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Computational Analysis of Spirulina Compounds as Potential Dual Inhibitors of Alpha-Amylase and DPP-4 for Type 2 Diabetes Management

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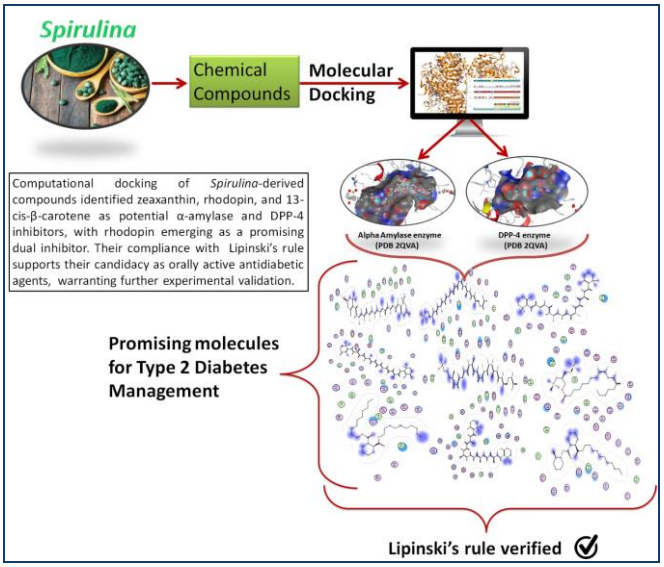
Abstract

Background: Diabetes is a growing global health concern, necessitating innovative approaches for management and treatment. Globally, type 2 diabetes mellitus (T2DM) has reached epidemic proportions, affecting over 500 million adults in 2021, with projections exceeding 780 million by 2050. The prevalence of T2DM is rising rapidly, particularly in low- and middle-income countries, driven by urbanization, sedentary lifestyles, and dietary changes. This growing burden poses a major public health challenge due to its strong association with cardiovascular disease, kidney failure, and increased mortality. Natural products, particularly plants and herbs, have shown promise in regulating blood glucose levels and improving insulin sensitivity. Spirulina, a blue-green cyanobacterium, has gained attention for its rich nutritional profile and potential health benefits, including possible antidiabetic effects. **Purpose:** This study aimed to investigate the interaction between Spirulina's chemical compounds and key enzymes involved in type 2 diabetes using computational methods. **Research Rationale:** Despite some promising studies on Spirulina's antidiabetic effects, research remains scarce, and the mechanisms of action are not fully understood. Computational methods offer an efficient approach to predict potential drug candidates and explore molecular interactions. **General Methods:** The study employed molecular docking techniques to analyze the interactions between Spirulina compounds and two key enzymes in diabetes management: alpha-amylase and dipeptidyl peptidase-4 (DPP-4). The 3D structures of molecules were obtained from PubChem and ChemSpider databases. Docking simulations were performed using Molecular Operating Environment (MOE) software. **Results:** Several Spirulina compounds showed higher binding affinities to alpha-amylase and DPP-4 compared to reference ligands (established inhibitors). Notably, zeaxanthin, rhodopin, and 13-cis-beta-carotene exhibited strong interactions with both enzymes. The physicochemical properties of these compounds were analyzed using SwissADME, indicating their potential as orally administered drugs. **Conclusions:** This computational study suggests that certain compounds in Spirulina, particularly carotenoids, may have potential as alpha-amylase and DPP-4 inhibitors. These findings provide a foundation

for further in-depth studies on Spirulina's role in diabetes management and highlight the potential of natural products in developing novel antidiabetic treatments.

Keywords: Spirulina. Molecular docking. Alpha-amylase. DPP-4. Type 2 diabetes mellitus. Zeaxanthin. Rhodopin. 13-cis-beta-carotene. Computational drug discovery.

Graphical Abstract



Introduction

As of 2025, Type 2 Diabetes Mellitus (T2DM) continues to pose a major global health challenge, with over 500 million individuals affected worldwide, and projections indicating a rise to 783 million by 2045 according to the *IDF Diabetes Atlas 2025* **[1]**. The burden is disproportionately high in low- and middle-income countries, where rapid urbanization and lifestyle changes have accelerated incidence rates. A recent study published in *BMC Public Health* highlights that between 1990 and 2021, the age-standardized incidence, prevalence, and disability-adjusted life years (DALYs) associated with T2DM have significantly increased across BRICS nations, with notable spikes in Russia and South Africa **[2]**. The disease burden is also shifting toward younger populations, especially in China, where incident risk among individuals aged 20–24 has risen sharply. These trends underscore the urgent need for targeted public health strategies, particularly in regions facing socioeconomic disparities and aging populations.

Research on diabetes is crucial for understanding its complex mechanisms and developing more effective treatments. As diabetes rates continue to rise globally, innovative approaches are needed to manage this chronic condition and improve patients' quality of life **[3]**.

