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GC/MS Profiling, In Vitro Antidiabetic Efficacy of *Origanum compactum* Benth. Essential Oil and In Silico Molecular Docking of Its Major Bioactive Compounds

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Abstract: Diabetes is a global health concern with significant implications for individuals and healthcare systems. Finding effective and safe antidiabetic agents is crucial for the management of this chronic disease. Natural products have emerged as potential alternatives to allopathic drugs, offering a vast source of bioactive compounds. In this study, we conducted an assessment of the antidiabetic potential of *Origanum compactum* essential oil, employing a two-pronged approach, i.e., experimental investigation and computational docking analysis. The results of gas chromatography–mass spectrometry (GC-MS) showed that thymol (54.6%), carvacrol (23.18%), and p-cymene (7.12%) were the major compounds. Experimental assessments revealed higher IC_{50} values (150 µg/mL for α-amylase; 120 µg/mL for α-glucosidase) of *O. compactum* oil, compared to the control drug acarbose. In silico analysis revealed the best binding affinity of the oil components (carvacrol and thymol) with human NADPH oxidase, while the lysosomal acid-α-glucosidase and salivary amylase also demonstrated good binding affinity towards carvacrol and thymol. Our findings highlight the translational potential of *O. compactum* oil-based treatment for diabetes mellitus and provide a basis for further studies on the modulation of NADPH oxidase, amylase inhibition, and α-glucosidase by antidiabetic natural products. However, further in vivo investigations are strongly required to confirm the results of in vitro antidiabetic effect of *O. compactum* EO.

Keywords: diabetes mellitus; *Origanum compactum*; molecular docking; α-glucosidase; α-amylase

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1. Introduction

Diabetes is a prevalent and rapidly growing health condition worldwide, characterized by elevated blood glucose levels [1]. According to global statistics, the prevalence

of diabetes has reached alarming levels, affecting millions of individuals across all age groups and socio-economic backgrounds [2]. This long-lasting illness carries important consequences for the well-being of the public, healthcare systems, and the overall quality of life for those affected. Its effects span the physical realm, as it raises the likelihood of experiencing serious complications like heart problems, impaired kidney function, nerve damage, and vision loss [3]. Finding effective and safe treatments for this disease is incredibly important in order to manage and control it. Common treatments include lifestyle modifications, oral medications, and insulin therapy [4,5]. Due to the commonly associated side effects of conventional treatments, there has been a growing interest in natural products as alternative options for diabetes treatment [6–8]. These are a colossal source of bioactive compounds, having therapeutic effects. In addition, natural products have a long history of traditional use and often exhibit fewer side effects. In the context of diabetes, natural products hold promise due to their potential to modulate glucose metabolism, enhance insulin sensitivity, protect pancreatic beta cells, and mitigate diabetic complications. Joshi et al. have reviewed the potential of berberine, quercetin, etc. for targeting the 5′-adenosine monophosphate-activated protein kinase (MAPK) pathway and management of diabetes [9]. *Cymbopogon jwarancusa*, an aromatic grass, has also exhibited antiglycemic effect in a rat model [10]. Trans-tiliroside from *Potentilla chinesis* has been reported to heal pancreatic tissue in diabetic rats [11]. Heghes et al. have reviewed the potential of oil-bearing plants, i.e., *Mentha x piperita* L., *Melissa officinalis* L., *Cuminum cyminum* L., or *Pistacia lentiscus* L. var. chia, in managing diabetes [12].

Modulation of α-amylase and α-glucosidase, the enzymes responsible for breaking down starch in the intestines, can be used to manage high blood sugar levels. Currently, acarbose, miglitol, and voglibose are the approved inhibitors for these enzymes [13]. However, the use of these inhibitors is impeded by gastrointestinal side effects, despite their ability to slow down glucose absorption [14]. Therefore, the development of efficacious and safe antidiabetic agents against the key enzymes, i.e., α-amylase and α-glucosidase, is highly desired. Exploring the potential of natural products and their essential oils (EOs) as alternative treatments for diabetes represents an exciting avenue in drug discovery and development. Eid et al. have reported a very good antiα-amylase activity of *Ocimum basilicum* seed EO, compared to the control acarbose [15]. EO from an ornamental plant from Egypt, *Myrtus communis* L. [16], and an evergreen Himalayan tree cone, *Cedrus deodara* [17], has also demonstrated good α-amylase activity. EO components of rosemary have also been reported to have antiglycemic effects [18]. A study by Capetti et al. has reported the metabolites from Eos of *Myristica fragrans*, *Eucalyptus radiata*, and *Laurus nobilis* to have antiglycemic effects equivalent to that of acarbose [19]. Daoudi et al. demonstrated that the inhibition activity of roasted *Argania spinosa* L. seed oils was higher against α-amylase and α-glucosidase compared to the unroasted seed oil. However, unroasted seed oil showed better antiglycemic activity against diabetic rat models compared to roasted seed oil [20].

O. compactum, commonly known as compact oregano, is an aromatic plant from Morocco (local name: *Zaatar*), that has been traditionally used for its medicinal properties [21]. Among the numerous other benefits, its oil has emerged as a promising candidate for antidiabetic therapy [22]. Preliminary studies have indicated that the oil also exhibits antioxidant activity in different biological systems [23]. Recent investigations have shown that *O. compactum* EOs possess promising health benefits and biological properties, including antioxidant, anticancer, insecticidal, and anti-inflammatory activities. Moreover, *O. compactum* EOs have also been found to exhibit interesting antimicrobial effects against yeast, molds, and Gram-positive and Gram-negative bacteria [21]. El Abdali et al. [24] have already demonstrated the antimicrobial and antioxidant potential of *O. compactum* EOs through in vitro and in silico approaches. However, there is no published work about the antidiabetic properties of this oil. Indeed, this exploratory investigation is the first to determine the antidiabetic activity of this oil through in vitro and in silico analysis.

