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Comparative Computational Analysis of Antidiabetic Potential of Diosgenin, Costunolide and Eremanthin from *Costus speciosus* (Sri Lankan Thebu)

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Costus speciosus is a medicinal plant used in Eastern medicine (also known as Asian medicine) due to its phytochemical content, which has been shown to significantly lower blood sugar levels in diabetic patients. The primary objective of this study was to conduct a comparative analysis of the antidiabetic potential of diosgenin, costunolide and eremanthin from *Costus speciosus*, in comparison to the standard drug, acarbose. Molecular docking studies revealed that diosgenin exhibited the highest potential as a natural antidiabetic agent by inhibiting four diabetic-regulating enzymes with docking score values ranging from -9.33 to -10.43 kcal/mol. Diosgenin also met Lipinski's rules with minimal violations, supporting its potential as a drug candidate. *In silico* ADMET analysis indicated that diosgenin had a favourable P value (5.01), suggesting high lipophilicity and membrane permeability. Toxicity analysis classified diosgenin in toxicity class 6 based on its LD_{50} value of 8000 mg/kg, indicating its potential as a safe and effective antidiabetic drug.

Keywords: *Costus speciosus*, Antidiabetic, Diosgenin, Molecular docking.

INTRODUCTION

Diabetes has become one of the most rapidly growing global health concerns in the 21st century [1]. According to the International Diabetes Federation (IDF), it was estimated that 537 million people worldwide had diabetes in 2021. This number is projected to rise to 643 million by 2030 and 783 million by 2045 [2]. A large national survey conducted in 2019 by researchers from several Sri Lankan universities, the Medical Research Institute (MRI) in Colombo and the Institute for Health Policy (IHP) found that nearly one in four adults in Sri Lanka (23%) had diabetes, with another one in three (31%) having elevated blood sugar levels. These findings identified Sri Lanka as a global hotspot for diabetes, with the highest prevalence in South Asia [3]. Type 2 diabetes mellitus (T2DM) is a major public health concern and is more prevalent than Type 1 diabetes, accounting for more than 90% of all diabetes cases worldwide [4]. In T2DM, individuals produce insulin, but either their pancreas produces insufficient amounts or their body cells cannot effectively use the insulin (insulin resistance). Key risk factors for T2DM include obesity, family history, a sedentary lifestyle, age, race and ethnicity. Poorly controlled diabetes can lead to serious complications, such as retinopathy, nephropathy, neuropathy and cardiovascular diseases [5]. Current treatments include oral medications and insulin injections. A variety of drugs, such as acarbose, sulfonylureas, meglitinides, biguanides (metformin), thiazolidinediones, GLP-1 analogues and incretin mimics, are used to manage diabetes [6]. However, the synthetic drugs can have harmful and sometimes dangerous side effects [7]. As a result, there has been a growing focus on developing natural herb-based treatments for diabetes.

Costus speciosus, a plant native to Sri Lanka, is an ornamental species with a long history of use in traditional medicine for its pharmacological properties [8]. Commonly known as canereed, crepe ginger, spiral fag and spiral ginger, *C. speciosus* thrives in moist, humid, evergreen environments typical of Sri Lanka and the Indo-Malayan region. Ayurvedic medicine has long recommended this plant for treating a variety of ailments.

Numerous pharmacological effects of *C. speciosus* have been reported, including anti-hyperglycemic, antibacterial, antidiuretic, anticancer, antioxidant, anti-inflammatory, antipyretic, larvicidal, antistress and antifungal activities [7,9,10].

Diosgenin, costunolide and eremanthin are the major phytochemicals found in *C. speciosus*, as shown in Fig. 1. According to *in vitro* studies [7], diosgenin is a phytosteroid sapogenin present in the rhizomes, leaves and seeds of the plant. Costunolide and eremanthin are naturally occurring sesquiterpene lactones primarily found in the rhizomes of *C. speciosus* [11]. *In vitro* studies have demonstrated a significant hypoglycemic effect of a methanolic extract of *C. speciosus* leaves in normal rats and alloxan-induced diabetic Wistar rats, with a 60% reduction in blood glucose levels at 90 min post-glucose loading, compared to the control group [12,13]. *C. speciosus* leaves were found to inhibit key carbohydrate-metabolizing enzymes, including α-amylase and α-glucosidase, *in vitro*. The bioactive substances, such as eremanthin, a sesquiterpene that enhances insulin secretion and glucose absorption, may be responsible for these effects [14]. The potential link between *C. speciosus*'s anti-diabetic properties and its costunolide content may be attributed to its ability to stimulate β-cell insulin release through the suppression of nitric oxide synthase (NOS) expression, thus promoting β-cell regeneration [15,16].

