model 5	-7.3	7.343	2.503
	Model 6	-7.2	3.799	1.984
	Model 7	-7.1	6.938	2.578
	Model 8	-7.1	3.443	2.159
	Model 9	-7	7.073	2.306
α -Glucosidase/C ₁₆ H ₁₆ O ₈	Model 1	-7.8	0	0
	Model 2	-7.4	2.277	1.741
	Model 3	-7.4	2.253	1.586
	Model 4	-7.3	1.945	1.367
	Model 5	-6.9	3.301	1.806
	Model 6	-6.8	6.784	4.429
	Model 7	-6.8	9.106	5.998
	Model 8	-6.7	5.254	3.677
	Model 9	-6.6	10.436	5.852

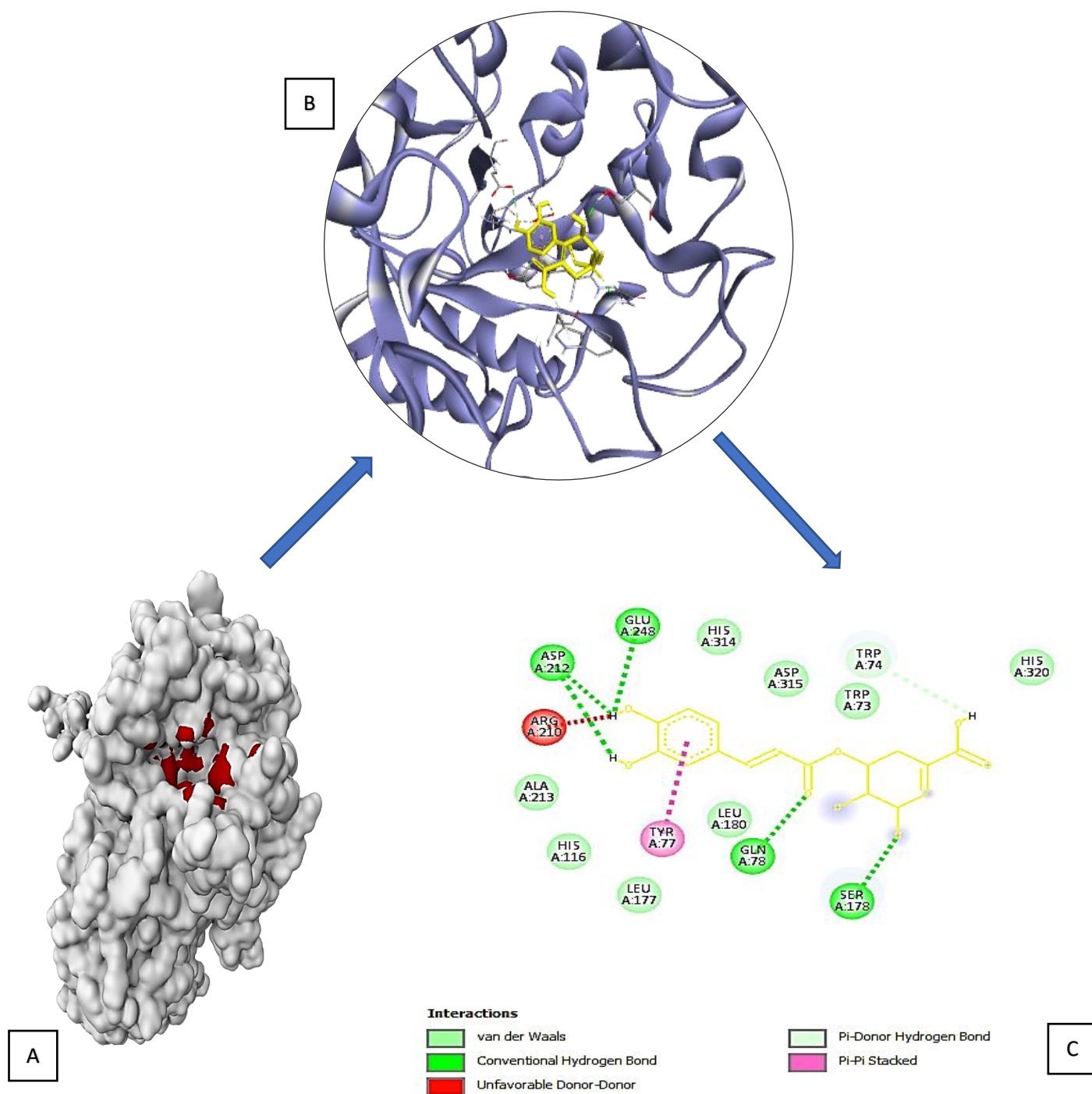


Figure 12 Molecular Docking of C16H16O8 with α -amylase. A) α -amylase protein showing active binding pocket. B) C16H16O8 attached to the active binding site. C) C16H16O8 bonds interactions with amino acids