The unique bioactive compounds present in *O. compactum* oil make it an intriguing subject for further investigation, particularly regarding its antidiabetic mechanisms and

potential therapeutic applications. *O. compactum* oil is generally rich in oxygenated monoterpenes such as thymol and carvacrol, which are known by their broad bioactivities [25–27]. They possess valuable antimicrobial, antioxidant, antidiabetic, anti-inflammatory, anticancer, and insecticidal activities [28,29].

Here, we utilized the EO of *O. compactum* for antienzymatic activity analysis (against α-amylase and α-glucosidase). In addition, we performed in silico analysis to find the essential residues of these enzymes involved in inhibition. We also utilized a docking approach to study the components of the oil binding with antioxidant NADPH oxidase to analyze the antioxidant activity at a fundamental level. Molecular docking is a useful in silico method employed for binding studies [30,31]. Molecular docking predicts the binding modes and affinities of compounds with target proteins, providing insights into their potential interactions and affinity [32]. It is a cost-effective and time-efficient approach to study binding [33]. Hence, we elucidated how the key components of the *O. compactum* oil exerted their antidiabetic effects. This work aims to provide valuable insights into the potential therapeutic benefits of *O. compactum* oil and its mechanisms of action against diabetes by analyzing the binding interactions. These findings can guide future studies and contribute to the development of safe and novel antidiabetic agents.

2. Results and Discussion

2.1. Chemical Composition

The identification of volatile components from *O. compactum* was performed using GC-MS analysis. The results are given in Table 1, which summarizes the chemical structure, molecular formula, Kovats index, and relative peak area of each component. A total of 10 components, accounting for 97.65% of the total peak area, were detected. Indeed, *O. compactum* EO was mainly represented by oxygenated monoterpenes (80.7%), while monoterpene hydrocarbons (8.24%), oxygenated sesquiterpenes (5.34%), and sesquiterpene hydrocarbons (3.37%) were revealed in the lowest percentages. The major identified components were thymol (54.6%), carvacrol (23.18%), and p-cymene (7.12%). Moreover, molecules such as caryophyllene oxide (5.34%), β-caryophyllene (3.37%), and linalool (2.64%) were also noticed in modest amounts.

Table 1. Chemical composition of *O. compactum* EO.

No [a]	Compounds [b]	KI [c]	Molecular Formula	% Relative Peak Area
1	α-Thujene	923	$C_{10}H_{16}$	0.41
2	α-Pinene	979	$C_{10}H_{16}$	0.17
3	p-Cymene	1026	$C_{10}H_{16}$	7.12
4	α-Phellandrene	1035	$C_{10}H_{16}$	0.54
5	Linalool	1098	$C_{10}H_{18}O$	2.64
6	α-Terpineol	1185	$C_{10}H_{18}O$	0.28
7	Thymol	1290	$C_{10}H_{14}O$	54.6
8	Carvacrol	1298	$C_{10}H_{14}O$	23.18
9	β-Caryophyllene	1494	$C_{15}H_{24}$	3.37
10	Caryophyllene oxide	1573	$C_{15}H_{24}O$	5.34
Total identified %				97.65
Monoterpene hydrocarbons				8.24
Oxygenated monoterpenes				80.7
Sesquiterpene hydrocarbons				3.37
Oxygenated sesquiterpenes				5.34

[a] In order of elution on HP-5Ms. [b] Compounds identified according to KI and MS. [c] Retention index established from alkane series on HP-5 MS capillary column (C8–C24).

These findings are in accordance with those indicated by numerous investigations. In fact, a recent exploration carried out by Hayani et al. [34] showed that the EO from *O. compactum* of the Ouazzane region (Morocco) was particularly rich in oxygenated monoterpene (60.9%) compounds, including thymol (38.59%) and carvacrol (26.65%). In addition, similar results were obtained by Bouyahya and his colleagues, who demonstrated

the abundance of the isomeric oxygenated monoterpenes carvacrol (24.71%) and thymol (15.32%) [26]. Additionally, Ouedrhiri et al. [35] indicated that oregano oil was rich in carvacrol (47.81%), γ-terpinene (17.25%), and thymol (15.70%). As evidenced in several works, carvacrol and thymol as well as their biosynthetic precursors, such as cymene and γ-terpinene, represent a major part of total oil components (more than 70%) [34,36,37]. Moreover, β-caryophyllene and caryophyllene oxide were also observed in significant amounts [25,38,39]. Therefore, the chemical composition may be related to biosynthetic pathways, promoting the overexpression of some components and blocking the synthesis of others [40,41].

On the other hand, it has been shown that *Origanum* species have substantial chemical variability in their volatile components, which could be explained by several factors, including plant origin, season and climatic conditions, soil constituents, precipitation, phenological stage, and drying process and also could be genetically determined [34,38,42,43]. Interestingly, the abundance of thymol and carvacrol in *O. compactum* oil, as known biologically active components, has made this oil a valuable source for development of novel pharmaceutical agents.