α-Amylase and α-glucosidase are essential enzymes involved in regulating postprandial blood glucose [17,18]. Natural inhibitors of these enzymes delay carbohydrate digestion, thereby slowing the rate of glucose absorption into the bloodstream [19]. Protein tyrosine phosphatase 1B (PTP1B) is a key negative regulator of the PI3K/AKT (phosphatidylinositol 3-kinase) insulin signaling pathway. In individuals with type 2 diabetes, PTP1B activity is often increased, leading to reduced expression of the insulin-regulated glucose transporter GLUT4 and elevated blood glucose levels [20]. GLUT4 is a crucial for glucose uptake in insulin-sensitive tissues, such as adipose tissue and muscle. Phytochemicals in *C. speciosus* (Thebu) stimulate the PI3K/AKT insulin signaling pathway by inhibiting PTP1B, thereby promoting GLUT4 translocation to the plasma membrane. This increase in GLUT4 expression in muscle and adipose tissues facilitates greater glucose uptake. In muscle cells, glucose is stored as glycogen, while in adipose tissue, it is stored as triglycerides, which helps lower blood glucose levels [21,22].

Additionally, patients with type 2 diabetes (T2D) exhibit increased levels of inducible nitric oxide synthase (NOS2) in their pancreatic islets. NOS2 catalyzes the production of nitric oxide (NO) radicals, which contribute to β-cell dysfunction and the progression of diabetes. Phytoconstituents in *C. speciosus* have the ability to bind to NOS2 and inhibit its activity, leading to the stimulation of β-cells, enhanced insulin secretion and β-cell regeneration [23]. Although several studies have investigated the antidiabetic effects of these phytochemicals individually [24] no comparative studies have been conducted using a well-known standard drug. To address this gap, we used acarbose, a US FDA-approved drug, as the standard in our comparative analysis.

This computational study aims to investigate the antidiabetic mechanistic activities of diosgenin, costunolide and eremanthin by examining their binding interactions with four key carbohydrate-metabolism enzymes–α-amylase, α-glucosidase, PTP1B and inducible nitric oxide synthase. This aspect has not been studied *in silico* before. Initially, geometry optimizations were performed to minimize unrealistic bond lengths,