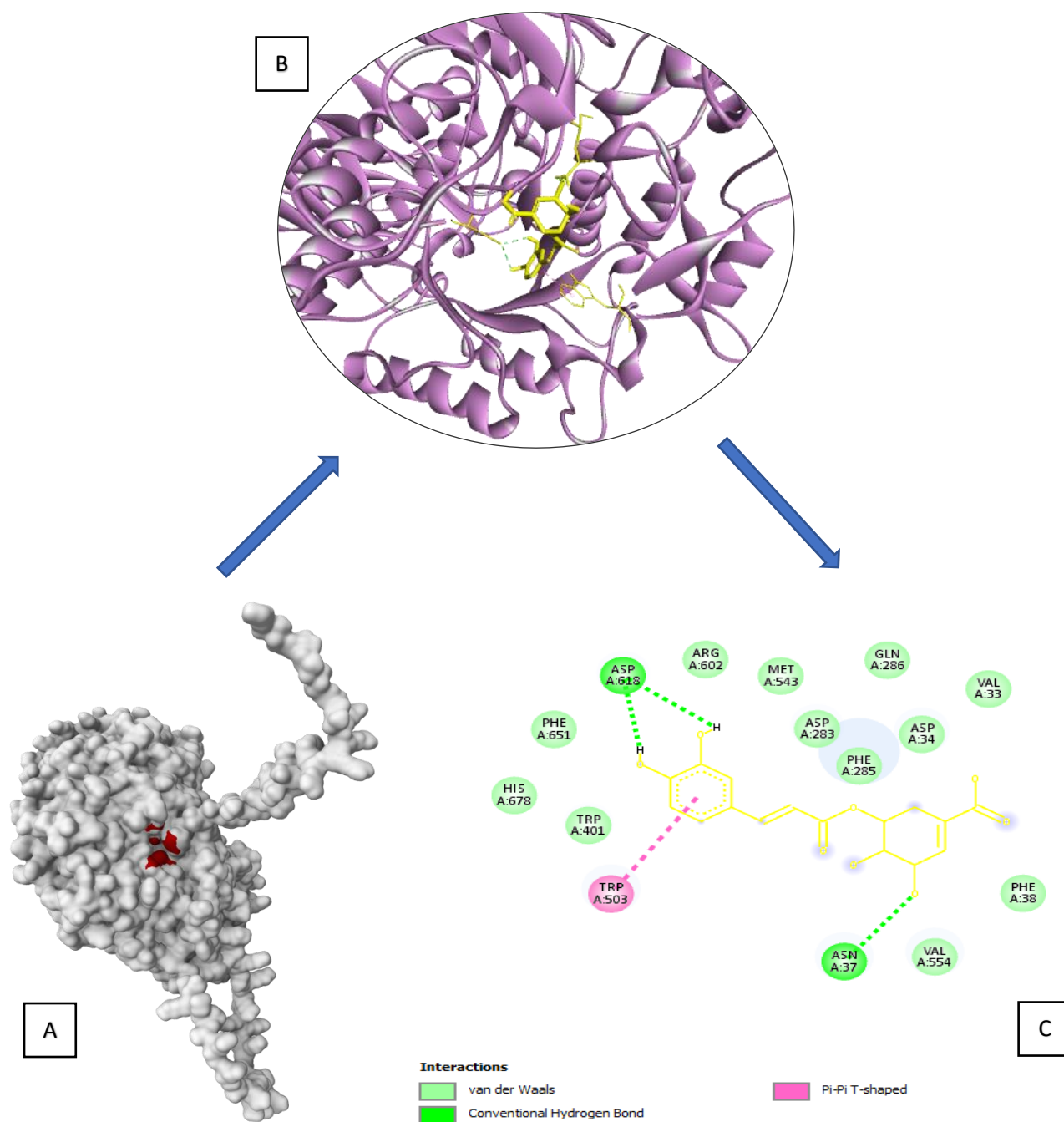


Figure 13 Molecular Docking of C16H16O8 with α -glucosidase. A) α -glucosidase protein showing active binding pocket. B) C16H16O8 attached to the active binding site. C) C16H16O8 bonds interactions with amino acids

2. *In vitro* Results

4.2.1. α -Glucosidase and α -Amylase Activity

5-O-caffeoylshikimic acid was purchased from the MERCK Republic of Korea “CAS Number:73263-62-4” to be examined against α -glucosidase and α -Amylase enzymes. Acarbose was the standard drug for results effectiveness comparison. As a result, to the inhibition percentages, the compound 5-O-caffeoylshikimic acid giving a promising result against the main diabetic related enzymes (Figure 14 A & B) comparing to Acarbose (Figure 14 C & D). The IC_{50} value of 5-O-caffeoylshikimic acid was determined to be 7.668 μ M ($R^2 = 0.74$) with α -glucosidase while Acarbose has demonstrated IC_{50} value of 8.621 μ M ($R^2 = 0.59$). Moreover, The IC_{50} value of 5-O-caffeoylshikimic was found to be 17.862 μ M ($R^2 = 0.79$) with α -amylase comparing to the reference drug Acarbose where it showed to be 30.978 μ M ($R^2 = 0.96$).

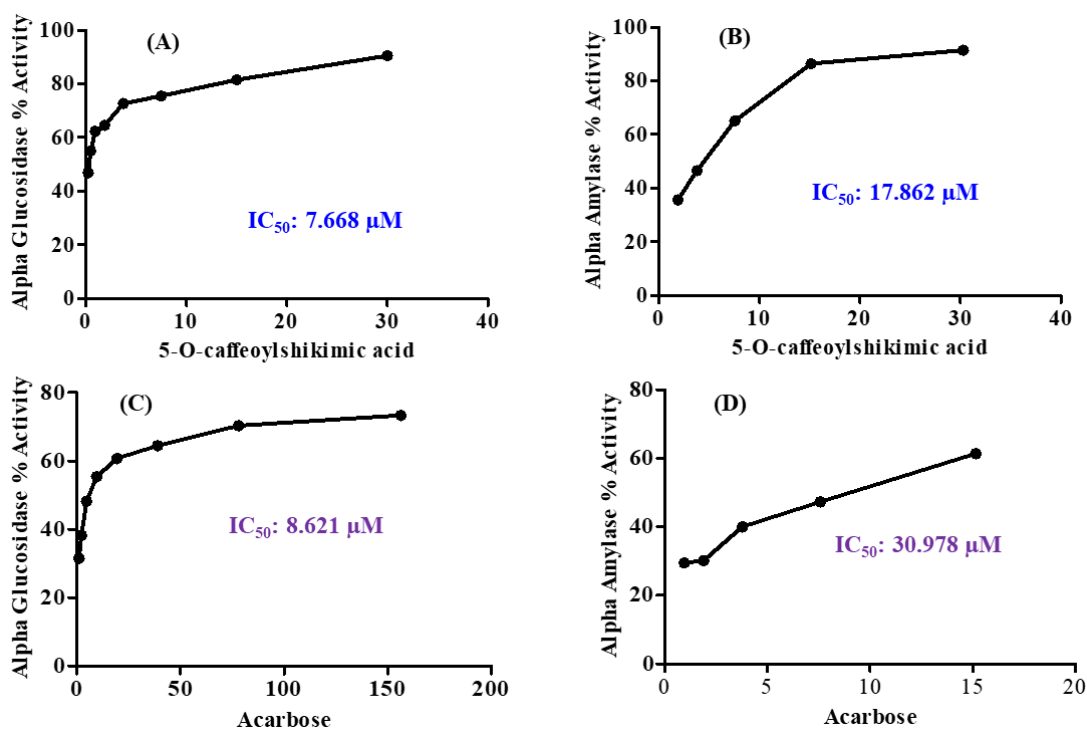


Figure 14 IC_{50} Curves of 5-O-caffeoylshikimic acid and Acarbose compound. (A & C) α -Glucosidase (B & D) α -Amylase.

4.3. Discussion

T2DM is a widespread long-lasting endocrinal condition classified by incompetence of body system to sensitise insulin due to resistance or improper functioning that leads to hyperglycaemic conditions. The initial therapeutic strategy against T2DM requires lifestyle practices management through physical activity and dietary intake along with synthetic oral administration and injectable drugs that stimulate insulin sensitivity, secretion, diabetic enzymes inhibition, and glucose excretions (Magkos et al., 2020). Various plants are highly concerned in the therapeutic medicine, to its high nutritional value that affects certain health conditions through their biological activities that targeting oxidative stress reactions, bacterial growth and cancerous cells proliferations. Such types of phytochemical compounds can be extracted from different plant parts. This study aimed to examine anti-hyperglycaemic effect of 5-O-caffeoylshikimic acid that can be found in *P. dactylifera*, a native Bahraini plant to provide a sustainable source of compound for treatment approaches to support the availability of compounds with fewer side effects hence it is plant-based therapy. According to the National Space Science Agency, around 257 thousand of *P. dactylifera* had been encountered all over Kingdom of Bahrain governorates via satellite image in 2018 (Amad, 2021). Current study emphasizes *in silico* approaches for proper compound selection followed by *in vitro* examinations of the chosen compound suppression of diabetic enzymes, α -glucosidase and α -amylase.