2.2. Antidiabetic Activity

One of the most widely used approaches to decrease the postprandial hyperglycemia (PPHG) in *diabetes mellitus* is by lowering the absorption of postprandial serum glucose levels via the inhibition of carbohydrate-hydrolyzing enzymes such as α-glucosidase and α-amylase [44,45]. In order to discover new and safer alternative compounds for *diabetes mellitus*, it is crucial to assess the antidiabetic properties of medicinal plants and their bioactive components. In the present study, EO of *O. compactum* was assessed for its inhibitory effect on α-amylase and α-glucosidase enzymes by an in vitro method. Our results show that *O. compactum* EO demonstrated an important ability to inhibit in vitro pancreatic α-amylase and α-glucosidase (Table 2). In the α-amylase inhibition assay, the EOs exhibited an interesting inhibitory effect on α-amylase activity with an IC_{50} value of 150.11 µg/mL. In the α-glucosidase inhibition assay, the studied EO has promising enzyme inhibitory activity against α-glucosidase with an IC_{50} value of 119.84 µg/mL. Acarbose was used as a standard reference drug, which showed α-amylase inhibitory activity with an IC_{50} value of 98.24 µg/mL and α-glucosidase inhibitory activity with an IC_{50} value of 62.31 µg/mL. From our results, EOs obtained from *O. compactum* have been shown to possess antidiabetic activity against the three selected enzymes. Furthermore, previous studies have reported that *Origanum* species from Tunisia exhibited stronger inhibitory activities against pancreatic α-amylase with a percentage of 90, 80, and 75%, respectively [46]. On the other hand, it has been reported that *Origanum* species from Turkey exhibit a potent inhibitory effect against α-amylase (0.13 mmol acarbose equivalent/g oil and 0.14 mmol acarbose equivalent/g oil) and α-glucosidase activity (6.04 mmol acarbose equivalent/g oil and 0.88 mmol acarbose equivalent/g oil) [47]. The antidiabetic activity of the EOs may be related to several bioactive compounds. Numerous studies have shown antidiabetic properties of thymol and carvacrol by in vitro or in vivo assays [48]. To our knowledge, this study is the first to report the α-amylase and α-glucosidase inhibitory activity of *O. compactum* EOs, suggesting their potential application as novel and effective sources of antidiabetic agents for the treatment of diabetes mellitus.

Table 2. Antidiabetic activity of *O. compactum* EOs against the enzymes α-amylase and α-glucosidase.

Samples	IC_{50} (µg/mL)	
	α-Amylase	**α-Glucosidase**
O. compactum EO	150.11 ± 0.12	119.84 ± 0.22
Acarbose (control)	98.24 ± 0.03	0.02

2.3. ADME and Toxicity Prediction

The use of computational drug-likeness assessments, conducted in silico, plays a crucial role in expediting the drug discovery process by effectively filtering and prioritizing potential candidates [29,49]. These computer-based methods not only save time and resources but also reduce the experimental workload, enabling the early identification of molecules with favorable pharmacokinetic properties and target interactions [50]. In order to adhere to Lipinski's rule of five, specific physical and chemical characteristics are required, including having fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, no more than 10 nitrogen or oxygen atoms, a molecular weight below 500 Da, and a MLOGP value less than or equal to 4.15 [51]. Notably, all the phytoconstituents satisfy Lipinski's rule of five (Table 3). Furthermore, the analysis of the ADME–toxicity profile provides further confirmation of the favorable characteristics of the small molecules under investigation. Firstly, all four small molecules exhibit a high level of predicted human intestinal absorption (HIA) exceeding 93%, indicating their potential to be well-absorbed in the human digestive system. Moreover, the assessment of their blood–brain barrier (BBB) permeability and central nervous system (CNS) permeabilities yields promising results, with all four small molecules demonstrating values exceeding −1 Log BB, falling within the range of −1 to −3 Log PS. These findings suggest that these compounds possess the ability to pass through the BBB and access the central nervous system, which is a crucial attribute for drugs targeting neurological conditions. Additionally, the metabolism test reveals specific interactions of the compounds with cytochrome enzymes. The C2 compound is predicted to be an inhibitor of cytochromes 1A2, 2C9, and 2C19, suggesting its potential to interfere with the metabolism of substances processed by these enzymes. On the other hand, C3 and C4 are predicted to be inhibitors of cytochrome 1A2, indicating their potential impact on the metabolic pathways involving this particular enzyme.

Table 3. The prediction of physicochemical properties of four compounds, based on Lipinski rules.

Compound Numbers	Physico-Chemical Properties						Lipinski Rules
	Molecular Weight (g/mol)	Molar Refractive Index	Rotatable Bonds	Log P (Octanol/Water)	H-BA	H-BD	Categorical (Yes/No)
Rule	≤500	40 ≤ MR ≤ 130	<10	<5	≤10	<5	Yes/No
C1	150.22	48.01	1	2.24	1	1	Yes
C2	220.35	68.27	0	3.15	1	0	Yes
C3	134.22	45.99	1	2.51	0	0	Yes
C4	150.22	48.01	1	2.32	1	1	Yes

The forecast of physicochemical characteristics provides validation that thymol, carvacrol, p-cymene, and caryophyllene oxide adhere to Lipinski's five rules (MW ≤ 500 g/mol, 40 ≤ MR ≤ 130, HBD < 5, Log P (octanol/water) < 5, and HBA ≤ 10) as depicted in Table 3 [29,49]. Furthermore, the ADME–toxicity profile examination verifies that the four volatile components were forecasted to exhibit favorable human intestinal absorption (HIA), surpassing 93%. Additionally, these components were projected to have blood–brain barrier (BBB) and central nervous system (CNS) permeabilities greater than −1 Log BB, falling within the range of −1 to −3 Log PS. The metabolism test declares that the C2 compound was predicted as an inhibitor of 1A2, 2C9, and 2C19 cytochromes, while C3 and C4 were predicted as inhibitors of 1A2 cytochrome [50].