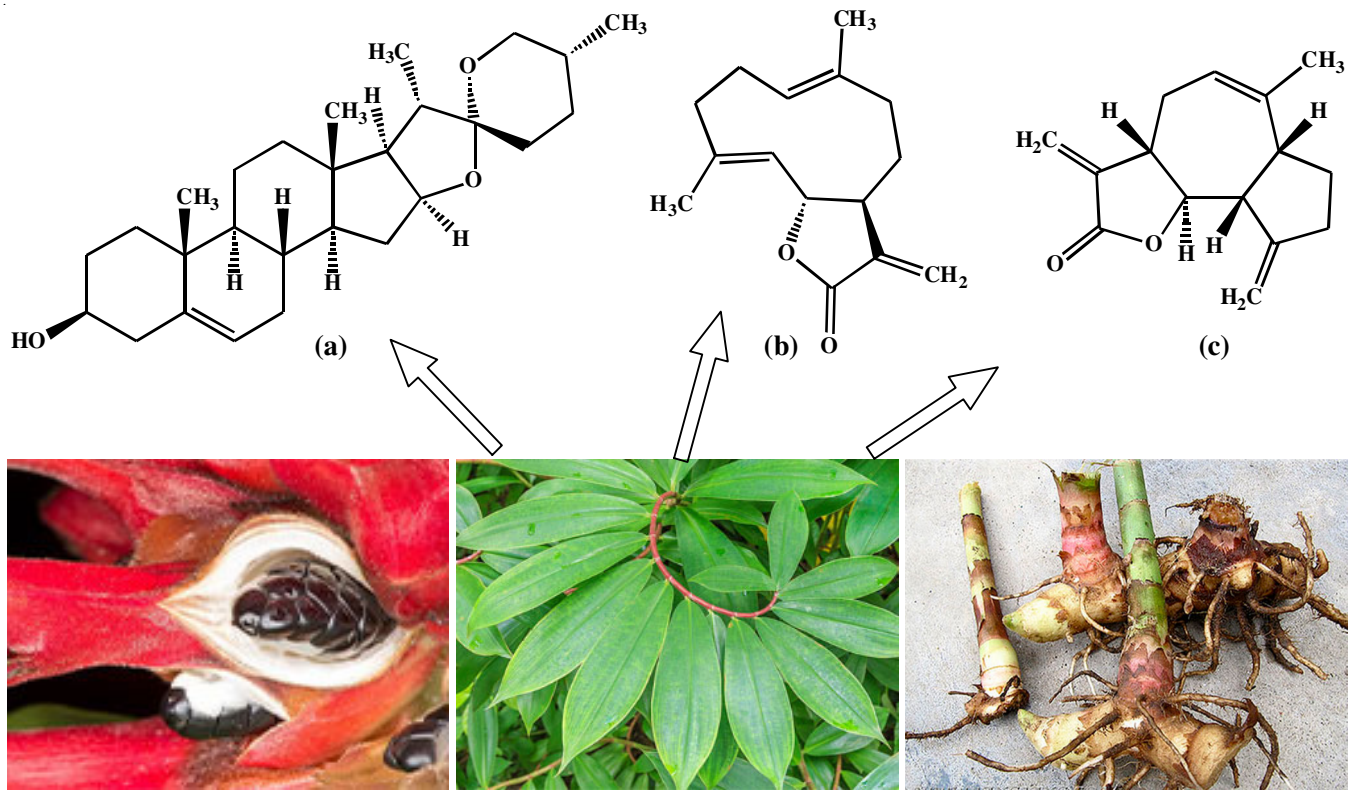


Fig. 1. Major antidiabetic phytochemicals in *Costus speciosus*: (a) diosgenin, (b) costunolide and (c) eremanthin

angles and geometries, ensuring more stable docking starting points and enhancing docking efficiency [25]. Protein-ligand docking is a powerful tool for identifying the best binding sites of a ligand (phytochemical) on a target receptor.

Furthermore, Lipinski's, Ghose's and Veber's principles were applied to assess the pharmacokinetic properties of these three phytochemicals. According to Lipinski, a good drug typically has no more than one violation of these parameters [26]. A ligand exhibiting favourable drug-like and pharmacokinetic characteristics is defined by a molecular weight under 500 g/mol, no more than five hydrogen bond donors, fewer than 10 hydrogen bond acceptors and an octanol-water partition coefficient (log P) no greater than 5. Additionally, the ADMET properties of diosgenin, costunolide and eremanthin were evaluated as these properties play critical roles in drug discovery and development.

EXPERIMENTAL

Ligand preparation: The structures of the ligands *viz*. diosgenin, costunolide and eremanthin were retrieved from the PubChem database (NCBI) in SDF format (https://www.ncbi.nlm.nih.gov). Ligands were geometrically optimized using Gaussian 09 software and GaussView 6.0. The structures were optimized using the density functional theory (DFT) method with the B3LYP functional and the 6-311G++ (d,p) basis set without solvation. After optimization, the vibrational frequencies of each molecule were analyzed. The ligands in SDF format were then converted into PDB files using Open Babel software.

Protein preparation: The 3D structures of the proteins were obtained from the Protein Data Bank (https://www.rcsb.org/): human pancreatic α-amylase (PDB: 4GQR), human intestinal α-glucosidase (PDB: 2QMJ), protein tyrosine phosphatase 1B (PTP1B, PDB: 2QBP) and human inducible nitric oxide synthase (PDB: 4NOS). Protein structures were selected based on resolution values close to 1 Å and the method used for protein determination (X-ray crystallography). Proteins were cleaned using Biovia Discovery Studio 2021 and all water molecules, ligands and heteroatoms were removed from the protein structures.

Molecular docking: Molecular docking studies were performed using AutoDock 4.2.6 and MGLTools 1.5.6. Polar hydrogen atoms and Kollman united atom charges were added to the macromolecule and the protein pdbqt file was prepared. Gasteiger charges were automatically added to the ligands and the ligand pdbqt file was prepared. In this study, blind docking was performed and grid parameters were set to cover the entire protein. A grid parameter file was then generated. The docking step involved opening the receptor and ligand files and setting the genetic algorithm (GA) with 300 populations and 100 GA runs. The docking output was set as Lamarckian GA and saved as a docking parameter file (DPF). Autogrid and AutoDock were then executed, generating GLG and DLG files [27]. Molecular interactions between the protein-ligand complexes, including bond lengths and amino acids in binding pockets, were analyzed using Biovia Discovery Studio and PyMOL. Six docking trials were performed for each protein-ligand complex [28,29] to obtain the best binding energies.

Analysis of ADMET properties of ligands: The ADMET properties of diosgenin, costunolide and eremanthin were analyzed, as well as those of the FDA-approved drug acarbose (for comparison). The ADMET properties of each ligand were investigated using SwissADME [30] and ProTox-3.0 software [31]. Canonical SMILES of each ligand were used to study physico-chemical properties, lipophilicity, water solubility, drug-likeness and pharmacokinetics. To validate the techniques used for molecular docking, the binding residues of the docked conformations were compared with the binding residues as mentioned in the literature [28].