Exploring natural products for diabetes management and treatment is particularly important, as they may offer new therapeutic options with potentially fewer side effects than synthetic drugs. Many plants and herbs have shown promising anti-diabetic properties, such as regulating blood glucose levels or improving insulin sensitivity **[4]**. Investigating these natural compounds could lead to the discovery of novel drug candidates or dietary interventions, potentially providing more accessible and affordable options for diabetes care, especially in regions with limited access to conventional medications. Spirulina, a blue-green cyanobacterium, has a rich history. It was rediscovered scientifically by botanist Jean Leonard in the 1960s during an expedition to Lake Chad. Leonard identified and named it Spirulina platensis **[5,6]**.

This discovery sparked renewed interest, leading to extensive research into its nutritional content and potential health benefits **[7-9]**. It is an edible, nontoxic, photoautotrophic cyanobacterium (blue-green alga) with dynamic metabolic composition. It is rich in proteins (60–70%), carbohydrates (8–25%), lipids (6–20%), essential vitamins, minerals, essential fatty acids, chlorophylls, carotenoids, and phycobiliproteins **[10]**. Spirulina is often referred to as the "food for the future" or a "superfood" due to its higher protein content and the presence of other bioactive compounds. It has been extensively used as a human dietary supplement and is also employed as poultry and aquaculture feed additive. NASA even used Spirulina as a food supplement in space **[11]**.

Spirulina is used worldwide as a nutraceutical food supplement. It lacks toxicity and offers therapeutic effects. Some potential health benefits include immune system support, antioxidant properties, anti-inflammatory effects, and probable effect as supplement in cardiovascular diseases management **[12-15]**.

The research on the therapeutic effects of Spirulina continues to pique the interest of researchers worldwide. It appears that this algae has not yet revealed all its secrets. Research on the antidiabetic effect of Spirulina is scarce, even if some studies have shown a promising effect in combating diabetes **[16-24]**.

The limited existing studies do not conclusively demonstrate the mechanism of action by which Spirulina exerts hypoglycemic effects. In our present study, we focused on investigating the interaction between Spirulina's chemical compounds and the key enzymes involved in type 2 diabetes using computational methods. Computational methods play a crucial role in studying the anti-diabetic effects of

natural compounds. These techniques allow researchers to predict potential drug candidates, assess pharmacokinetic properties, and explore molecular interactions.

For instance, recent studies have employed molecular docking to evaluate antidiabetic effects **[25-28]**. These computational approaches help identify promising compounds, predict their absorption profiles, and understand their interactions with relevant proteins. By combining computational insights with experimental data, researchers gain valuable insights into potential therapeutic mechanisms and optimize drug design for diabetes treatment.

Material and Methods

Selection of phytochemical molecules and ligand preparation

For the purposes of this study, we have identified the chemical compounds constituting spirulina by compiling information from various scientific articles, particularly those focusing on studies conducted in the Mediterranean basin. The 3D structures of the molecules constituting Spirulina were obtained from the PubChem and ChemSpider. PubChem is a comprehensive resource maintained by the National Center for Biotechnology Information (NCBI). It houses information on chemical compounds, substances, and biological assays. Researchers can explore physical properties, structures, and biological activities of various molecules.

ChemSpider, on the other hand, is a valuable database managed by the Royal Society of Chemistry. It provides access to over 100 million chemical structures from diverse data sources. Researchers can search by names, explore properties, and find associated literature references. Other molecules were drawn using ChemDraw software. To achieve the most energetically favorable conformation of molecules derived from Spirulina, ligand energies were minimized using the Molecular Operating Environment software (MOE) **[29]**.

The minimization process used the Molecular Orbital PACkage (MOPAC) integrated within MOE, a versatile tool for predicting and analyzing electronic structures, chemical properties, and reactions. MOPAC operates as a semi-empirical quantum chemistry program based on the Dewar and Thiel NDDO approximation. In electronic calculations, the semi-empirical Hamiltonians MNDO, MINDO/3, AM1, and PM3 were applied to determine molecular orbitals, heat of formation, and geometry derivatives. Additionally, the Merck Molecular Force Field (MMFF94x) was utilized in this study.

MMFF94x is an extension of the original MMFF94 force field introduced in 1994, designed to precisely accommodate organic compounds, especially small and medium-sized molecules. This force field includes various chemical bonds, bond angles, torsions, Van der Waals interactions, and electrostatic interactions, providing a superior depiction of molecular structures and relative energies. MMFF94x's enhanced accuracy makes it crucial for obtaining energetically stable ligands. The molecules (ligands) identified in essential oil were compiled into a database in *mdb format for subsequent docking studies. Reference ligands (drugs) were optimized using the same method as the molecules from Spirulina.

Enzyme preparation (receptor)

Alpha-amylase and DPP-4 (Dipeptidyl peptidase-4) play significant roles in diabetes management and treatment due to their effects on carbohydrate metabolism and blood glucose regulation. Alpha-amylase is an enzyme that breaks down complex dietary carbohydrates (such as starch) into smaller sugars. Inhibiting alpha-amylase activity can reduce the amount of glucose available for absorption from the small intestine, thus controlling postprandial hyperglycemia in diabetic patients **[30,31]**.