The AMES toxicity test confirms that the four chemical compounds were predicted to not have toxicity in the human body, and none of these compounds showed a hepatotoxic effect, but they all present a positive skin sensitization, as listed in Table 4 [52]. The BOILED-Egg predictive model, primarily used for bioactive compounds, indicates that every chemical compound belongs to the yellow Egan egg category, leaving no exceptions. This suggests that they are anticipated to passively traverse the blood–brain barrier (BBB) [29,49]. Consequently, they are predicted to be agents affecting the central nervous system (CNS) with the highest likelihood of crossing the BBB (Figure 1) [53]. The

assessment of bioavailability indicates that all the compounds fall within the favorable range for oral bioavailability, as evident by their consistent placement within the pink zone on the bioavailability radar (as depicted in Figure 2). This positioning unequivocally categorizes them as drug-like substances, with no exceptions.

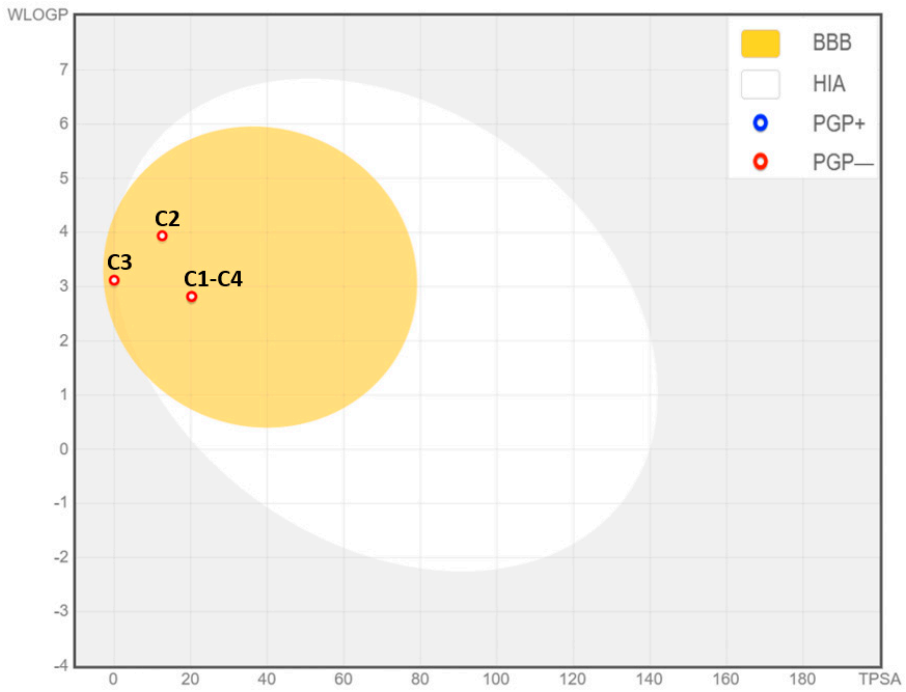

Figure 1. The BOILED-Egg model of four chemical compounds.

Figure 2. The bioavailability radars of the studied molecules, taking into account six physicochemical properties ideal for oral bioavailability, namely lipophilicity (LIPO), polarity (POLAR), size (SIZE), solubility (INSOLU), saturation (INSATU), and flexibility (FLEX).

Table 4. The prediction of ADMET in silico pharmacokinetic properties of four compounds.

Hepatotoxicity	Absorption	Distribution		Metabolism							Excretion	Toxicity		
	Intestinal Absorption	BBB Permeability	CNS Permeability	Substrate		Inhibitor					Total Clearance	AMES Toxicity	Hepatotoxicity	Skin Sensitization
				Cytochromes										
				2D6	3A4	1A2	2C19	2C9	2D6	3A4				
	Numeric (% Absorbed)	Numeric (Log BB)	Numeric (Log PS)	Categorical (Yes/No)							Numeric (Log mL/min/kg)	Categorical (Yes/No)		
C1	93.712	0.381	−1.438	No	No	No	No	No	No	No	0.243	No	No	Yes
C2	96.066	0.654	−2.525	No	No	Yes	Yes	Yes	No	No	0.905	No	No	Yes
C3	95.52	0.541	−1.348	No	No	Yes	No	No	No	No	0.239	No	No	Yes
C4	93.24	0.366	−1.349	No	No	Yes	No	No	No	No	0.259	No	No	Yes

2.4. Molecular Docking

Two- and three-dimensional visualizations of intermolecular interactions shown in Figure 3 confirm that carvacrol (C2) and thymol (C3), as major compounds of the studied plant, were docked to antioxidant protein. Initially, C2 and C3 compounds were docked in the active sites of NADPH oxidase protein encoded as 2CDU.pdb with binding energies of −6.26 Kcal/mol and −5.83 Kcal/mol, respectively. Both compounds share a number of common intermolecular interactions such as the pi–sigma bond type with His10 amino acid and two hydrogen bonds with Leu299 and Asp282 amino acid residues. In addition, there are alkyl and pi–alkyl bonds with Ala11, Ala300, and Ala303 amino acid residues.

Thereafter, the candidate ligands were docked to antidiabetic protein of human salivary amylase (PDB ID:1SMD) as shown in Figure 4. The compound C2 formed one hydrogen bond with Ala128 amino acid and one pi–pi T-shaped bond with Tyr67 amino acid in the A chain of the targeted protein with a binding energy of −5.42 Kcal/mol. Meanwhile, the C3 compound was docked to the same protein, forming two hydrogen bonds with Arg346 and Gln302 residues and two pi–pi T-shaped bonds with Arg303 and Phe348 amino acid residues, with binding energy of −4.96 Kcal/mol.