RESULTS AND DISCUSSION

Diosgenin, costunolide and eremanthin from *C. speciosus* were computationally optimized using the density functional theory (DFT) method with the B3LYP functional and 6-311G++ (d,p) basis set. The results presented in Table-1 demonstrate that diosgenin exhibited the most negative Gibbs free energy of formation (-8.03×10^5 kcal/mol) compared to the other two phytochemicals. This is consistent with the findings of Michalak *et al*. [32], who investigated the higher stability of diosgenin and its derivatives; however, their study did not include a comparison with the other two phytochemicals (costunolide and eremanthin) analyzed in the present work. The relatively higher stability of diosgenin can be attributed to its unique and highly stable molecular structure.

Molecular docking results, as presented in Table-2, show the averaged binding energies of diosgenin, costunolide, eremanthin and the standard drug acarbose with four enzyme-proteins (α-amylase, α-glucosidase, protein tyrosine phosphatase 1B and inducible nitric oxide synthase). These results agreed with the findings of Morris *et al*. [33], who reported similar docking studies to determine the best possible binding sites for ligand-protein complexes.

The effectiveness of the binding interactions was determined by comparing the magnitude of the inhibition constant (Ki value) obtained from the molecular docking of these phytochemicals with the four enzymes. The inhibition constant (Ki) can also be described as the dissociation constant of the docked protein-inhibitor complex (enzyme-phytochemical complex). A lower dissociation constant (*i.e.* a smaller Ki value) indicates a stronger inhibition potential [34]. The inhibition constant (dissociation constant) was calculated using the following formula:

$$Ki = e^{-\Delta G/(RT)}$$

where ΔG is the free energy of binding; R is the gas constant (1.987 cal K^{-1} mol^{-1}) and T is the absolute temperature (298.15 K).

The docking results (Table-2) indicated that ligands (three phytochemicals) with more negative free energies of binding exhibit a higher binding affinity with the enzymatic proteins. Diosgenin exhibited the most negative binding energies with α-amylase (-9.36 kcal/mol), α-glucosidase (-9.79 kcal/mol), protein tyrosine phosphatase (PTP1B) (-9.33 kcal/mol) and inducible nitric oxide synthase (iNOS) (-10.43 kcal/mol), demonstrating the strongest inhibitory activity compared to the other two phytochemicals (costunolide and eremanthin).

TABLE-1
OPTIMIZED STRUCTURES AND ENERGIES OF THE PHYTOCHEMICALS FROM *Costus speciosus*

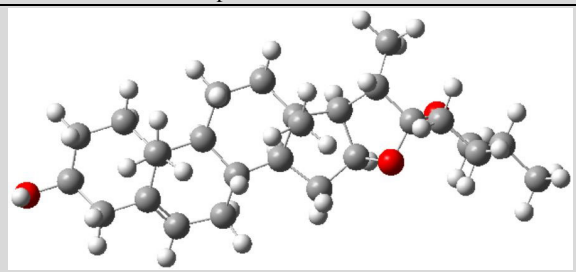
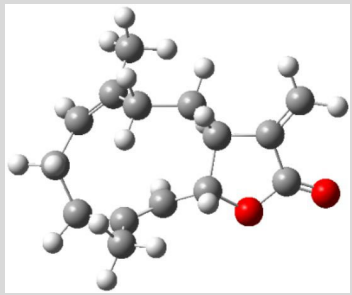
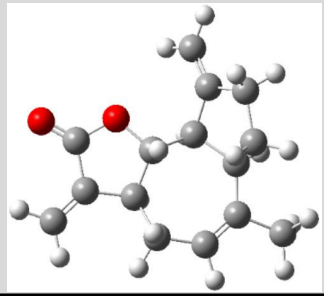
Phytochemical	Optimized structure	Optimized energy (kcal/mol)
Diosgenin		-8.03×10^5
Costunolide		-4.61×10^5
Eremanthin		-4.59×10^5

TABLE-2
PROTEIN-LIGAND BINDING ENERGIES, MODES OF ACTION AND INHIBITION CONSTANTS

Ligand	Protein	Binding energy (kcal/mol)	Action	Inhibition constant, Ki (μM)
Diosgenin	α-Amylase	-9.36	Inhibitor	0.136
	α-Glucosidase	-9.79	Inhibitor	0.067
	PTP1B	-9.33	Inhibitor	0.146
	NOS	-10.43	Inhibitor	0.023
Costunolide	α-Amylase	-6.78	Inhibitor	10.71
	α-Glucosidase	-7.00	Inhibitor	7.38
	PTP1B	-7.39	Inhibitor	3.86
	NOS	-7.68	Inhibitor	2.36
Eremanthin	α-Amylase	-6.93	Inhibitor	8.29
	α-Glucosidase	-8.46	Inhibitor	0.632
	PTP1B	-7.32	Inhibitor	4.28
	NOS	-8.02	Inhibitor	1.33
Acarbose (standard)	α-Amylase	-8.73	Inhibitor	0.399
	α-Glucosidase	-7.44	Inhibitor	3.54
	PTP1B	-7.59	Inhibitor	2.71
	NOS	-8.22	Inhibitor	0.949