The Bioactive compounds are plants secondary metabolites that have ecological roles regardless to nutritional primary metabolites that are crucial to basic life support of the plants. Date fruits of *P.dactylifera* is has high nutritional value due to different phytochemical classified as carotenoids, polyphenols, tannins and sterols. However, cultivation practices, origin, fruit

type, maturation status and storage can affect the significance concentration of compounds in the plant parts (Al-Alawi, 2017). In the present study, 17 bioactive constituents of *P.dactylifera* were found to meet drug-like properties. Compounds with high oral bioavailability and drug likeness score are expected to have pharmaceutical effect according to their structure, physical and chemical properties. Table 2 emphasizes ADMET properties of 5-O-caffeoylshikimic acid for pharmaceutical use. Caffeoyl shikimic acid is a combined structure of phenolic hydroxy group of the shikimic acid by an ester bond to carboxyl group of caffeic acid (Yu et al., 2024). High oral bioavailability exhibits the efficiency of the effective required concentration to enter the systemic circulation and reach to their biological destination in spite of distribution and clearance conditions (Price and Patel, 2023). Therefore, the poor bioavailability compounds require higher dosages and side effects are more expected. In the current study, 5-O-caffeoylshikimic acid was chosen with drug likeness score 0.65 and oral bioavailability of 56%. According to this study, 5-caffeoylshikimic acid has low solubility (-2.13), which is a key factor in the effectiveness of drug bioavailability. It is stated in the study carried out by Pinho et al. (2014) that low solubility and stability is expected from caffeic acid compounds which is encounter difficulties in drug discovery, therefore, axillary factors as cyclodextrin was tested to improve characteristics of the compound for their antibacterial treatment. Due to limited absorption of the esterified form of caffeic acid by human tissues and fluids, the absorption of the compound by intestinal mucosa is expected after hydrolysis of esterase enzyme by intestinal microflora (Ganguly et al., 2023). Study results predict intestinal absorption of 5-O-caffeoylshikimic acid to reach 51.654%.

The ability of the compound to cross cell membranes is essential to be addressed to prevent unfavorable and adverse side effects of therapeutic

applications on central nervous system. The number of hydroxyl groups in the compound structure maintain low lipophilicity that reduce compound penetration to brain and nervous system (Liu et al., 2020). 5-caffeoylshikimic acid has five hydroxyl groups and ADMET profile suggest a low possibility of the compound to cross BBB (-1.222 log BB) and CNS (-3.613 log PS) due to its hydrophilic properties. Moreover, total clearance rate of the drug from the bloodstream is crucial for effective dose levels. According to the study conducted by Benet et al (2021), the rate of drug excretion needs to be considered according to organ health. Indeed, drug concentration beside the drug total clearance rate in blood both contribute to an effective dose administration to reduce the risk of improper dosing. However, traditional methods of drug clearance calculations are still reliable to be used for pharmaceuticals (Rowland et al., 2022).

5-O-caffeoylshikimic acid is a secondary metabolite assigned under the phenolic bioactive compounds. 5-O-caffeoylshikimic acid can be extracted from different plants such as *P.dactylifera*, *Smilacis Glabrae* rhizoma (Zhang et al., 2021), *Solanum somalense* leaves (Chideh et al., 2014), *Eriobotrya japonica* leaves (Park et al., 2019), and *Stevia rebaudiana* leaves (Pacifico et al., 2018). Several researches were conducted targeting efficiency of 5-O-caffeoylshikimic acid against diseases through its biological functions. In the study performed by Zhang et al (2021), 5-O-caffeoylshikimic acid has potentially antagonist Xanthine oxidase to avoid high serum and hepatic uricemia considering it as a novel Xanthine oxidase inhibitor (Zhang et al., 2021). The phenolic extraction of *Smilax glabra* has shown a remarkable antioxidant and inflammatory mediators inhibition effect in the study conducted by Lu et al. (2014), where 5-O-caffeoylshikimic acid was one of the phenolic compounds found in the plant extract.

According to the study undertaken by Xu et al. (2020), streptozotocin-induced diabetic rats had been treated with caffeic acid for 5 weeks, their findings showed a significant decreasing in sugar concentrations, rising insulin levels and enhancement in lipid profile. This enhancement was either due to insulin secretion or improvement in pancreatic β -cells formation. Therefore, phenolic compounds have an impact on antagonizing insulin resistance and augmenting glucose uptake through targeting phosphorylation of INSR and signaling proteins that consequently targets metabolic pathways (Huang et al., 2009). Ihara et al (2023), had conducted a research on the effect of caffeine and chlorogenic acid on T2DM, their results had shown a promising effect of the compound on restoring endoplasmic reticulum from stress due to its antioxidant properties and its protective action on pancreatic β cells by triggering insulin receptors substrate. Hence 5-O-caffeoylshikimic acid shares structure with the previous compounds, it is expected to have similar effects on T2DM. During glucotoxicity condition, glucose-stimulated insulin secretion by genes expression and triggers to increase secretion of insulin after the use of phenolic compounds due to their effective protection and influencing functional role in pancreatic β cell (Bhattacharya, 2013).

According to study results, MMP2 is one of the closest proteins that may interact with the compound through lipid and atherosclerosis pathways. Increased activity of MMP2 due to hyperglycemia, mediated by oxidative stress and glycation end products, can serve as a diagnostic marker for diabetes and atherosclerosis progression, thereby increasing the risk of thrombosis (Das and Maiti, 2013). Therefore, the compound's capability to interact with MMP2 may downregulate its expression, thereby promoting its protective effects. Another biomarker protein is CASP3; increasing CASP3 levels leads to the destruction of pancreatic β -cells, which is directly linked

to elevated HbA1c levels, causing hyperglycemia and ultimately leading to T2DM (Sun et al., 2021). A previous study carried out by Sato et al (2015) highlights the critical role of SRC in stimulating glucose-induced insulin secretion to maintain glucokinase activity. Indeed, suppression of SRC corresponds to a reduction in glucose metabolism, as the enzyme shifts toward intracellular organelles without affecting its expression.

In fact, hyperglycemia is expected after carbohydrates rich diet due to their conversion into monosaccharides, controlling the digestion enzymes can ultimately reduce the risk high circulating glucose levels through prolonging digestion process (Molehin, 2019). From the inhibition *in vitro* assay, 5-O-caffeoylshikimic acid successfully resembled as diabetic enzymes inhibitor based on inhibitor concentration variation in the assays. Accordingly, the lead compound has antidiabetic efficiency to interact as enzyme substrate in order to down regulate its activity. 5-O-caffeoylshikimic acid has effectively inhibited α -glucosidase with IC_{50} of 7.668 μ M and α -amylase with IC_{50} of 17.862 μ M compared to acarbose the standard inhibitor that had higher IC_{50} than the lead compound which indicates a suggestive pharmaceutical hypoglycemic significance. This result suggest that compound has a modest potency against the activity of α -glucosidase as it requires lower concentrations compared to acarbose. However, the interaction of the compound with α -amylase is slightly more potential than acarbose. However, previous studies had stated the effect of α -amylase inhibition on gastrointestinal distribution as it was demonstrated to cause unfavorable side effects, therefore considering pharmaceutical drugs with potential α -glucosidase inhibitors rather than α -amylase (Zengin et al., 2019). A study performed by Cao et al (2023), had investigated the impact of shikimic acid on α -amylase where compounds are found to change the protein by decreasing α -helix structure and increasing β -sheet by

uncompetitive interaction of the compound into non-active pocket and leads to inhibit starch digestion. Oboh et al. (2014), stated the potency of phenolic acids specially caffeic acid in targeting diabetic related enzymes by reducing glucose absorption in the digestive tract, however, complex of compounds as chlorogenic acids that are structurally composed from two compounds will undergo hydrolysis into caffeic acid during metabolism to be absorbed by passive diffusion and active transport proportionally inducing its inhibitory activity action.

This inhibitory effect of 5-O-caffeoylshikimic acid aligns with the suppression effect of *P. dactylifera* extracts in the previous studies. In the study conducted by Ajiboye, Oloyede, and Salawu (2020), *P. dactylifera* fruits phenolic and flavonoid extracts had a significant inhibitory action against α -amylase and α -glucosidase comparing to standard diabetic drug. Moreover, they confirmed their results through *in-vivo* experiment which indicated decreasing fasting glucose, increased insulin, and glycogen in albino rats administered the extract compared to diabetic control rats. Correspondingly, the study undertaken by Chakroun et al. (2016) on *P.dactylifera* leaves extracts had potential enzyme inhibition effect more significantly against α -glucosidase, therefore, blood glucose were found to decrease after oral extract administration by the albino mice. According to this study result, 5-O-caffeoylshikimic acid in plants extract is one of the main anti diabetic enzyme inhibitors that caused decreasing in blood glucose of the clinical trials on albino mice in the previous studies.