Lastly, the second antidiabetic protein of human lysosomal acid α-glucosidase, encoded as 5NN5.pdb, was reacted with carvacrol (C2) and thymol (C3) with binding energies of −5.29 Kcal/mol and −5.72 Kcal/mol, respectively. One hydrogen bond and one pi–pi T-shaped bond were detected for carvacrol towards Leu868 and His717 amino acid residues, respectively. Three hydrogen bonds were created between thymol and Glu866, Ser864, and Arg594 amino acid residues of the targeted protein in the A chain, as shown in Figure 5.

Natural product-based treatments often have a favorable safety profile compared to synthetic drugs. EOs from various plants have shown potential in modulating glucose metabolism and improving insulin sensitivity, which are key factors in diabetes management [54]. These oils offer a natural approach to regulate blood glucose levels due to the metabolites present in them [55]. By exploring the antidiabetic properties of metabolites in the EOs, we can identify key residues that bind compounds in EOs and exhibit antiglycemic effects. This can pave the way for the development of therapies against diabetes mellitus. Since the antioxidant potential of *O. compactum* has also been demonstrated, this study also provides mechanistic information on the potential of *O. compactum* to manage oxidative-stress-allied diabetic issues. Natural products and, hence, EOs are generally well-tolerated and have fewer adverse effects, which is particularly beneficial for long-term use in chronic conditions like diabetes. They also offer the advantage of being easily accessible, cost-effective, and readily available in many regions, making them suitable for widespread use. Therefore, understanding the antidiabetic properties and binding mechanisms of *O. compactum* oil can pave the way for further exploration, potential optimization, and translation of its therapeutic benefits into clinical practice, offering new possibilities for managing and treating diabetes.

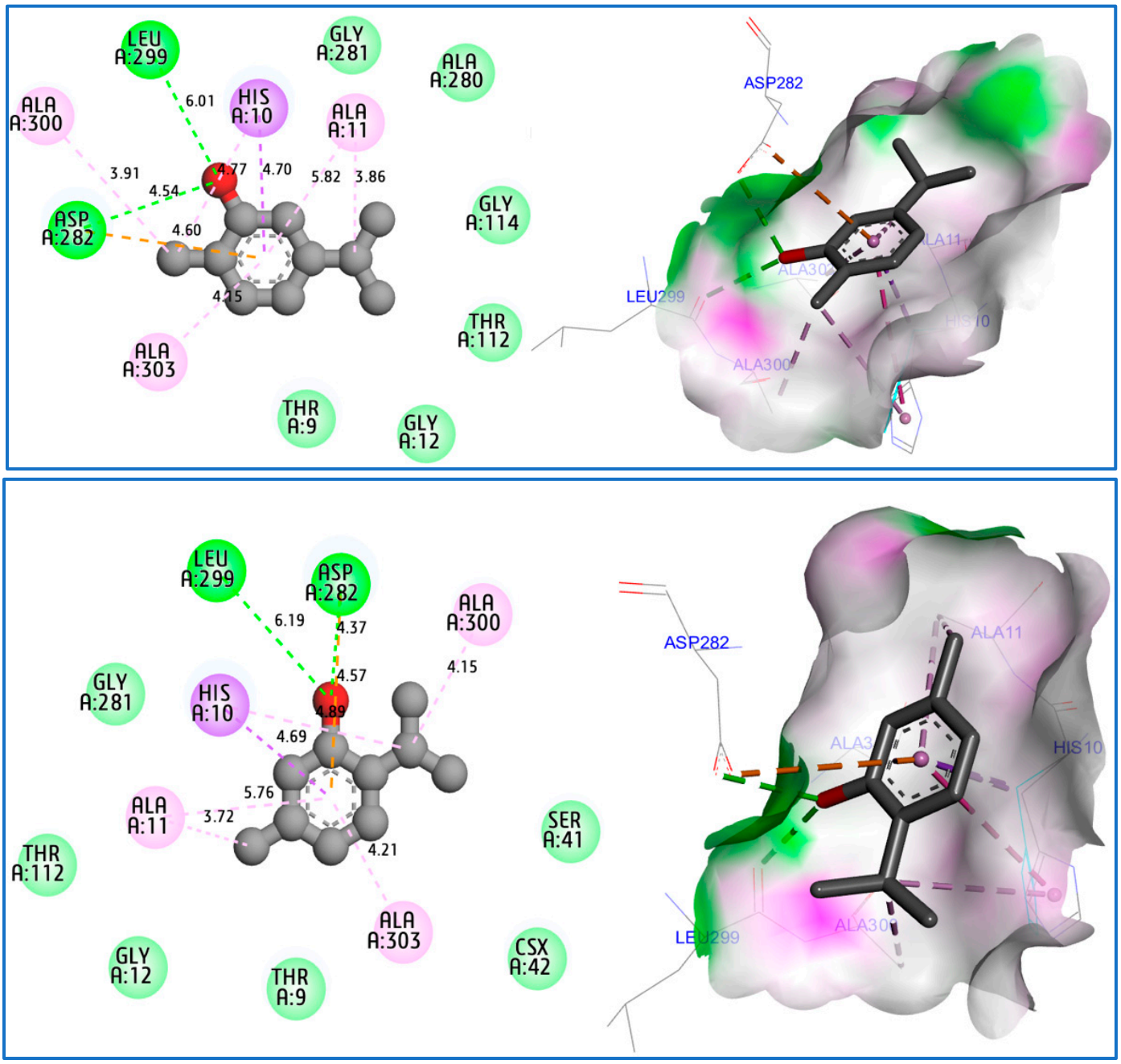

Figure 3. The 2D and 3D visualizations of intermolecular interactions established between NADPH oxidase protein (2CDU.pdb) towards carvacrol (C2) and thymol (C3) compounds, with binding energies of −6.26 Kcal/mol and −5.83 Kcal/mol, respectively.