The lower values for the inhibition constant (Ki) of diosgenin, ranging from 0.023 μM for iNOS to 0.146 μM for PTP1B, supported this hypothesis.

Further investigations revealed that diosgenin's inhibition potential surpassed that of acarbose, the reference inhibitor used in this study. Although Rocha *et al*. [24] has investigated the antidiabetic effects of these phytochemicals individually, no comparative study has been conducted using a well-known standard drug. The second phytochemical, costunolide, exhibited relatively less negative binding energies and higher Ki values compared to diosgenin with all four enzymatic proteins. The binding energies ranged from -6.78 kcal/mol for α-amylase to -7.68 kcal/mol for iNOS. Costunolide demonstrated significantly higher Ki values of 10.71 μM for α-amylase and 7.38 μM for α-glucosidase, indicating lower inhibitory activity. However, its inhibition capability was more prominent with the other two enzymes, PTP1B and iNOS (protein tyrosine phosphatase and inducible nitric oxide synthase).

The third phytochemical, eremanthin, displayed binding free energies and Ki values that were intermediate between those of diosgenin and costunolide. The binding energies ranged from -6.93 kcal/mol for α-amylase to -8.46 kcal/mol for α-glucosidase, while the Ki values ranged from 0.632 μM for α-glucosidase to 8.29 μM for α-amylase. Therefore, eremanthin exhibited stronger inhibitory activity compared to costunolide, though it was less potent than diosgenin. Additionally, eremanthin demonstrated more negative binding energy with iNOS (-8.02 kcal/mol) and higher inhibition potential (Ki = 1.33 μM). The presence of a lower inhibition constant (Ki) and higher binding affinity indicates that diosgenin requires a lower dosage to block diabetes-related receptors effectively. Fig. 2

summarizes the binding energies of each phytochemical with the four different enzymes (protein-ligand complexes).

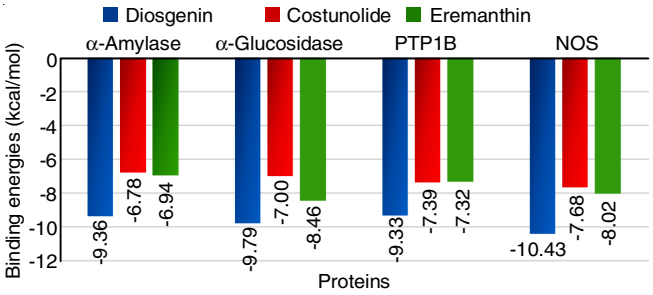


Fig. 2. Binding free energies of three ligands (diosgenin, costunolide and eremanthin) with four human enzymatic proteins

In addition to investigating binding free energies, it is important to visualize the amino acid residues present in the active site and their secondary interactions, such as hydrogen bonding, dipole-dipole interactions and van der Waals forces. These secondary interactions are crucial for predicting the ligand-binding function at the molecular level. They reveal the strength, specificity and orientation of the ligand within the protein's binding pocket. The precise positioning of the ligand allows it to interact correctly with the protein, thereby activating or inhibiting its function. Fig. 3 illustrates the molecular docking complexes of the phytochemical diosgenin with four enzymatic proteins involved in diabetes metabolism. Diosgenin interacts with α-amylase by forming hydrogen bonds with the amino acids Glu 233 and Asn 298, hydrophobic interactions with the amino acids Trp 58, Trp 59, Leu 165 and His 299 and van der Waals forces with the amino acids Tyr 62, Gln 63, Thr 163, Arg 195, Asp 197, Ile 235, Phe 256 and Asp 300 in the active site.