Molecular docking displays the interaction between the small ligand compounds to protein molecules as enzymes in their active binding pockets to predict the ideal interaction pose and affinity that stimulate or inhibit the biological enzymatic action for an optimal pharmaceutical activity (Roy et al., 2015). Through molecular simulation, the lead compound interacts with

amino acids in the active site pockets with the highest affinity to both α -amylase and α -glucosidase to obtain a potential suppression rate. According to α -amylase, 5-O-caffeoylshikimic acid has four conventional hydrogen bonds with amino acids residues such as GLU-A248, ASP-A212, GLN-A78, and SER-A178 that forms strong bonds to stabilize the binding affinity of intermolecular interactions. On the other hand, α -glucosidase had formed double hydrogen bonds through AS-A618 and one hydrogen bond by ASN-A37 with the ligand compound. Other interactions are also presented in the compound-ligand molecular associations through van der waals, and pi-pi stacking. Although these non-covalent interactions help the ligand to interact with the binding sites of the corresponding proteins and affect target their biological activity, they are considered as weak interactions in comparison to hydrogen bonds as they are fundamental to sustain the functional stability of the complex (Mir et al., 2022). In accordance with the study of Safitri et al. (2020), hydrogen bonds are crucial in estimating the strength of compound-ligand complex as the lowest number of hydrogen bonds were most likely to have weakest binding energy. Similar findings were also reported in the study of Nyathi et al. (2023). Based on the strength and number of hydrogen bonds, the affinity gets higher leading more stable interaction. Despite that, acarbose that had high number of hydrogen bonds, however, some bonds were formed with water molecules.

4.4 Conclusion

The current study declared the anti-hyperglycemic effect of the phenolic compound 5-O-caffeoylshikimic acid that is presented in a Bahraini indigenous referred as *P.dactylifera* to treat T2DM. The compound shown optimistic results to target the main gastrointestinal enzymes α -amylase and α -glucosidase that increase postprandial monosaccharaides in blood leading to high circulating blood glucose. *In vitro* findings suggest compound

effectiveness in delaying digestion, therefore, it can reduce blood glucose levels in blood. According to molecular docking simulations, high binding affinity cause effective interaction between the 5-O-caffeoylshikimic acid and α -amylase and α -glucosidase. Therefore, this research serves as a cornerstone for future researches to explore 5-O-caffeoylshikimic acid as a plant-based therapeutic drug against T2DM. However, *in vivo* confirmation of the current research findings is required to ensure safeness the capability of the compound in managing T2DM.

4.5 Research Study's Limitation and Recommendations

The composition and nutritional value of date fruits vary according to environmental and maturation stages, further research is necessary to determine the optimal maturation stage for extracting a compound. This study is prospective research for further *in vivo* examination trials to confirm it biological effectiveness in reducing blood glucose, along with biochemical analysis for additional investigations on its effect against diabetic related complications. Indeed, evaluating the efficacy and safety of 5-O-caffeoylshikimic acid in animal models, performing absorption, distribution, metabolism, and excretion (**ADME**) studies to confirm the compound's bioavailability and metabolic stability.

References

- Abbas, Q., Hassan, M., Raza, H., Kim, S. J., Chung, K. W., Kim, G. H., & Seo, S. Y. (2017). In vitro, in vivo and in silico anti-hyperglycemic inhibition by sinigrin. *Asian Pacific Journal of Tropical Medicine*, 10(4), 372–379. <https://doi.org/10.1016/j.apjtm.2017.03.019>
- Abdelaziz, D. H. A., Ali, S. A., & Mostafa, M. M. A. (2015). Phoenix dactylifera seeds ameliorate early diabetic complications in streptozotocin-induced diabetic rats. *Pharmaceutical Biology*, 53(6), 792–799. <https://doi.org/10.3109/13880209.2014.942790>
- Abdullah, Ahlam & Al-bokai, Nuha. (2022). Effects of Methanolic extract of *Phoenix dactylifera* L. seeds (PDS) on blood glucose levels of normoglycemic and Dexamethasone induced Diabetic rabbits. 10.21203/rs.3.rs-1400576/v1.
- Amad, R. A. (2021, June 9). *About 257 Thousand Palm Trees in Bahrain and the Western Regions are on Top*. NSSA. <https://www.nssa.gov.bh/about-257-thousand-palm-trees-in-bahrain-and-the-western-regions-are-on-top/>
- Al-Alawi, R. A., Al-Mashiqri, J. H., Al-Nadabi, J. S. M., Al-Shihi, B. I., & Baqi, Y. (2017). Date Palm Tree (*Phoenix dactylifera* L.): Natural Products and Therapeutic Options. *Frontiers in Plant Science*, 8. <https://doi.org/10.3389/fpls.2017.00845>
- Al-Abachi, S., Yaseen, S., & Shihab, G. (2022). Biochemical Study of Consumption Zahdi Dates (*Phoenix dactylifera*) in Type 2 Diabetic Patients. *Rafidain Journal of Science*, 31(1), 11–22. <https://doi.org/10.33899/rjs.2022.172924>

- AlFaris, N. A., AlTamimi, J. Z., AlGhamdi, F. A., Albaridi, N. A., Alzaheb, R. A., Aljabryn, D. H., Aljahani, A. H., & AlMousa, L. A. (2021). Total phenolic content in ripe date fruits (*Phoenix dactylifera* L.): A systematic review and meta-analysis. *Saudi Journal of Biological Sciences*, 28(6), 3566–3577. <https://doi.org/10.1016/j.sjbs.2021.03.033>
- Alkhoori, M. A., Kong, A. S., Aljaafari, M. N., Abushelaibi, A., Erin Lim, S. H., Cheng, W. H., Chong, C. M., & Lai, K. S. (2022). Biochemical Composition and Biological Activities of Date Palm (*Phoenix dactylifera* L.) Seeds: A Review. *Biomolecules*, 12(11), 1626. <https://doi.org/10.3390/biom12111626>
- Almansoori, T. A., Al-Khalifa, M. A., & Mohamed, A. M. (2015). Date palm status and perspective in Bahrain. *Date Palm Genetic Resources and Utilization*: Volume 2, 353-386.
- Asonganyi, T. (2013). In vivo, in vitro and in silico. *Health Sciences and Disease*, 10(2). <https://doi.org/10.5281/hsd.v10i2.262>
- Awad, S., Zhou, Y., Katsou, E., Li, Y., & Fan, M. (2020). A Critical Review on Date Palm Tree (*Phoenix dactylifera* L.) Fibres and Their Uses in Bio-composites. *Waste and Biomass Valorization*, 12(6), 2853–2887. <https://doi.org/10.1007/s12649-020-01105-2>
- Ajiboye, B. O., Oloyede, H. O. B., & Salawu, M. O. (2020). Phoenix dactylifera Linn fruit based-diets palliate hyperglycemia in alloxan-induced diabetic rats. *Journal of Basic and Clinical Physiology and Pharmacology*, 32(1). <https://doi.org/10.1515/jbcpp-2019-0185>
- Bhattacharya, S., Oksbjerg, N., Young, J. F., & Jeppesen, P. B. (2014). Caffeic acid, naringenin and quercetin enhance glucose-stimulated insulin secretion and glucose sensitivity in INS-1E cells. *Diabetes, obesity & metabolism*, 16(7), 602–612. <https://doi.org/10.1111/dom.12236>