DPP-4 (dipeptidyl peptidase-4) is an enzyme that rapidly breaks down incretin hormones, such as GLP-1 (glucagon-like peptide 1) and GIP (glucose-dependent insulinotropic polypeptide). By inhibiting DPP-4, we can prolong the action of these hormones, leading to improved glycemic control. DPP-4 inhibitors are considered effective and safe for managing type 2 diabetes **[32-34]**. The inhibition of both alpha-amylase and DPP-4 addresses different aspects of glucose metabolism: slowing glucose absorption from the gut and enhancing insulin secretion while suppressing glucagon.

The 3D crystal structure of alpha-amylase and DPP-4 were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) under the code 2QV4 for alpha-amylase and 1X70 for DPP-4 with three-dimensional resolutions of 1.97 Å and 2.10Å, respectively. Following the approach detailed by Soga et al. **[35]**, we utilized the MOE software to identify and isolate the enzyme's largest active site, where we later attached specific substrates using the "site finder" module. Before initiating the docking procedure, we performed a critical validation step by re-docking the reference ligand.

This validation is essential to ensure the accuracy of the following operations. The success of the docking

process during the re-docking stage was determined by the percentage of instances in which the Root Mean Square Deviation (RMSD) of the top-ranked pose was less than 2 Å **[36,37]**. In this study, the RMSD values obtained were 1.41Å for alpha-amylase and 0.39Å for DPP-4, respectively, confirming the validity of our procedure. Figures 1 and 2 illustrate the overlap of the co-crystallized ligand within the active site of each enzyme following the re-docking process.

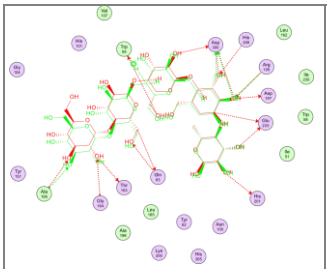


Figure 1. Overlay of the co-crystallized ligand within the active site of alpha-amylase (PDB ID 2QVA).

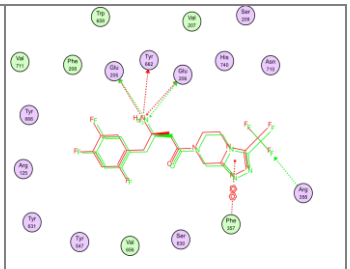


Figure 2. Overlay of the co-crystallized ligand within the active site of DPP-4(PDB ID 1X70).

Source: Own authorship.

Molecular docking results

Docking plays a vital role in virtual screening methodologies, where it involves the precise placement of a ligand within the active site of an enzyme. This process is essential for understanding the interactions between the ligand and the enzyme's specific amino acids within this site. The affinity between the ligand and the enzyme is quantified using a scoring function, ΔG [U total in kcal/mol], which combines the contributions of electrostatic and Van der Waals energies. By calculating this affinity, different ligand conformations, or poses, can be ranked, facilitating the identification of promising ligand-receptor interactions **[38,39]**.

Docking algorithms utilize an integrated approach that combines scoring functions with search strategies to predict ligand binding with precision. The effectiveness of these algorithms hinges on two key theoretical aspects of the scoring function. The first aspect is the existence of a global extremum in the landscape of ligand poses at the correct binding site, indicating the scoring function's capability to accurately pinpoint the binding location. The second aspect concerns the precision of the scoring function's value at this extremum, which is crucial for accurately predicting the binding affinity between the ligand and the target. Together, these factors determine the overall reliability and performance of docking algorithms in virtual screening **[40]**.

The scoring value acts as an essential thermodynamic metric for assessing and contrasting different ligands. Remarkably, some scoring functions possess the ability to estimate the dissociation constant between two molecules, offering deeper insights into their interaction dynamics. These scoring functions are instrumental in evaluating ligand-receptor interactions, as they can accurately predict binding affinities. This information is critical in the selection and prioritization of promising candidate molecules, guiding the decision-making process in drug discovery and development **[41-43]**.

These interactions are subsequently analyzed to assess the ligand's potential to inhibit the enzyme. Docking simulations were performed under standard conditions, with a temperature set at 300 K and a pH of 7. To provide a basis for comparison, reference ligands, which are approved drugs with well-documented efficacy and high affinity for a specific receptor, were used. This approach enabled a direct comparison of the binding affinities between the ligands under study and the established medicaments targeting the same receptor. Tables 1 and 2 include the docking results of the Spirulina compounds and the reference ligands. Only the best scores in comparison to the reference ligands have been reported. Table 3 shows the type of interactions between the enzyme's active site and the ligands with best scores.

Table 1. Docking results of Spirulina compounds and reference ligands with the alpha-amylase enzyme (PDB ID 2QVA).

Compound	PubChem	ChemSpider	Score
Reference ligands			
Miglitol	441314	390074	-4.52174234
Voglibose	444020	392046	-5.51595259
Acarbose	41774	392239	-7.4075799
Spirulina Compounds			
Zeaxanthine	5280899	4515242	-8.35746479
Rhodopin	5365880	4517822	-8.09853172
13-Cis-Beta-	10256668	8432151	-8.0846405
Canthaxanthine	5281227	4444639	-7.86633158

Source: Own authorship.

Table 2. Docking results of Spirulina compounds and reference ligands with the DPP-4 enzyme (PDB ID 1X70).