Diosgenin and α-glucosidase interacted through hydrogen bonds with amino acids Ile 13 and Tyr 46, hydrophobic interactions with amino acids Val 7, Lys 48 and Pro 137 and van der Waals interactions with amino acids Glu 11, Arg 12, Tyr 45, Ser 47, Ser 134, Arg 135, Gln 136, Ser 155, Ile 156, Gly 157, Pro 158, Arg 171 and Ser 139. Diosgenin with PTP1B displayed hydrogen bonds with amino acids Ser 80 and Glu 75, hydrophobic interactions with amino acids Val 211, Leu 204, Pro 206, Arg 79 and Lys 73 and van der Waals forces with amino acids Ser 205, His 208, Gln 208 and Pro 210. Further studies revealed that diosgenin bound to nitric oxide synthase (NOS) *via* hydrogen bonds with amino acids Leu 125, hydrophobic interactions with amino acids Phe 369, Arg 199, Phe 488, Trp 463, Tyr 491A, Pro 198, Met 355, Ala 197, Val 352, Tyr 373, Pro 350 and van der Waals interactions with amino acids Trp 372, Glu 377, Met 374, Cys 200, Tyr 489 and Tyr 490.

A comparative study was conducted to investigate the secondary interactions of the other two phytochemicals, costunolide and eremanthin, with these four enzymes involved in diabetes regulation. The results demonstrated that diosgenin exhibited a broad range of binding interactions across all four enzymes, compared to costunolide and eremanthin, which showed relatively lower binding affinities. To validate these results, acarbose was docked with the four proteins using the same procedure. Acarbose exhibited various interactions with the main enzymatic proteins, α-amylase and α-glucosidase. In case of α-glucosidase, acarbose interacted through multiple conventional hydrogen bonds at binding sites Arg 189, Asn 667, Asp 667, Gln 187 and Lys 715 and carbon-hydrogen bonds with Val 668 and Ser 664. His 668 also showed pi-alkyl interactions with the protein, along with van der Waals interactions with Gln 670. For acarbose-α-amylase docked complex, hydrogen bonds were formed at the active site with Asn 53, Gln 63, Ser 112 and Trp 59, carbon-hydrogen bonds with Ala 50, Trp 357 and alkyl interactions with Pro 54. Additionally, Acarbose displayed van der Waals interactions with Asp 356, Ala 106, Gly 104, Ser 108, Val 354, Tyr 52 and Phe 119.

The pharmacokinetic properties, based on Lipinski's rule of five, were investigated for these three phytochemicals from *C. speciosus* to assess their potential pharmaceutical applications. According to the computationally investigated results in Table-3, diosgenin contains one hydrogen bond donor and three hydrogen bond acceptors. The presence of hydrogen bond donors enhances its ability to interact with biological targets such as proteins and enzymes. The three acceptors suggest that diosgenin forms multiple hydrogen bonds, which may contribute to its stability and binding affinity with target proteins. In contrast, costunolide and eremanthin lack hydrogen bond donors and possess only two hydrogen bond acceptors. This limitation may reduce their ability to form stable interactions with biological targets compared to diosgenin. These findings align with the observations by [26] regarding the applications of Lipinski's rule.

Diosgenin has the highest molecular weight among the three ligands. A higher molecular weight generally increases the chances of binding due to a larger surface area, but it may also pose challenges in terms of absorption and distribution. In contrast, costunolide and eremanthin have lower molecular weights, which may enhance their absorption and bioavailability. However, their smaller molecular sizes might lead to fewer interactions with larger biological targets.

The Log P value is a critical factor in Lipinski's rule of five, which helps assess the drug-likeness of a compound. *In silico* analysis revealed that diosgenin has a Log P value of 5.01, indicating its high lipophilicity. This characteristic enhances

TABLE-3
PHARMACOKINETIC PROPERTIES ANALYSIS OF THREE PHYTOCHEMICALS AND THE STANDARD BASED ON LIPINSKI'S RULE OF FIVE

Ligand	H bond donors (≤ 5)	H bond acceptors (≤ 10)	Molecular weight (g/mol)	C log P (≤ 5)	Number of rotatable bonds
Diosgenin	1	3	414.62	5.01	0
Costunolide	0	2	232.32	2.97	0
Eremanthin	0	2	230.30	2.93	0
Acarbose	14	19	645.60	-6.22	9