Benet, L. Z., Sodhi, J. K., Makrygiorgos, G., & Mesbah, A. (2021). There is Only One Valid Definition of Clearance: Critical Examination of Clearance Concepts Reveals the Potential for Errors in Clinical Drug Dosing Decisions. *The AAPS journal*, 23(3), 67. <https://doi.org/10.1208/s12248-021-00591-z>

Cao, J., Zhang, J., Cao, R., Li, W., Wang, G., Cui, L., & Sun, L. (2023). Elucidation of α -amylase inhibition by natural shikimic acid derivatives regarding the infrequent uncompetitive inhibition mode and structure–activity relationship. *Food Frontiers*, 4(4), 2058–2069. <https://doi.org/10.1002/fft2.278>

Chakroun, M., Khemakhem, B., Mabrouk, H. B., El Abed, H., Makni, M., Bouaziz, M., Drira, N., Marrakchi, N., & Mejdoub, H. (2016). Evaluation of anti-diabetic and anti-tumoral activities of bioactive compounds from *Phoenix dactylifera* L's leaf: In vitro and in vivo approach. *Biomedicine & Pharmacotherapy*, 84, 415–422. <https://doi.org/10.1016/j.biopha.2016.09.062>

Chang, C. L. T., Lin, Y., Bartolome, A. P., Chen, Y. C., Chiu, S. C., & Yang, W. C. (2013). Herbal Therapies for Type 2 Diabetes Mellitus: Chemistry, Biology, and Potential Application of Selected Plants and Compounds. *Evidence-Based Complementary and Alternative Medicine*, 2013, 1–33. <https://doi.org/10.1155/2013/378657>

Chaari, A., Abdellatif, B., Nabi, F., & Khan, R. H. (2020). Date palm (*Phoenix dactylifera* L.) fruit's polyphenols as potential inhibitors for human amylin fibril formation and toxicity in type 2 diabetes. *International Journal of Biological Macromolecules*, 164, 1794–1808. <https://doi.org/10.1016/j.ijbiomac.2020.08.080>

Chideh, S., Pilard, S., Attoumbré, J., Saguez, R., Hassan-Abdallah, A., Cailleu, D., Wadouachi, A., & Baltora-Rosset, S. (2014). 5-O-Caffeoylshikimic acid from *Solanum somalense* leaves: Advantage of centrifugal partition chromatography over conventional column chromatography. *Journal of Separation Science*, 37(17), 2331–2339. <https://doi.org/10.1002/jssc.201400226>

Chin, C. H., Chen, S. H., Wu, H. H., Ho, C. W., Ko, M. T., & Lin, C. Y. (2014). cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Systems Biology*, 8(S4). <https://doi.org/10.1186/1752-0509-8-s4-s11>

Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research*, 47(W1), W357–W364. <https://doi.org/10.1093/nar/gkz382>

Das, S., & Maiti, A. (2013). *Matrix Metalloproteinases in Subjects with Type 2 Diabetes Mellitus: Pattern of MMP-2 and MMP-9 Profile in Diabetes Mellitus Type-2 Patients*. <http://iasir.net/AIJRFANSpapers/AIJRFANS13-219.pdf>

Diabetes. (2023, April 5). Retrieved from <https://www.who.int/news-room/fact-sheets/detail/diabetes>

Djaoud, K., Muñoz-Almagro, N., Benítez, V., Martín-Cabrejas, M. N., Madani, K., Boulekbache-Makhlouf, L., & Villamiel, M. (2022). New valorization approach of Algerian dates (*Phoenix dactylifera* L.) by ultrasound pectin extraction: Physicochemical, techno-functional, antioxidant and antidiabetic properties. *International Journal of Biological Macromolecules*, 212, 337–347. <https://doi.org/10.1016/j.ijbiomac.2022.05.115>

- Doncheva, N. T., Morris, J. H., Gorodkin, J., & Jensen, L. J. (2018). Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data. *Journal of Proteome Research*, 18(2), 623–632. <https://doi.org/10.1021/acs.jproteome.8b00702>
- Echegaray, N., Pateiro, M., Gullón, B., Amarowicz, R., Misihairabgwi, J. M., & Lorenzo, J. M. (2020). *Phoenix dactylifera* products in human health – A review. *Trends in Food Science & Technology*, 105, 238–250. <https://doi.org/10.1016/j.tifs.2020.09.017>
- Fernández-López, J., Viuda-Martos, M., Sayas-Barberá, E., De Vera, C. N., & Pérez-Álvarez, J. Á. (2022). Biological, Nutritive, Functional and Healthy Potential of Date Palm Fruit (*Phoenix dactylifera* L.): Current Research and Future Prospects. *Agronomy*, 12(4), 876. <https://doi.org/10.3390/agronomy12040876>
- Ferrari, I. V., De Gregorio, A., Fuggetta, M. P., & Ravagnan, G. (2023). PharmMapper Server and Molecular Docking Study Focusing on Polydatin to Identify Potential Targets. *European Journal of Applied Sciences–Vol, 11* (Iss5).
- García-Pérez, L. E., Álvarez, M., Dilla, T., Gil-Guillén, V., & Orozco-Beltrán, D. (2013). Adherence to Therapies in Patients with Type 2 Diabetes. *Diabetes Therapy*, 4(2), 175–194. <https://doi.org/10.1007/s13300-013-0034-y>
- Ganguly, R., Singh, S. V., Jaiswal, K., Kumar, R., & Pandey, A. K. (2023). Modulatory effect of caffeic acid in alleviating diabetes and associated complications. *World journal of diabetes*, 14(2), 62–75. <https://doi.org/10.4239/wjd.v14.i2.62>
- Glüge, J., McNeill, K., & Scheringer, M. (2023). Getting the SMILES right: identifying inconsistent chemical identities in the ECHA database, PubChem

and the CompTox Chemicals Dashboard. *Environmental Science: Advances*, 2(4), 612–621. <https://doi.org/10.1039/d2va00225f>

Gothai, S., Ganesan, P., Park, S. Y., Fakurazi, S., Choi, D. K., & Arulselvan, P. (2016). Natural Phyto-Bioactive Compounds for the Treatment of Type 2 Diabetes: Inflammation as a Target. *Nutrients*, 8(8), 461. <https://doi.org/10.3390/nu8080461>

Hameed, I., Masoodi, S. R., Mir, S. A., Nabi, M., Ghazanfar, K., & Ganai, B. A. (2015). Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. *World Journal of Diabetes*, 6(4), 598. <https://doi.org/10.4239/wjd.v6.i4.598>

Hosseini Nasab, N., Raza, H., Eom, Y. S., Shah, F. H., Kwak, J. H., & Kim, S. J. (2024). Exploring chalcone-sulfonyl piperazine hybrids as anti-diabetes candidates: design, synthesis, biological evaluation, and molecular docking study. *Molecular diversity*, 10.1007/s11030-024-10831-x. Advance online publication. <https://doi.org/10.1007/s11030-024-10831-x>

Huang, D., Shen, S., & Wu, J. S. (2009). Effects of Caffeic Acid and Cinnamic Acid on Glucose Uptake in Insulin-Resistant Mouse Hepatocytes. *Journal of Agricultural and Food Chemistry*, 57(17), 7687–7692. <https://doi.org/10.1021/jf901376x>

Ihara, Y., Asahara, S., Inoue, H., Seike, M., Ando, M., Kabutoya, H., Kimura-Koyanagi, M., & Kido, Y. (2023b). Chlorogenic Acid and Caffeine in Coffee Restore Insulin Signaling in Pancreatic B Cells. *PubMed*, 69(1), E1–E8. <https://pubmed.ncbi.nlm.nih.gov/37088693>

International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: 2021. Available at: <https://www.diabetesatlas.org>

Kahn, S. E., Cooper, M. E., & Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future.