Compound	PubChem ID	ChemSpider ID	Score (kcal/mol)
Reference ligands			
Sitagliptin	4369359	3571948	-7.60182381
Vildagliptin	6918537	5293734	-5.61681652
Saxagliptin	11243969	9419005	-6.2126298
Alogliptin	11450633	9625485	-5.8204546
Anagliptin	44513473	28492667	-7.52611971
Linagliptin	10096344	8271879	-7,9265
Gemigliptin	11953153	48054670	-6.99343967
Teneligliptin	11949652	10123963	-7.3002634

Spirulina compounds			
Rhodopin	5365880	4517822	-8.99357319
1-Monolinoleoylglycerol trimethylsilyl ether	87962319	4518457	-8.79675674
Didecyl1,2-cyclohexanedicarboxylate	593102	515583	-8.60941029
13-Cis-Beta-Carotene	10256668	8432151	-8.55139351
1,2-Cyclohexanedicarboxylic acid, 2- Methylcyclohexyl undecyl ester	91721833	/	-7.87258768

Source: Own authorship.

Table 3. Types of interactions of the best selected ligands docked in the active site of the Alpha amylase enzyme (PDB ID 2QVA) and DPP4 enzyme (PDB ID 1X70).

Ligands ID	Atoms of compound	Involved Receptor atoms	Involved Receptor residues	Type of interaction bond	Distances (Å)	Energy (kcal/mol)
Bonds between atoms of compounds and residues of the alpha-amylase active site						
5280899 (PubChem)	O3	NZ	LYS 200	H-acceptor	3.10	-2.5
5365880 (PubChem)	Only electrostatic interactions are perceptible					
10256668 (PubChem)	Only electrostatic interactions are perceptible					
5281227 (PubChem)	Only electrostatic interactions are perceptible					
Bonds between atoms of compounds and residues of the DPP-4 active site						
5365880 (PubChem)	O37	O	TRP 305	H-donor	3.24	-1.0
87962319 (PubChem)	Only electrostatic interactions are perceptible					
593102 (PubChem)	Only electrostatic interactions are perceptible					
10256668 (PubChem)	Only electrostatic interactions are perceptible					
91721833 (PubChem)	O3 O3	NE NH2	ARG 358 ARG 358	H-acceptor H-acceptor	3.10 3.05	-2.8 -3.1

Source: Own authorship.

Discussion

For the alpha-amylase enzyme

The results indicate that several natural compounds derived from Spirulina achieved higher scores than established alpha-amylase inhibitors. Among these, zeaxanthin (PunChem ID 5280899) produced the best score compared to reference ligands and other Spirulina compounds, with a binding energy of -8.35746479 kcal/mol. It forms one H-acceptor bonds to LYS200 with distance of 3.10 Å and energy equal to -2.5 kcal/mol. Figures 3 (a) and 3(b) show the 2D and 3D interactions between Zeaxanthin and residues of the alpha-amylase active site.

Zeaxanthin, a xanthophyll carotenoid found in various foods, has been studied for its potential effects on diabetic retinopathy (DR). Oxidative stress and inflammation contribute to retinal neurodegeneration in diabetic patients. Zeaxanthin, along with lutein, promotes retinal health and visual function. Observational studies suggest that depletion of xanthophyll carotenoids (including zeaxanthin) in the macula may be a novel feature of DR, especially in poorly managed type 1 or type 2 diabetes. Early interventional trials with dietary carotenoid supplementation show promise in improving serum levels and macular pigments, leading to benefits in visual performance **[44–46]**.

We also note that Rhodopin (PubChem ID 5365880) exhibits a binding energy with alpha-amylase of -8.09853172 kcal/mol, as shown in Figures 4(a) and 4(b), were only electrostatic interactions are perceptible. Rhodopin is a carotenoid pigment found in some photosynthetic bacteria, particularly in purple non-sulfur bacteria like Rhodospirillum rubrum. There is no specific literature information about a direct, established relationship between Rhodopin and diabetes; it's only known that some carotenoids have been studied for their potential beneficial effects on diabetes risk and management.

Rhodopin is not as well-studied as some other carotenoids in relation to human health. Most research on Rhodopin focuses on its role in bacterial photosynthesis rather than its potential effects on human metabolism. Given the interest in carotenoids for their health benefits and the present docking results, it's possible that Rhodopin could be a subject of future studies related to diabetes.

The 13-Cis-Beta-Carotene (PubChem ID 10256668) gives binding energy to the active site of alpha amylase equal to -8.0846405 kcal/mol. According to Figures 5(a) and 5(b) only electrostatic interactions are perceptible. β-Carotene, a prominent carotenoid found in fruits and vegetables, has been studied for its potential benefits in type 2 diabetes mellitus (T2DM) and obesity. β-Carotene acts as an antioxidant, protecting cells from oxidative stress. It may help counteract insulin resistance, dyslipidemia, and inflammation associated with T2DM and obesity. Consuming carotenoids, including β-carotene, is encouraged for their potential preventive effects **[47]**.