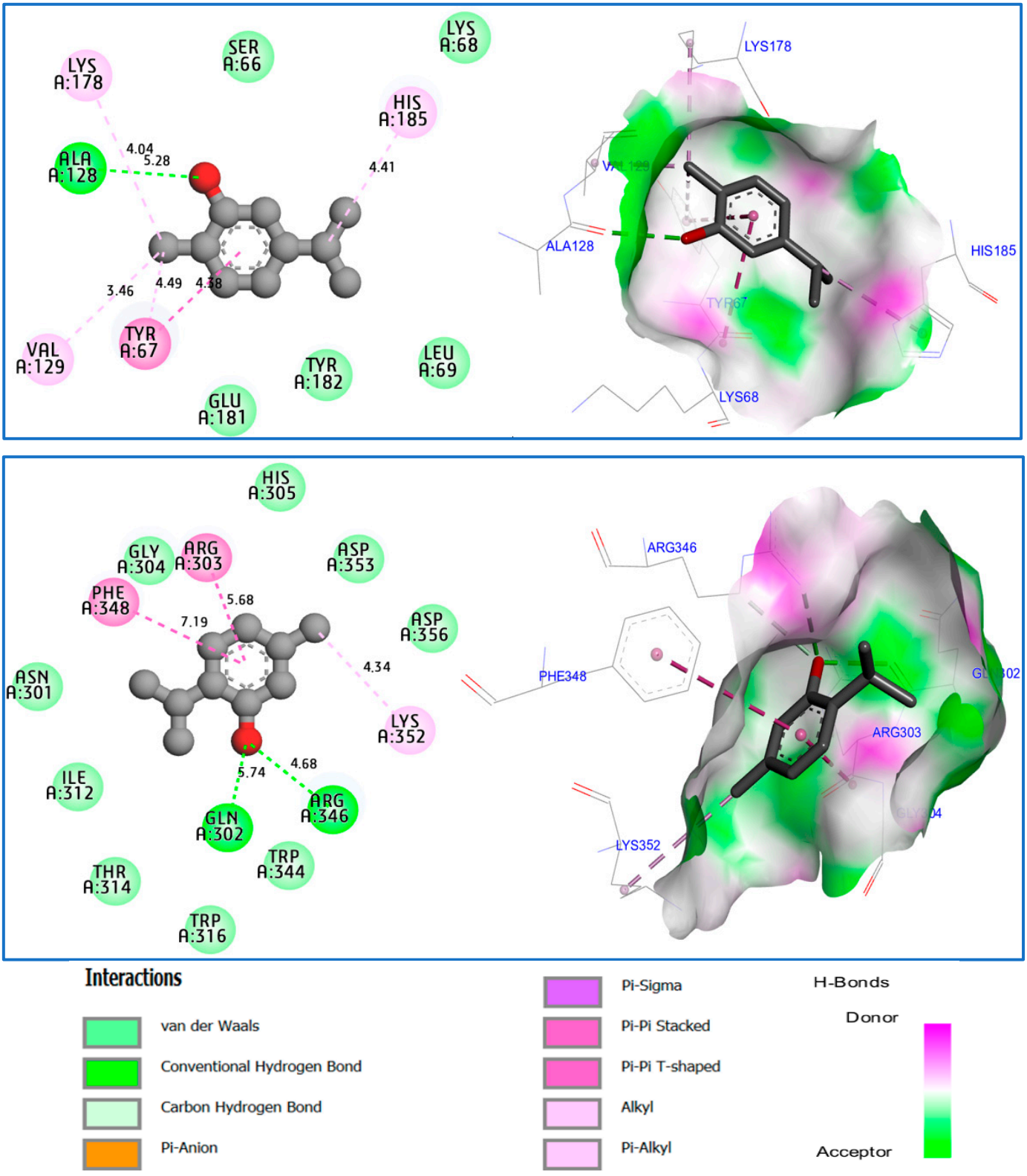

Figure 4. The 2D and 3D visualizations of intermolecular interactions established between human salivary amylase protein (1SMD.pdb) towards carvacrol (C2) and thymol (C3) compounds, with binding energies of −5.42 Kcal/mol and −4.96 Kcal/mol, respectively.