The Lancet, 383(9922), 1068–1083. [https://doi.org/10.1016/s0140-6736\(13\)62154-6](https://doi.org/10.1016/s0140-6736(13)62154-6)

Khursheed, R., Singh, S. K., Wadhwa, S., Kapoor, B., Gulati, M., Kumar, R., Ramanunni, A. K., Awasthi, A., & Dua, K (2019). Treatment strategies against diabetes: Success so far and challenges ahead. *European Journal of Pharmacology*, 862, 172625. <https://doi.org/10.1016/j.ejphar.2019.172625>

Khaliq, T., Sarfraz, M., & Ashraf, M. (2016). Recent Progress for the Utilization of *Curcuma longa*, *Piper nigrum* and *Phoenix dactylifera* Seeds against Type 2 Diabetes. *West Indian Medical Journal*. <https://doi.org/10.7727/wimj.2016.176>

Kumar, V., Sachan, R., Rahman, M., Sharma, K., Al-Abbasi, F. A., & Anwar, F. (2020). *Prunus amygdalus* extract exert antidiabetic effect via inhibition of DPP-IV: in silico and in-vivo approaches. *Journal of Biomolecular Structure and Dynamics*, 39(11), 4160–4174. <https://doi.org/10.1080/07391102.2020.1775124>

Li, L., Yang, L., Yang, L., He, C., He, Y., Chen, L., Dong, Q., Zhang, H., Chen, S., & Li, P. (2023) Network pharmacology: a bright guiding light on the way to explore the personalized precise medication of traditional Chinese medicine. *Chinese Medicine*, 18(1). <https://doi.org/10.1186/s13020-023-00853-2>

Liu, W., Li, J., Zhang, X., Zu, Y., Yang, Y., Liu, W., Xu, Z., Gao, H., Sun, X., Jiang, X., & Zhao, Q. (2020). Current advances in naturally occurring caffeoylquinic acids: Structure, bioactivity, and synthesis. *Journal of agricultural and food chemistry*, 68(39), 10489-10516.

Lu, C., Zhu, W., Wang, M., Xu, X., & Lu, C. (2014). Antioxidant and Anti-Inflammatory Activities of Phenolic-Enriched Extracts of *Smilax glabra*.

Evidence-based Complementary and Alternative Medicine, 2014, 1–8.
<https://doi.org/10.1155/2014/910438>

Magkos, F., Hjorth, M. F., & Astrup, A. (2020). Diet and exercise in the prevention and treatment of type 2 diabetes mellitus. *Nature Reviews Endocrinology*, 16(10), 545–555. <https://doi.org/10.1038/s41574-020-0381-5>

Magliano, D. J., Sacre, J. W., Harding, J. L., Gregg, E. W., Zimmet, P. Z., & Shaw, J. E. (2020). Young-onset type 2 diabetes mellitus — implications for morbidity and mortality. *Nature Reviews Endocrinology*, 16(6), 321–331. <https://doi.org/10.1038/s41574-020-0334-z>.

Meng, E. C., Goddard, T. D., Pettersen, E. F., Couch, G. S., Pearson, Z. J., Morris, J. H., & Ferrin, T. E. (2023). UCSF ChimeraX: Tools for structure building and analysis. *Protein Science*, 32(11). <https://doi.org/10.1002/pro.4792>

Mir, W. R., Bhat, B. A., Rather, M. A., Muzamil, S., Almilaibary, A., Alkhanani, M., & Mir, M. A. (2022). Molecular docking analysis and evaluation of the antimicrobial properties of the constituents of *Geranium wallichianum* D. Don ex Sweet from Kashmir Himalaya. *Scientific Reports*, 12(1). <https://doi.org/10.1038/s41598-022-16102-9>

Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. P. B., Aparna, S. R., Mangalapandi, P., & Samal, A. (2018). **IMPPAT**: A curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics. *Scientific Reports*, 8(1). <https://doi.org/10.1038/s41598-018-22631-z>

Molehin, O. R. (2019). Phytochemical screening and in vitro α -amylase and α -glucosidase inhibitory potential of *Clerodendrum volubile* leaf extract. *Archives*, 1, 198-206.

- Mousavi, L., Mohd Salleh, R., & Murugaiyah, V. (2020). Antidiabetic and In Vitro Enzyme Inhibition Studies of Methanol Extract of *Ocimum tenuiflorum* Linn Leaves and Its Fractions. *Tropical Life Sciences Research*, 31(1), 141–158. <https://doi.org/10.21315/tlsr2020.31.1.9>
- Neelawala, D., Rajapakse, S., & Kumbukgolla, W. W. (2019). Potential of medicinal plants to treat dengue. *International Journal of One Health*, 86–91. <https://doi.org/10.14202/ijoh.2019.86-91>
- Nyathi, B., Bvunzawabaya, J. T., Venissa P Mudawarima, C., Manzombe, E., Tsotsoro, K., Selemani, M. A., Munyuki, G., & Rwere, F. (2023). Inhibitory and in silico molecular docking of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa phytochemical compounds on human α -glucosidases. *Journal of Ethnopharmacology*, 312, 116501. <https://doi.org/10.1016/j.jep.2023.116501>
- Oboh, G., Agunloye, O. M., Adefegha, S. A., Akinyemi, A. J., & Ademiluyi, A. O. (2014). Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (in vitro): a comparative study. *Journal of Basic and Clinical Physiology and Pharmacology*, 26(2), 165–170. <https://doi.org/10.1515/jbcpp-2013-0141>
- Oyebode, O. A., Erukainure, O. L., Chukwuma, C. I., Ibeji, C. U., Koorbanally, N. A., & Islam, S. (2018). *Boerhaavia diffusa* inhibits key enzymes linked to type 2 diabetes in vitro and in silico; and modulates abdominal glucose absorption and muscle glucose uptake ex vivo. *Biomedicine & Pharmacotherapy*, 106, 1116–1125. <https://doi.org/10.1016/j.biopha.2018.07.053>
- Pacifico, S., Piccolella, S., Nocera, P., Tranquillo, E., Poggetto, F. D., & Catauro, M. (2018). New insights into phenol and polyphenol composition

of *Stevia rebaudiana* leaves. *Journal of Pharmaceutical and Biomedical Analysis*, 163, 45–57. <https://doi.org/10.1016/j.jpba.2018.09.046>

Park, B., Nomura, T., Fukudome, H., Onjo, M., Shimada, A., & Samejima, H. (2019). Chemical Constituents of the Leaves of *Eriobotrya japonica*. *Chemistry of Natural Compounds*, 55(5), 942–944. <https://doi.org/10.1007/s10600-019-02854-w>

Pinho, E., Henriques, M., & Soares, G. (2014). Caffeic acid loading wound dressing: physicochemical and biological characterization. *Therapeutic Delivery*, 5(10), 1063–1075. <https://doi.org/10.4155/tde.14.77>

Price G, Patel DA. Drug Bioavailability. [Updated 2023 Jul 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557852/>