Canthaxanthine (PubChem ID 5281227) gives interaction energy with alpha-amylase of -7.86633158

kcal/mol. As shown in Figures 6(a) and 6(b) only electrostatic interaction are perceptible. Canthaxanthine**,** a natural carotenoid, has been studied in various contexts. Canthaxanthine is found naturally in sources like mushrooms (such as chanterelles), flamingos, and other exotic birds. It also contributes to the red color of coral (Corallium rubrum) and is detected in crustaceans and certain fish (like trout and salmon). As an additive in food, it appears in products like Strasbourg sausages (coded as E161g). Canthaxanthine is authorized for animal feed, including fish, poultry (enhancing egg yolk color), and pets (cats, dogs, fish, and birds). It serves as a tanning agent and is even used to treat vitiligo.

High doses of canthaxanthine (e.g., from self-tanning pills) can lead to retinopathy. The acceptable daily intake for humans is 0.03 mg/kg of body weight **[48,49]**. While direct research is scarce, there might be some indirect connections worth exploring. Canthaxanthine, like other carotenoids, possesses antioxidant properties. Some studies have suggested that antioxidants might play a role in preventing or managing diabetes-related complications. However, more research is needed to confirm this.

Canthaxanthine is sometimes used as a food additive in products aimed at weight loss. Obesity is a known risk factor for type 2 diabetes. Therefore, any potential link between canthaxanthine and diabetes might be indirect, mediated through its impact on weight management.

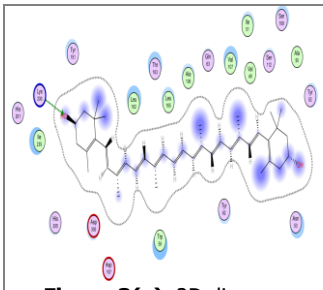


Figure 3(a). 2D diagram interaction between Zeaxanthin and alpha-amylase.

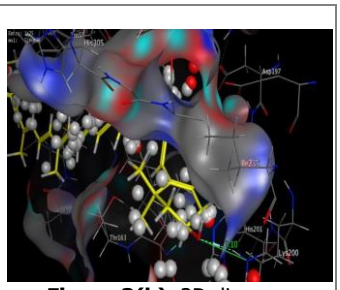


Figure 3(b). 3D diagram interaction between Zeaxanthin and alpha-amylase.

Source: Own authorship.

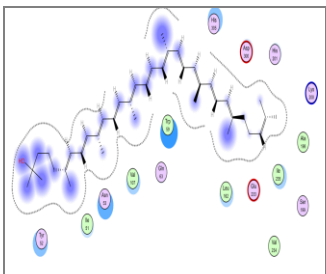


Figure 4(a). 2D diagram interaction between Rhodopin and alpha-amylase.

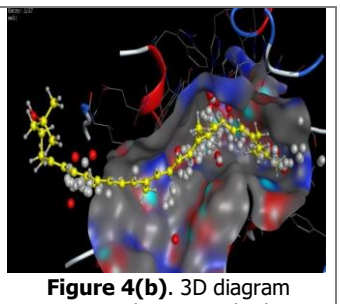


Figure 4(b). 3D diagram interaction between Rhodopin and alpha-amylase.

Source: Own authorship.

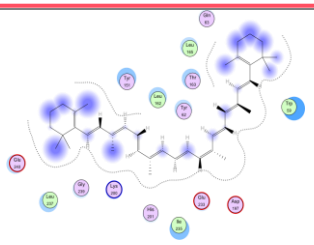


Figure 5(a). 2D diagram interaction between 13-Cis-Beta-Carotene and alpha-amylase.

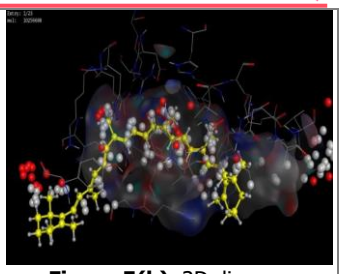


Figure 5(b). 3D diagram interaction between 13-Cis-Beta-Carotene and alpha-amylase.

Source: Own authorship.

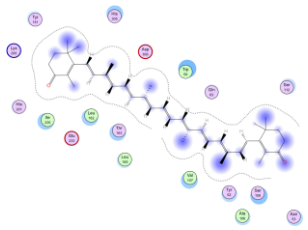


Figure 6(a). 2D diagram interaction between Canthaxanthine and alpha-amylase.

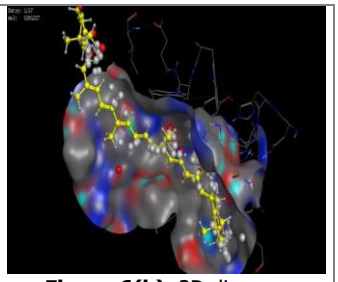


Figure 6(b). 3D diagram interaction between Canthaxanthine and alpha-amylase.

Source: Own authorship.