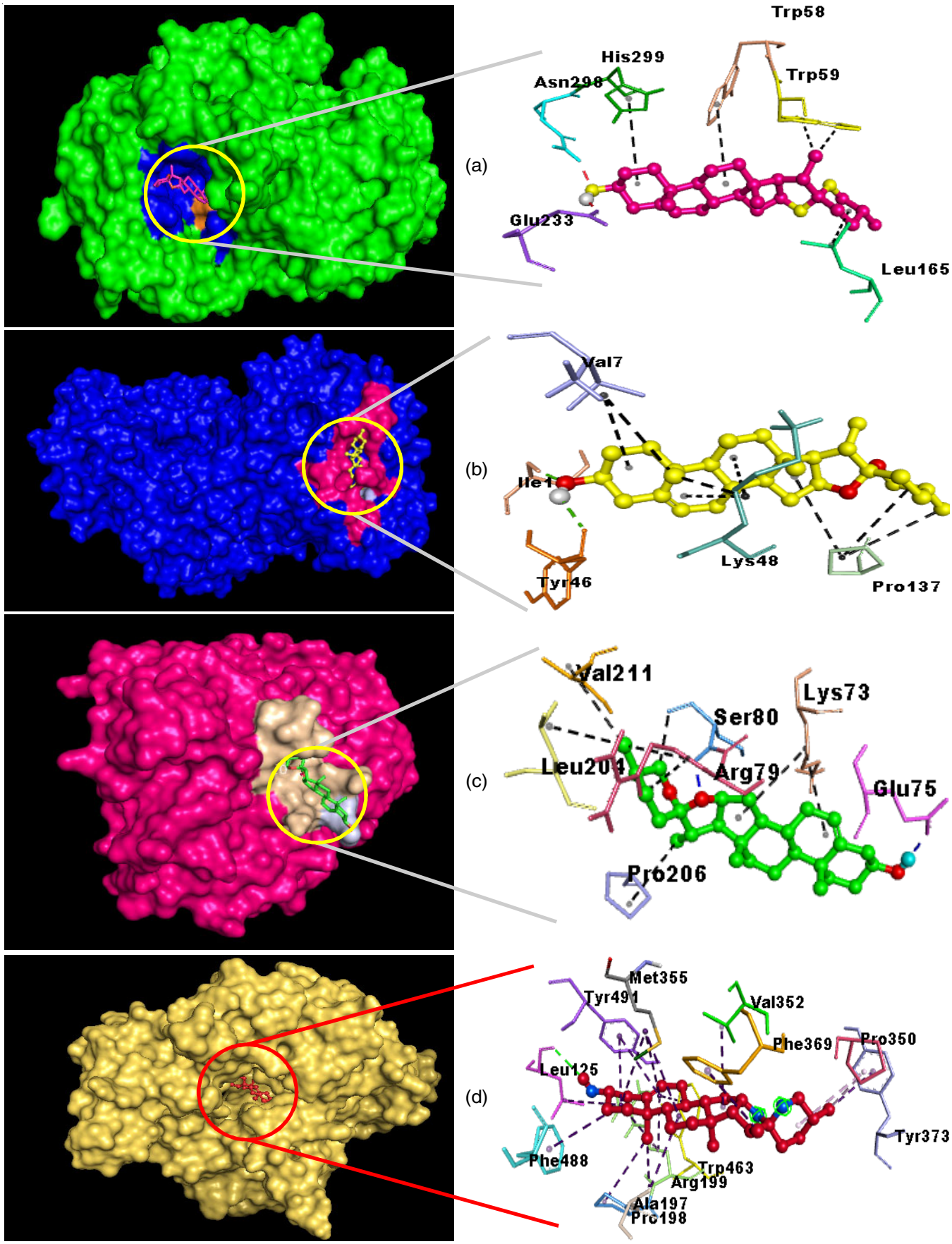


Fig. 3. Docked complexes of diosgenin with (a) α-amylase, (b) α-glucosidase, (c) protein tyrosine phosphatase 1B (PTP1B) and (d) inducible nitric oxide synthase (NOS)

membrane permeability, promoting more efficient absorption in biological systems. On the other hand, costunolide has a lower Log P value, reflecting its reduced lipophilicity compared to diosgenin. This may result in higher solubility in aqueous environment, potentially improving bioavailability but limiting its ability to penetrate lipid membranes. Eremanthin displayed similar lipophilicity and solubility properties to costunolide. These findings are consistent with the evidence provided by [35], offering valuable insights for the pharmaceutical industry.

In silico analysis of the ADMET properties of these three phytochemicals is presented in Table-4. The capacity of a compound to pass through biological membranes is correlated with its molar refractivity [36]. Diosgenin, along with acarbose (standard), demonstrated significantly higher molar refractivity values ranging from 40 to 130 cm^3/mol, which are typically linked to oral bioavailability and efficient absorption, compared to costunolide and eremanthin. The topological polar surface area (TPSA) serves as an indicator of liposolubility and the ability to penetrate biological membranes. The significantly higher TPSA value (321.17 $Å^2$) of acarbose suggests its more polar nature and its higher potential for hydrogen bonding. In contrast, diosgenin (38.69 $Å^2$), costunolide (26.30 $Å^2$) and eremanthin (26.30 $Å^2$) exhibited lower TPSA values, indicating that they are less polar and may have distinct absorption characteristics. A lower TPSA suggests better membrane permeability.