Ratna Wulan, D., Priyo Utomo, E., & Mahdi, C. (2015). Antidiabetic Activity of *Ruellia tuberosa* L., Role of α -Amylase Inhibitor: In Silico, In Vitro, and In Vivo Approaches. *Biochemistry Research International*, 2015, 1–9. <https://doi.org/10.1155/2015/349261>

Ríos, J., Francini, F., & Schinella, G. (2015). Natural Products for the Treatment of Type 2 Diabetes Mellitus. *Planta Medica*, 81(12/13), 975–994. <https://doi.org/10.1055/s-0035-1546131>

Roy, K., Kar, S., & Das, R. N. (2015). Other related techniques. In *Elsevier eBooks* (pp. 357–425). <https://doi.org/10.1016/b978-0-12-801505-6.00010-7>

Rowland, M., Roberts, M. S., & Pang, K. S. (2022). In Defense of Current Concepts and Applications of Clearance in Drug Development and Therapeutics. *Drug metabolism and disposition: the biological fate of chemicals*, 50(2), 187–190. <https://doi.org/10.1124/dmd.121.000637>

- Safitri, A., Fatchiyah, F., Ratih, D., Sari, T., & Roosdiana, A. (2020). Phytochemical screening, in vitro anti-oxidant activity, and in silico anti-diabetic activity of aqueous extracts of *Ruellia tuberosa* L. *Journal of Applied Pharmaceutical Science*, 10(3), 101–108. <https://doi.org/10.7324/japs.2020.103013>
- Sato, H., Nagashima, K., Ogura, M., Sato, Y., Tahara, Y., Ogura, K., ... & Inagaki, N. (2016). Src regulates insulin secretion and glucose metabolism by influencing subcellular localization of glucokinase in pancreatic β -cells. *Journal of diabetes investigation*, 7(2), 171-178.
- S., L. S., Raghu, C., H.A., A., & P., A. (2019). In vitro and in silico inhibition properties of fucoidan against α -amylase and α -D-glucosidase with relevance to type 2 diabetes mellitus. *Carbohydrate Polymers*, 209, 350–355. <https://doi.org/10.1016/j.carbpol.2019.01.039>
- Shahzad, D., Saeed, A., Larik, F. A., Channar, P. A., Abbas, Q., Alajmi, M. F., Arshad, M. I., Erben, M. F., Hassan, M., Raza, H., Seo, S. Y., & El-Seedi, H. R. (2019). Novel C-2 Symmetric Molecules as α -Glucosidase and α -Amylase Inhibitors: Design, Synthesis, Kinetic Evaluation, Molecular Docking and Pharmacokinetics. *Molecules (Basel, Switzerland)*, 24(8), 1511. <https://doi.org/10.3390/molecules24081511>
- Smirnovienė, J., Baranauskienė, L., Zubrienė, A., & Matulis, D. (2021). A standard operating procedure for an enzymatic activity inhibition assay. *European Biophysics Journal*, 50(3–4), 345–352. <https://doi.org/10.1007/s00249-021-01530-8>
- Sun, J., Singh, P., Österlund, J., Orho-Melander, M., Melander, O., Engström, G., & Edsfeldt, A. (2021). Hyperglycaemia-associated Caspase-3 predicts diabetes and coronary artery disease events. *Journal of Internal Medicine*, 290(4), 855–865. <https://doi.org/10.1111/JOIM.13327>

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L. J., & von Mering, C. (2021). Correction to ‘The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets.’ *Nucleic Acids Research*, 49(18), 10800–10800. <https://doi.org/10.1093/nar/gkab835>

Thomas, P. D. (2016). The gene ontology and the meaning of biological function. *Methods in Molecular Biology*, 15–24. https://doi.org/10.1007/978-1-4939-3743-1_2

Tomic, D., Shaw, J. E., & Magliano, D. J. (2022). The burden and risks of emerging complications of diabetes mellitus. *Nature Reviews Endocrinology*, 18(9), 525–539. <https://doi.org/10.1038/s41574-022-00690-7>

Tolmie, M., Bester, M. J., & Apostolides, Z. (2021). Inhibition of α -glucosidase and α -amylase by herbal compounds for the treatment of type 2 diabetes: A validation of in silico reverse docking with in vitro enzyme assays. *Journal of Diabetes*, 13(10), 779–791. <https://doi.org/10.1111/1753-0407.13163>

Type 2 Diabetes. (2023, April 18). Retrieved from <https://www.cdc.gov/diabetes/basics/type2.html>

UniProt Consortium. (2019). UniProt: a worldwide hub of protein knowledge. *Nucleic acids research*, 47(D1), D506-D515.

Vijayakumar, K., Prasanna, B., Rengarajan, R. L., Rathinam, A., Velayuthaprabhu, S., & Vijaya Anand, A. (2020). Anti-diabetic and hypolipidemic effects of Cinnamon cassia bark extracts: an in vitro, in vivo, and in silico approach. *Archives of Physiology and Biochemistry*, 129(2), 338–348. <https://doi.org/10.1080/13813455.2020.1822415>

- Wu, F., Zhou, Y., Li, L., Shen, X., Chen, G., Wang, X., Liang, X., Tan, M., & Huang, Z. (2020). Computational Approaches in Preclinical Studies on Drug Discovery and Development. *Frontiers in Chemistry*, 8. <https://doi.org/10.3389/fchem.2020.00726>
- Xu, W., Luo, Q., Wen, X., Xiao, M., & Mei, Q. (2020). Antioxidant and anti-diabetic effects of caffeic acid in a rat model of diabetes. *Tropical Journal of Pharmaceutical Research*, 19(6), 1227–1232. <https://doi.org/10.4314/tjpr.v19i6.17>
- Yu, J., Xie, J., Sun, M., Xiong, S., Xu, C., Zhang, Z., Li, M., Li, C., & Lin, L. (2024). Plant-Derived Caffeic Acid and Its Derivatives: An Overview of Their NMR Data and Biosynthetic Pathways. *Molecules*, 29(7), 1625. <https://doi.org/10.3390/molecules29071625>
- Zengin, G., Mahomoodally, M. F., Paksoy, M. Y., Picot-Allain, C., Glamocilja, J., Sokovic, M., Diuzheva, A., Jekő, J., Cziáky, Z., Rodrigues, M. J., Sinan, K. I., & Custodio, L. (2019). Phytochemical characterization and bioactivities of five Apiaceae species: Natural sources for novel ingredients. *Industrial Crops and Products*, 135, 107–121. <https://doi.org/10.1016/j.indcrop.2019.04.033>
- Zhang, D., Zhao, M., Li, Y., Zhang, D., Yang, Y., & Li, L. (2021). Natural xanthine oxidase inhibitor 5-O-caffeoylshikimic acid ameliorates kidney injury caused by hyperuricemia in mice. *Molecules*, 26(23), 7307. <https://doi.org/10.3390/molecules26237307>
- Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., . . . Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications*, 10(1). <https://doi.org/10.1038/s41467-019-09234-6>

Appendix A

In Vitro α -Glucosidase Enzyme Inhibition Assay

Table 7 Inhibition of α -Glucosidase by 5-O-Caffeoylshikimic Acid Concentrations.

Concentrations	% Inhibition			Means
30	92.0956	90.3255	89.3257	90.5823
15	81.1538	83.2448	80.3257	81.5748
7.5	76.9841	74.397	75.3659	75.5823
3.75	74.397	72.3366	71.3259	72.6865
1.875	64.8871	62.3659	66.3258	64.5263
0.9375	62.5506	60.3654	64.3258	62.414
0.46875	58.5417	54.3254	52.3259	55.0643
0.23438	48.3801	45.3687	46.8746	46.8745

Table 8 Inhibition of α -Glucosidase by Acarbose Concentrations.