For DPP-4 enzyme

The results also indicate that several compounds achieve better scores than the reference ligands. Rhodopin, in particular, exhibits a relatively high binding energy of -8.99357319kcal/mol compared to the reference ligands. It forms a hydrogen bond donor interaction with a distance of 3.24 Å and an energy of -0.1 kcal/mol, as illustrated in Figures 7(a) and 7(b). Given Rhodopin's interaction energies with both alpha-amylase and DPP-4, it can be suggested that this ligand may function as a dual inhibitor, which enhances its significance. This reinforces the need for an in-depth study of the potential of Rhodopin in the treatment and management of type 2 diabetes. The 1-Monolinoleoylglycerol trimethylsilyl ether (PubChem ID 87962319) gives energy interaction with DPP-4 equal to -8.79675674 kcal/mol. Only electrostatic interactions are perceptible as shown in Figures 8(a) and 8(b).

1-Monolinoleoylglycerol trimethylsilyl ether is a compound with several biological properties. While it's not directly linked to diabetes, it has been studied for its anti-inflammatory, hepatoprotective, and antioxidant effects **[50]** (antioxidant may affect diabetes management). For specific deep information on its impact on diabetes, further research would be needed.

The Didecyl1,2-cyclohexanedicarboxylate (Pub-Chem ID 593102) shows energy interaction of -8.60941029 kcal/mol. According to Figures 9(a) and 9(b), only electrostatic interactions are possible. The compound Didecyl1,2-cyclohexanedicarboxylate (DCHDC) doesn't appear to have a direct link to diabetes in the available

literature. The product in question deserves to be studied more thoroughly to highlight its potential role in the treatment and management of diabetes, particularly by investigating its inhibitory effect on DPP-4.

The 13-cis-Beta-caroten indicates an energy interaction of -8.55139351 kcal/mol with DPP-4. As shown in Figures 10(a) and 10(b) only electrostatic interactions are perceptible. Beta-Carotene has been studied for its effects on diabetes. Higher cis-β-carotene levels are associated with lower fasting blood glucose levels and improved insulin sensitivity. In diabetic rats, β-carotene treatment reduces total triglycerides and LDL-cholesterol, improving cardiac function.

Beta-carotene shows promise in treating metabolic disorders by inhibiting the insulin-resistance pathway in diabetes **[51-53]**. In addition to the interaction energy of 13-cis-beta-carotene with alpha-amylase demonstrated above, it can be suggested that this compound may also serve as a potential common inhibitor of both alpha-amylase and DPP-4.

Concerning the 1,2-Cyclohexanedicarboxylic acid, 2-Methylcyclohexyl undecyl ester (PubChem ID 91721833), the energy given by the interaction with DPP-4 is of -7.87258768 kcal/mol. As shown in Figures 11(a) and 11(b), the ligand forms two hydrogen bonds as an acceptor with ARG 358, with bond distances of 3.10 Å and 3.05 Å, and corresponding binding energies of -2.8 kcal/mol and -3.1 kcal/mol, respectively. The literature does not specify any relationship between this compound and diabetes. In-depth studies are necessary. Figure 12 represents the 2D diagram legend.

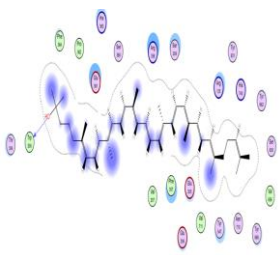


Figure 7(a). 2D diagram interaction between Rhodipin and DPP-4.

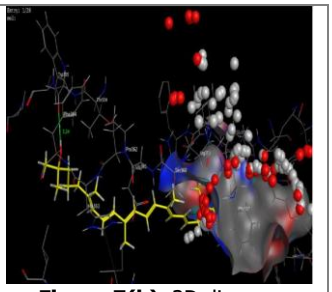


Figure 7(b). 3D diagram interaction between Rhodipin and DPP-4.

Source: Own authorship.

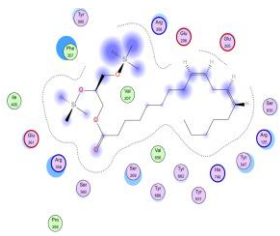


Figure 8(a). 2D diagram interaction between 1-Monolinoleoylglycerol trimethylsilyl ether and DPP-4.

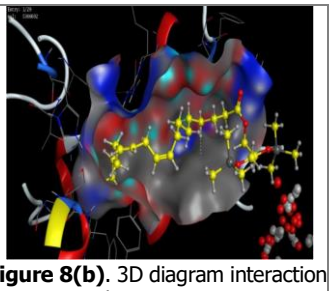


Figure 8(b). 3D diagram interaction between 1-Monolinoleoylglycerol trimethylsilyl ether and DPP-4.

Source: Own authorship.

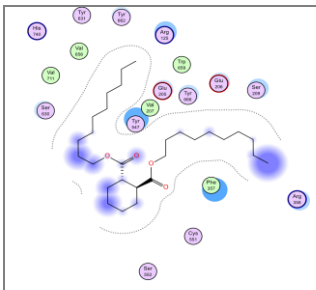


Figure 9(a). 2D diagram interaction between Didecyl1,2-cyclohexanedicarboxylate and DPP-4.