In improving the *in silico* prediction of drug bioavailability, the FDA-approved standard drug, acarbose, exhibited relatively low bioavailability (0.17), which may limit its effectiveness despite its high solubility. However, the phytochemicals present in *C. speciosus* (diosgenin, costunolide and eremanthin) demonstrated significantly higher absorption characteristics with a bioavailability value of 0.55. These three ligands exhibited high gastrointestinal absorption, a crucial feature for their effectiveness as therapeutic agents. Furthermore, diosgenin, costunolide and eremanthin were found to have the ability to cross the blood-brain barrier (BBB), making them potential candidates for applications in the central nervous system [36].

Toxicity analysis *via* ProTox 3.0 indicated that eremanthin has a moderate toxicity level with an LD_{50} value, while diosgenin and acarbose exhibited comparatively lower toxicity (Class 6). Costunolide (Class 5) showed slight toxicity, which is supported by studies indicating its non-toxic nature at the tested doses. When compared to other drugs, acarbose exhibited the highest LD_{50}, indicating the least toxicity. Diosgenin also demonstrated a higher LD_{50} value compared to costunolide and eremanthin, indicating it has lower toxicity. Skin penetration refers to the amount of active ingredient retrieved in tape strips (from the superficial layers of stratum corneum), urine and sweat. Diosgenin exhibited the greatest potential for skin penetration (-4.08 cm/s), making it a better candidate for transdermal distribution, compared to acarbose, which has a skin penetration rate of -6.18 cm/s. These findings align with the conditions outlined in the Lipinski, Ghose and Veber principles, as reported by Benet *et al*. [26]. These phytochemicals possess pharmacokinetic properties that need to be considered for their potential pharmaceutical applications.

Conclusion

In this study, a comparative computational analysis of three major phytochemicals from *Costus speciosus* (Sri Lankan Thebu) revealed that diosgenin is the most thermodynamically stable molecular structure, with a Gibb's free energy of formation of -8.03×10^5 kcal/mol, compared to costunolide and eremanthin. The molecular docking studies demonstrated that diosgenin exhibited the strongest potential as a natural antidiabetic agent by inhibiting four key enzymes (human pancreatic α-amylase, human intestinal α-glucosidase, human protein tyrosine phosphatase 1B and human inducible nitric oxide synthase), with docking scores ranging from -9.33 to -10.43 kcal/mol. This was further supported by its relatively lower Ki values (ranging from 0.023 to 0.146 μM) with these four key enzymatic proteins. While both costunolide and eremanthin showed similar binding affinities with four enzymes, they were less significant compared to diosgenin. *In silico* analysis of secondary interactions revealed that diosgenin exhibited the highest degree of bond strength, specificity and proper orientation within the protein-binding pockets of all four diabetes-regulating enzymes. In comparison, eremanthin demonstrated greater stability only with α-glucosidase and inducible nitric oxide synthase. The standard drug, acarbose showed binding affinities in the range of -7.44 to -8.73 kcal/mol with these enzymes. Therefore, the enhanced binding affinity of diosgenin underscores its potential as a lead compound in the fight against diabetes. Pharmacokinetic analysis based on Lipinski's rule indicated that diosgenin has higher lipophilicity and membrane permeability (log P value of 5.01) compared to costunolide and eremanthin. *In silico* ADMET analysis revealed that all three phytochemicals diosgenin, costunolide and eremanthin demonstrated significantly higher absorption features, with a bioavailability value of 0.55, compared to acarbose. Toxicity analysis indicated that diosgenin,

TABLE-4
ANALYSIS OF ADMET PROPERTIES OF 3 PHYTOCHEMICALS AND THE STANDARD DRUG

Ligand (phytochemical)	Diosgenin	Costunolide	Eremanthin	Acarbose
Molar refractivity (cm^3/mol)	121.59	69.85	67.74	136.69
TPSA ($Å^2$)	38.69	26.30	26.3	321.17
Lipinski's rule	Yes	Yes	Yes	NO
Bioavailability	0.55	0.55	0.55	0.17
Gastrointestinal absorption	High	High	High	Low
Blood-brain barrier (BBB)	Yes	Yes	Yes	Yes
Toxicity class	6	5	4	6
LD_{50} (mg/kg)	8000	3140	1330	24000
Log Kp (skin permeation in cm/s)	-4.08	-6.23	-5.88	-6.18

with an LD_{50} value of 8000 mg/kg, belongs to toxicity class 6 (the same as acarbose), suggesting the lowest toxicity risk when compared to costunolide and eremanthin. This study concludes that diosgenin holds promise as an effective pharmaceutical agent for the treatment of type-2 diabetes mellitus.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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