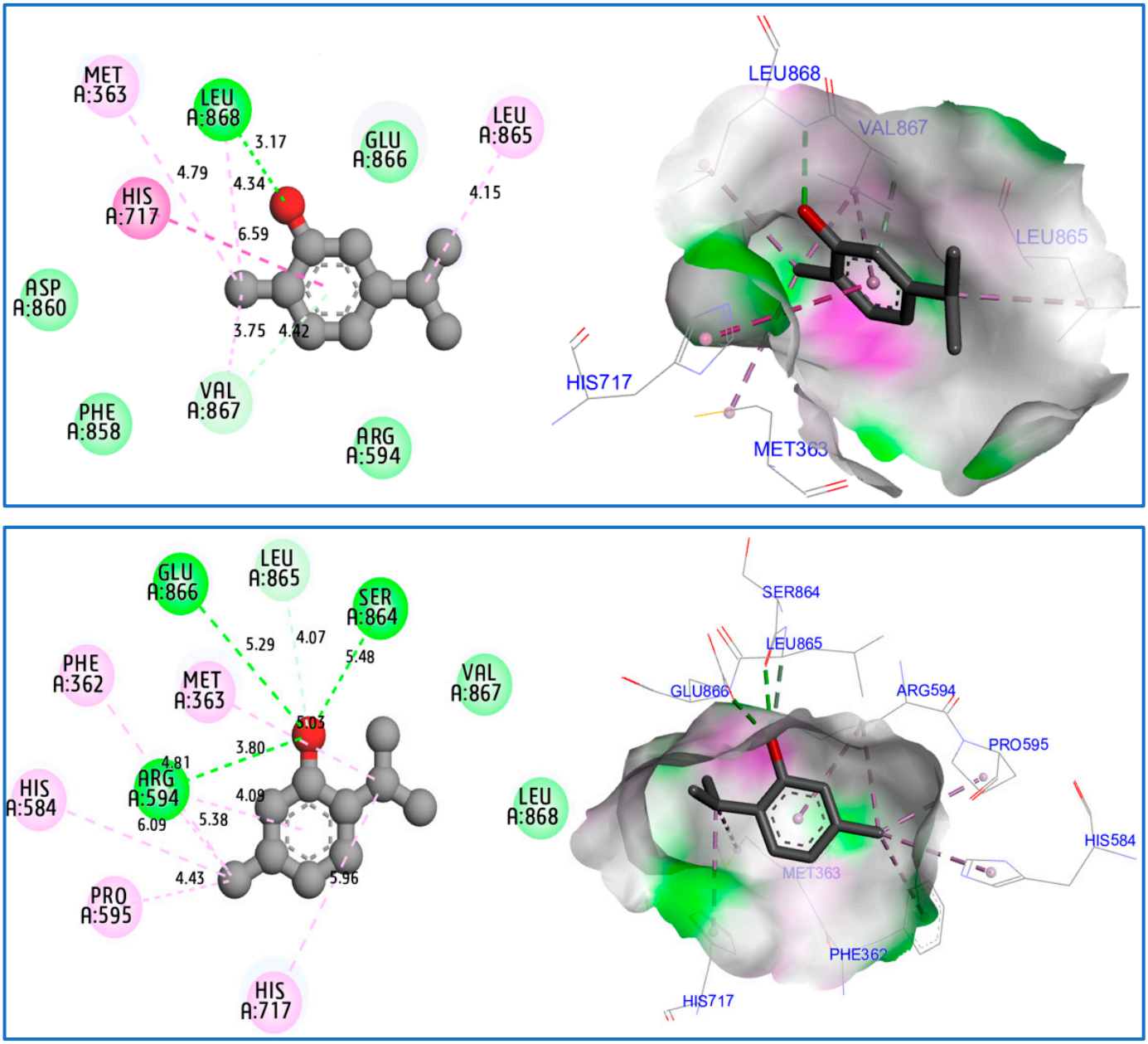

Figure 5. The 2D and 3D visualizations of intermolecular interactions established between human lysosomal acid α-glucosidase protein (5NN5.pdb) towards carvacrol (C2) and thymol (C3) compounds, with binding energies of −5.29 Kcal/mol and −5.72 Kcal/mol, respectively.

3. Materials and Methods

3.1. Chemicals

Anhydrous sodium sulfate (Na_2SO_4), α-amylase, α-glucosidase, acarbose, and dinitrosalicylic acid (DNS) were purchased from Sigma-Aldrich, Saint-Louis, American. All other used chemicals were of analytical grade.

3.2. Plant Material and EO Extraction

The aerial parts of *Origanum compactum* Benth. were collected from its natural environment in the province of Thar Es-Souk, north Morocco (34°39′03″ N, 4°16′40″ W). The botanical authenticity was confirmed by botanists from the Scientific Institute, University Mohamed V and a voucher number was provided (RAB 11421). The plant was dried at room temperature and the then extraction of oil was started. An amount of 100 g of dried aerial parts was distillated by hydro-distillation for 180 min using a Clevenger-type device. The obtained oil was recovered and dehydrated by anhydrous sodium sulfate, filtered, and then maintained at 4 °C, pending future assays.

3.3. GC-MS Analysis

The volatile content of *O. compactum* oil was characterized using chromatography (Trace GC-Ultra(Santa Clara, CA, USA) coupled with mass spectrometry (Quadrapole, Polaris Q (Santa Clara, CA, USA) (GC-MS) as described by Al-Mijalli [50,56,57]. The system is well-appointed with a non-polar HP-5MS capillary column (30 m, 0.32 mm × 0.25 µm). The temperature of the injector and detector was fixed at 280 and 300 °C, respectively. The column temperature was set at 50 °C for 5min and then at 180 °C for 4 °C/min. Helium (He) served as carrier gas (1.2 mL/min). A sample volume of 0.9 µL was injected manually. The chemical characterization of *O. compactum* oil constituents was undertaken via the comparison of its retention index (RI) (detected using a homologous series of C_8–C_{24} alkanes) and its mass spectra (MS) fragmentation patterns to those described in the literature catalogues [58,59]. Furthermore, each component was measured based on internal normalization of the total area of peaks observed in each chromatogram. Then, component MS was completed as illustrated in chemical libraries (NIST LIBRARY Version 2.0, 1 July 2002) through computer matching.

3.4. In Vitro Antidiabetic Assay

The antidiabetic potential of *O. compactum* EO was assessed through antidiabetic assays, i.e., through inhibition of the enzymatic activity of α-amylase and α-glucosidase. This is a target-based standard method of determining antidiabetic potential of plant oil. The control drug acarbose (IUPAC name: O-4,6-dideoxy-4-[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl] amino]-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose) was purchased from Sigma-Aldrich. The procedure for antidiabetic assays is described below:

3.4.1. α-Amylase Inhibition Assay

The α-amylase activity was determined by modifying a previously described method [50]. First, 200 µL of EOs was mixed with 200 µL of substrate starch solution (1%) at 37 °C for 20 min. Then, 200 µL of α-amylase solution and 200 µL of 0.02 M phosphate buffer (pH = 6.9) were added. The mixture was incubated for 10 min at 37 °C. Then, 500 µL of dinitrosalicylic acid solution (DNS) was added to the mixture and boiled in a water bath at 100 ∘C for 8 min. Then, the solution was cooled by adding 2000 µL of distilled water. Absorbance of the solution was read at 540 nm. The α-amylase inhibitory activity was expressed as IC_{50} value (µg/mL).