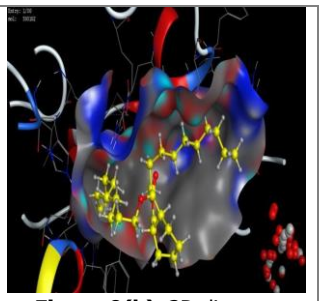


Figure 9(b). 3D diagram interaction between Didecyl1,2-cyclohexanedicarboxylate and DPP-4.

Source: Own authorship.

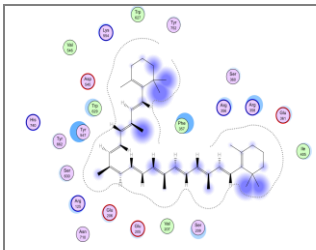


Figure 10(a). 2D diagram interaction between 13-cis-Beta-caroten and DPP-4.

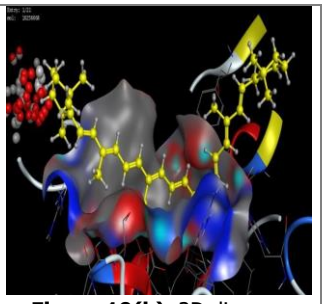


Figure 10(b). 3D diagram interaction between 13-cis-Beta-caroten and DPP-4.

Source: Own authorship.

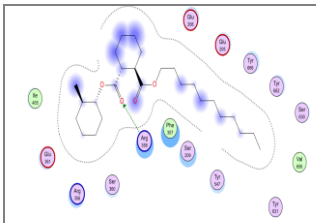


Figure 11(a). 2D diagram interaction between 2-Cyclohexanedicarboxylic acid, 2-Methylcyclohexyl undecyl ester and DPP-4.

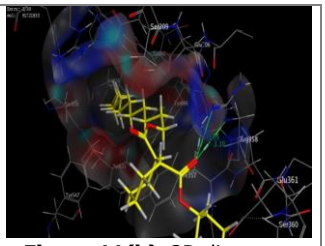


Figure 11(b). 3D diagram interaction between 2-Cyclohexanedicarboxylic acid, 2-Methylcyclohexyl undecyl ester and DPP-4.

Source: Own authorship.

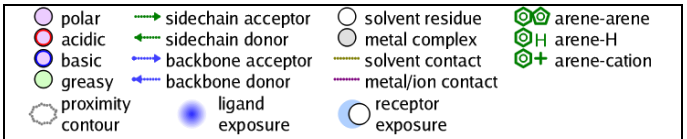


Figure 12. 2D diagrams legend. Source: Own authorship.

Source: Own authorship.

Spirulina, a cyanobacterium rich in bioactive compounds, has been extensively studied for its nutritional and therapeutic properties. However, its potential as a source of alpha-amylase and DPP-4 inhibitors remains underexplored. This study is among the first to systematically evaluate the binding affinities of *Spirulina*-derived compounds, shedding light on their potential role in diabetes management.

Key novel contributions of this research

Elucidating mechanisms of action: Zeaxanthin, identified in this study as a potent alpha-amylase inhibitor, has previously been recognized for its antioxidant properties and potential benefits in diabetic retinopathy **[45]**. Our findings build upon this knowledge by demonstrating its strong binding affinity to alpha-amylase, suggesting a novel mechanism through which zeaxanthin may aid in glycemic control. This dual functionality—addressing both oxidative stress and carbohydrate metabolism—enhances its therapeutic potential.

Comparative analysis with previous literature: While previous studies have explored the antidiabetic effects of *Spirulina*, few have unexplored the specific interactions between its bioactive compounds and key diabetic enzymes using computational methods. For example, Hatami et al. (2021) **[16]** conducted a systematic review and meta-analysis on *Spirulina*'s effects on type 2 diabetes, highlighting its potential without identifying specific compounds or mechanisms. Our study advances this understanding by pinpointing individual bioactive molecules and elucidating their interactions with alpha-amylase and DPP-4, providing a clearer foundation for future research and therapeutic applications.

Unique Contributions to the Field: A particularly novel aspect of our study is the identification of rhodopin as a dual inhibitor of both alpha-amylase and DPP-4. This finding underscores the multifaceted role of *Spirulina* compounds in diabetes management. The dual inhibitory effect of rhodopin on key enzymes involved in carbohydrate metabolism and incretin degradation has not been previously reported, making this a significant contribution to the field.

Additionally, our study explores lesser-known carotenoids, such as rhodopin and canthaxanthin, in the context of diabetes management. While carotenoids like β-carotene and lutein have been widely studied for their antioxidant and antidiabetic properties, the therapeutic potential of these understudied carotenoids remains largely unexplored. Our findings highlight the need for further research into their mechanisms of action, which may provide novel strategies for diabetes treatment.

Finally, the identification of rhodopin and 13-cis-beta-carotene as potential dual inhibitors of alpha-amylase and DPP-4 represents a significant advancement in diabetes research. Multi-target therapies are increasingly recognized as promising approaches for managing complex diseases like T2DM, which involve multiple pathological pathways. By simultaneously targeting carbohydrate digestion and incretin hormone regulation, these compounds could offer a more holistic approach to diabetes management, potentially reducing reliance on polypharmacy and minimizing side effects.