Concentrations	% Inhibition			Means
156.25	71.5651	75.3659	73.2589	73.3966
78.125	68.4748	70.2366	72.3987	70.3701
39.0625	63.7678	65.3687	64.3987	64.5117
19.5313	59.4184	61.8745	60.9553	60.7494
9.76563	55.1544	56.2366	54.9632	55.4514
4.88281	49.1972	48.3659	47.1853	48.2494
2.44141	38.207	37.3659	39.3641	38.3123
1.2207	29.6849	31.3659	33.7416	31.5975

Appendix B

In Vitro α -amylase Enzyme Inhibition Assay

Table 9 Inhibition of α -Amylase by 5-O-Caffeoylshikimic Acid Concentrations.

Concentrations	% Inhibition			Means
30.303	90.3587	92.6587	91.3857	91.4677
15.1515	86.354	87.9587	84.9658	86.4262
7.57576	65.3287	66.9855	62.9562	65.0901
3.78788	47.8965	45.3985	46.3215	46.5388
1.89394	35.9857	33.9857	36.985	35.6521

Table 10 Inhibition of α -Amylase by Acarbose Concentrations.

Concentrations	% Inhibition			Means
15.1515	60.396	62.3654	61.2547	61.3387
7.57575	48.2548	47.3259	46.3587	47.3131
3.78788	38.4837	40.3254	41.3257	40.0449
1.89394	28.2116	30.3258	31.9874	30.1749
0.94697	30.8701	29.3659	28.3257	29.5206

Abstract

Type 2 diabetes mellitus (T2DM) is a widely spread chronic disease threatening individual health and increasing mortality due to complications. *Phoenix dactylifera*, is an indigenous Bahraini plant referred as palm tree, that has phytochemicals with high medicinal effect against different disease including diabetes. Plant-derived antihyperglycemic agents represent a promising direction for novel drug discovery, offering a cost-effective, accessible, and potentially safer alternative to synthetic drugs. The current research aims to analyze the phytochemical compounds of *P. dactylifera*, and assess the inhibition kinetic mechanism of the lead compound against diabetic related enzymes.

The *in silico* network pharmacology and molecular simulation are carried out with multiple bioinformatical software considering **ADMET** profile for a potential therapeutic effect. A total of 17 bioactive compounds retrieved from **IMPPAT** were found with high drug likeness and oral bioavailability extracted from *P. dactylifera*. Exploring compound characteristics by **ADMET** profile eliminates poor pharmacokinetic properties at early development stages. 5-O-caffeoylshikimic acid is a phenolic compound interacts with proteins enrolled in diabetic related pathways according to **Metascape** and **ShinyGo** databases. Molecular docking in **PyRx** revealed strong binding affinities of 5-O-caffeoylshikimic acid to α -amylase (-7.7 kcal/mol) and α -glucosidase (-7.8 kcal/mol), indicating a stable and potent interaction.

In vitro enzyme inhibition kinetics are implemented to affirm the effectiveness of *in silico* findings of the compound against α -amylase and α -glucosidase and determine the required IC₅₀ value. 5-O-caffeoylshikimic acid was found to have a strong inhibition with IC₅₀ of 17.862 μ M against α -amylase and 7.668 μ M against α -glucosidase in contrast to acarbose, the positive control with IC₅₀ of 8.621 μ M and 30.978 μ M. 5-O-caffeoylshikimic acid has a significant inhibitory effect against the main digestive enzymes that triggers T2DM progression as anti-hyperglycaemic compound suggesting a promising compound for diabetic patients' pharmaceutical use.

الملخص

داء السكري من النوع الثاني (T2DM) مرض مزمن منتشر على نطاق واسع، يُهدد صحة الفرد ويزيد من معدل الوفيات بسبب مضاعفاته. فينيكس داكليليفيرا، وهو نبات بحريني أصلي يُعرف باسم شجرة النخيل، يحتوي على مواد كيميائية نباتية ذات تأثير علاجي قوي ضد أمراض مختلفة، بما في ذلك داء السكري. تُمثل العوامل الخافضة لسكر الدم المشتقة من النباتات توجهاً واعداً لاكتشاف أدوية جديدة، حيث تُقدم بديلاً فعالاً من حيث التكلفة، وسهل المنال، وربما أكثر أماناً من الأدوية الاصطناعية. يهدف البحث الحالي إلى تحليل المركبات الكيميائية النباتية لنبات فينيكس داكليليفيرا، وتقييم آلية تثبيط المركب الرئيسي ضد الإنزيمات المرتبطة بداء السكري.

أُجريت محاكاة جزيئية ودوائية حاسوبية باستخدام برامج معلوماتية حيوية متعددة، مع الأخذ في الاعتبار ملف تعريف **ADMET** للحصول على تأثير علاجي محتمل. وُجد أن 17 مركباً حيوياً نشطاً، تم استخلاصها من **IMPPAT**، تتمتع بتشابه دوائي عالٍ وتوافر حيوي فموي عالٍ، مستخلصة من *P. dactylifera*. يُسهم استكشاف خصائص المركبات من خلال ملف تعريف **ADMET** في استبعاد ضعف خصائص الحركة الدوائية في مراحل التطوير المبكرة. حمض O-caffeoylshikimic-5 هو مركب فينولي يتفاعل مع البروتينات المسجلة في مسارات مرتبطة بمرض السكري، وفقاً لقواعد بيانات **ShinyGo** و **Metascape**. أظهر الالتحام الجزيئي في **PyRx** ألفة ارتباط قوية لحمض O-caffeoylshikimic-5 مع ألفا-أميلاز (-7.7 كيلو كالوري/مول) وألفا-غلوكوزيداز (-7.8 كيلو كالوري/مول)، مما يشير إلى تفاعل مستقر وفعال.

تم تطبيق حركية تثبيط الإنزيم في المختبر لتأكيد فعالية النتائج الحاسوبية للمركب ضد ألفا أميليز وألفا جلوكوزيداز وتحديد قيمة IC_{50} المطلوبة. وُجد أن حمض O-caffeoylshikimic-5 له تثبيط قوي مع IC_{50} يبلغ 17.862 ميكرومولار ضد ألفا أميليز و7.668 ميكرومولار ضد ألفا جلوكوزيداز على عكس الأكاربوز، التحكم الإيجابي مع IC_{50} يبلغ 8.621 ميكرومولار و30.978 ميكرومولار. يتمتع حمض O-caffeoylshikimic-5 بتأثير مثبط كبير ضد الإنزيمات الهضمية الرئيسية التي تحفز تقدم مرض السكري من النوع 2 كمركب مضاد لفرط سكر الدم مما يشير إلى مركب واعد للاستخدام الدوائي لمرضى السكري.

جامعة البحرين

قسم علم الأحياء

كلية العلوم



تقييم الفعالية المضادة لداء السكري في السيليكو و المختبر بواسطة مركبات كيميائية مستخلصة من التمر

أطروحة مقدمة كجزء من متطلبات الحصول على درجة ماجستير العلوم في علوم الحياة

إعداد

إيمان عبدالله حسن غلوم

٢٠١٧٢٢٧٩

إشراف

الدكتور قمر عباس سولانجي

أستاذ مساعد

جامعة البحرين

مملكة البحرين

يونيو ٢٠٢٥ م