3.4.2. α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity was determined according to a previously described method [60], with slight modifications. EO (150 µL) was incubated with 100 µL of the enzymatic solution (α-glucosidase, 0.1 U/mL) for 10 min at 37 °C with an additional 200 µL of 1 mM *p*NPG (substrate) solution dissolved in 0.1 M sodium phosphate buffer

and the mixture was incubated for 30 min at 37 °C. The reaction was terminated with the addition of 0.1 M of Na_2CO_3 (1 mL). The absorbance was measured at 405 nm. The α-glucosidase inhibitory activity was expressed as IC_{50} value (mg/mL).

3.5. ADMET Prediction

The effectiveness of a medication can be compromised by its restricted absorption, distribution, metabolism, elimination, and toxicity features, collectively referred to as its ADMET properties [61]. Additionally, the principal obstacle faced in drug development during clinical studies is the drug's pharmacokinetic features, which can lead to significant expenses [62]. As a result, computational methods were utilized to evaluate the ADME characteristics of *O. compactum* essential oil to forecast its suitability as a potential candidate for drug development. The physicochemical properties of the studied molecules were initially predicted and Lipinski's rule of five parameters [60] also validated. Subsequently, we examined the pharmacokinetic properties of absorption, distribution, metabolism, excretion, and toxicity (three toxicological features were chosen, AMES toxicity, hepatotoxicity, and skin sensitization of each chemical compound), using SwissADME [62] and pkCSM [63] online servers. Afterwards, we utilized a predictive model for the BOILED-Egg plot, which relied on calculating lipophilicity through the logarithm of the partition coefficient between n-octanol and water (Log P O/W), as well as assessing polarity using the topological polar surface area (TPSA) of small molecules. This approach was employed to identify highly probable central nervous system (CNS) agents capable of crossing the blood–brain barrier (BBB). Furthermore, we evaluated the oral bioavailability of the analyzed molecules. This was depicted as radar plots, comprising six physicochemical properties crucial for optimal oral bioavailability, i.e., lipophilicity (LIPO), polarity (POLAR), size (SIZE), solubility (INSOLU), saturation (INSATU), and flexibility (FLEX).

3.6. Molecular Docking Study

The molecular docking technique was applied to the candidate molecules of the *O. compactum* EO and the targeted human proteins in order to study the type of intermolecular interactions and their binding energies. Initially, the 3D crystal structures of three proteins, namely NADPH oxidase, human salivary amylase, and human lysosomal acid α-glucosidase, identified by the PDB IDs 2CDU, 1SMD, and 5NN5, were sourced from the Protein Data Bank (PDB). Specifically, the NADPH oxidase structure was selected for the investigation of antioxidant properties of major *O. compactum* compounds. These protein structures had been determined using X-ray diffraction techniques, resulting in resolutions of 1.80 Å, 1.60 Å, and 2.00 Å, respectively. The proteins of interest were initially processed using AutoDock 4.2 software [64]. This involved the removal of all co-crystallized ligands bound to the respective proteins, the addition of Gasteiger charges, and the elimination of any solvent water molecules. Subsequently, these prepared proteins were subjected to docking with the primary compounds from *O. compactum* EO that met Lipinski's five rules. The docking procedure employed a grid box centered on the target protein, with dimensions set at a maximum of 126 units in each of the three dimensions and a spacing of 0.375 Å. Finally, the strongest ligand–protein complexes' two- and three-dimensional interactions were visualized using Discovery Studio 2021 software [65].

3.7. Strengths and Weaknesses of Methodology

This study on the translational potential of *O. compactum* EO for diabetes mellitus treatment provided a coherent justification for further in vivo studies on the modulation of the activities of α-glucosidase and α-amylase by the oregano oil constituents. However, it would be beneficial to test the in vitro antidiabetic activities of single compounds of this oil to clearly elucidate their antidiabetic efficacy.

The original algorithm used in the present study that required various software techniques to predict the physio-chemical, toxicological, and biological properties highlighted the inner mechanisms involved in the induction of these characteristics and may represent

a useful evaluation tool to be tested in in vivo evaluations of other natural products with biological activity. However, further in silico investigations targeting all *compactum* EO components are strongly required to fill knowledge gaps in the antidiabetic potential of this oil.

4. Conclusions

Research on EOs derived from plants and their metabolites has major implications in the field of therapeutic research. These EOs have been implicated to affect glucose metabolism, improve insulin sensitivity, and offer various therapeutic effects. This underscores their importance for further exploration. Our objective was to delve into the potential antidiabetic properties of *O. compactum* EO and understand the binding mechanism of its key metabolites, carvacrol and thymol, with human enzymes such as NADPH oxidase, α-glucosidase, and salivary amylase. The EO showed promising results due to its positive impact on glucose metabolism, insulin sensitivity, and related parameters and could serve as an effective antidiabetic agent. ADMET and binding residue information was also derived for the metabolites through computational assays. Binding site residues were identified through molecular docking. Such findings open avenues for future research to investigate this oil and its major bioactive compounds for their potential antidiabetic properties. Our findings can contribute to the development of natural-product-based interventions that are safe, effective, and targeted specifically towards key biological pathways involved in diabetes. We also propose similar studies on other unexplored EOs of plants used for antidiabetic treatment in traditional medicine. However, further in vivo investigations are strongly required to confirm the results of in vitro antidiabetic effect of *O. compactum* EO.

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