Physicochemical properties of ligands with high score

To identify the most promising ligands for potential drug development, as guided by Lipinski's rule, we analyzed the physicochemical properties of the top molecules, as shown in Table 4. The evaluation focused on ensuring these molecules met the extended Lipinski's rule of five criteria, which include: molecular weight (MW) of 500 g/mol or less, hydrophobicity (log P) of 5 or lower, solubility (log S) greater than -4, a maximum of 5 hydrogen bond donors (HBD), no more than 10 hydrogen bond acceptors (HBA), and a topological polar surface area (TPSA) of 140 Å² or less. For a ligand to be considered suitable for oral administration, it must meet at least two of these criteria **[54,55]**.

Although Lipinski's rules serve as a useful guideline, they are not absolute, and several orally active drugs exist that do not conform entirely to these rules **[56]**. To perform this analysis, we utilized SwissADME, an open-access tool designed for calculating the physicochemical, pharmacokinetic, and drug-like properties of molecules intended for drug development. It has been shown that the studied compounds satisfied Lipinski's rules with at most one violation. This indicates that these compounds could potentially be developed into orally administered drugs, provided that further in-depth studies are conducted.

Table 4. Main physicochemical properties of the selected ligands.

Compound (PubChem ID)	Toxicity	Weight (g/mol)	LogP	LogS	Hbond donor	H-bond acceptor	TPSA Å2	Drug likeness
5280899	No	568.89	10.55	-14.68	2	2	40.46	1 Violation
5365880	No	554.90	12.13	-17.26	1	1	20.23	1 Violation
10256668	No	536.89	12.61	-18.00	0	0	00.00	1 Violation
5281227	No	564.85	10.96	-15.75	0	2	34.14	1 Violation
87962319	No	498.89	8.41	-6.94	0	3	44.76	0 Violation
593102	No	452.72	8.16	-9.77	0	2	52.60	0 Violation
91721833	No	396.61	6.60	-7.21	0	2	52.60	0Violation

Source: Own authorship.

Conclusion

Our computational analysis suggests that Spirulina-derived compounds, particularly zeaxanthin, rhodopin, and 13-cis-beta-carotene, show strong potential as inhibitors of alpha-amylase and DPP-4, with rhodopin emerging as a promising dual inhibitor.

Their physicochemical properties align with Lipinski's rule of five, supporting their candidacy for oral drug development. While these results are encouraging, they remain preliminary; *in vitro* and *in vivo* validation, alongside studies on bioavailability, metabolism, and safety, are essential. This study highlights Spirulina as a valuable source of novel anti-diabetic agents and demonstrates the utility of computational approaches in guiding drug discovery for type 2 diabetes.

Study Limitations

This study is limited by its reliance on computational approaches, which, although effective for predicting molecular interactions, cannot fully reflect the complexity of biological systems. The absence of *in vitro* and *in vivo* experiments prevents confirmation of the predicted binding affinities and biological activities of the Spirulina-derived compounds. Moreover, pharmacokinetic aspects such as bioavailability, metabolism, and potential toxicity were not assessed. Future experimental investigations are therefore required to validate these findings and to determine the translational potential of these compounds in diabetes management.

Abbreviations

T2DM-Type 2 diabetes mellitus; NCBI- National Center for Biotechnology Information; MOE- Molecular Operating Environment; MOPAC- Molecular Orbital PACkage; NDDO- Neglect of Diatomic Differential Overlap; MNDO- Modified Neglect of Diatomic Overlap; MINDO/3- Modified Intermediate Neglect of Differential Overlap, version 3; AM1- Austin Model 1; PM3- Parametric Method 3; MMFF94x- Merck Molecular Force Field; mdb-Microsoft Database; DPP-4-Dipeptidyl peptidase-4; GLP-1- glucagon-like peptide 1; GIP- glucose-dependent insulinotropic polypeptide; DCHDC-Didecyl1,2-cyclohexanedicarboxylate; MW-molecular weight; log P-hydrophobicity; log S-solubility; HBD -hydrogen bond donors; HBA-hydrogen bond acceptors; TPSA- topological polar surface area.

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Author contributions: **Conceptualization-** Hicham Bousekkine, Salim Bouchentouf, Fatima Nehal; **Data curation-** Hicham Bousekkine, Salim Bouchentouf, Fatima Nehal; **Formal Analysis-** Hicham Bousekkine, Salim Bouchentouf, Fatima Nehal; **Investigation-** Hicham Bousekkine, Salim Bouchentouf, Fatima Nehal; **Methodology-** Hicham Bousekkine, Salim Bouchentouf, Fatima Nehal; **Project administration-** Hicham Bousekkine, Salim Bouchentouf, Fatima Nehal; **Supervision**Salim Bouchentouf; **Writing - original draft-** Hicham Bousekkine, Salim Bouchentouf, Fatima Nehal; **Writing-review & editing-** Hicham Bousekkine, Salim Bouchentouf, Fatima Nehal.

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Not applicable.

Informed Consent

Not applicable.

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Data Sharing Statement

The data and supportive information are available within the article.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate@.

Application of Artificial Intelligence (AI)

Not applicable.

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It was performed.

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