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Computational Studies on *Diospyros Ebenum* and *Oldenlandia Umbellata* Phytochemicals as Novel Fatty Acid Synthase Inhibitors

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Abstract

Obesity is a global health concern associated with severe diseases, including atherosclerosis, osteoarthritis, coronary artery disease, and cerebrovascular disorders. These conditions pose significant risks such as restricted blood flow, coronary heart disease, and mortality. Fatty acid synthase (FAS), a key enzyme in lipogenesis, plays a pivotal role in fat accumulation and is a primary target for therapeutic intervention. Orlistat, a widely used drug that targets and inhibits fatty acid synthase activity to treat obesity; however, prolonged use may result in unintended health issues. This study aimed to identify natural compounds from *Diospyros ebenum* (Ceylon ebony) and *Oldenlandia umbellata* (chay root) that inhibit fatty acid synthase. Through comprehensive *in silico* analyses, including ADMET studies, toxicity profiling, pharmacophore analysis, ligand-receptor interactions, and molecular simulations, we found phytochemicals from these plants to be non-toxic with favourable pharmacological properties. Molecular interaction studies demonstrated efficient binding to the fatty acid synthase site, suggesting potential for inhibiting enzyme activity and reducing fat accumulation. In conclusion, *Oldenlandia umbellata* exhibited greater efficacy compared to *Diospyros ebenum*, suggesting its ability as a promising source of anti-obesity agents. These findings offer valuable insights for further experimental investigation.

Keywords: *Diospyros ebenum, Oldenlandia umbellata,* Anti-obesity, Lipogenesis, Fatty acid synthase, Molecular docking

Introduction

Obesity, characterized by an imbalance between energy intake and expenditure resulting in excess fat accumulation, has become a global public health concern affecting nearly one-third of the world's population. This condition, associated with metabolic disorders such as hypertension, dyslipidemia, and hyperglycemia, poses a significant risk for diseases like diabetes, cardiovascular disorders, and cancer. The escalating prevalence of obesity underscores the urgency to address its detrimental health effects. While surgical weight loss interventions effectively reduce morbidity and mortality risk, they may evoke negative emotions. Some anti-obesity drugs exist, but they come with potential side effects. The need for developing safe and efficient weight management treatments to prevent obesity (1) requires a comprehensive understanding of the mechanism.

Adipocytes play a crucial role in maintaining nutritional homeostasis by storing triglycerides (TG) when there's an energy surplus and releasing fatty acids during fasting or periods of heightened energy demand to support other tissues. The process of adipocyte differentiation from multipotent mesenchymal stem cells is guided by specific adipogenic transcription

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factors. Mature adipocytes can expand in size by synthesizing and storing triglycerides through the process of lipogenesis when there is an excess of available nutrients. This facilitates the storage of fat in existing fat cells (2). To maintain physiological conditions, it is essential to control the transcription of genes associated with lipid synthesis and storage. This includes overseeing the activity of crucial genes such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and genes involved in the accumulation of lipids (3).

Orlistat, an FDA-approved weight loss drug available globally, targets fatty acid synthase (FAS), a key enzyme for lipid synthesis and an important anti-obesity drug target (4). By inhibiting FAS, orlistat reduces the breakdown of dietary triglycerides into absorbable free fatty acids and monoglycerides, preventing fat absorption. Although side effects, notably gastrointestinal, such as oily stool, may occur early in treatment, they often diminish as therapy progresses (5). Hence, there is a growing interest in exploring alternative approaches for treating and managing the disease. Natural compounds derived from sources such as plants are gaining attention due to their rich repository of structurally diverse metabolites, holding potential applications ranging from medicine to industry(6). We computationally screened phytochemicals from medicinal plants *Diospyros ebenum,* (*D.ebenum*) and *Oldenlandia umbellata* (*O.umbellata*) to assess their potential to inhibit the activity of the enzyme FAS. We compared their efficacy with the standard drug, orlistat, to analyze their inhibitory capabilities.

D. ebenum, traditionally used for treating diarrhoea and dyspepsia, possesses leaves with diuretic, laxative, carminative, and styptic properties, making them beneficial for digestive and wound-healing conditions. Additionally, its dried flowers have been employed in treating urinary and skin infections, showcasing hepatoprotective and antidiabetic properties(7). In Indian Siddha medicine, *O.umbellata* is traditionally employed to address health issues such as bronchitis, asthma, tuberculosis, and hemoptysis. Scientific research has highlighted its pharmacological effects, revealing potential antibacterial, anti-inflammatory, antipyretic, hepatoprotective, antioxidant, and antitussive activities(8).

Utilizing computational screening expedites drug discovery by efficiently analyzing extensive compound libraries, predicting ligand properties, and pinpointing potential therapeutics. Recent advances in ligand discovery enhance the development of safer and more effective small-molecule treatments. Employing computational technologies enables cost-effective and rapid identification of diverse, potent, and target-specific ligands, transforming the landscape of drug discovery. Molecular docking and simulation play crucial roles, enabling prediction of molecular interactions, aiding in drug candidate identification, and advancing our understanding of biochemical processes for novel treatments (9,10)

Evaluating the inhibitory binding potential of phytochemicals from *D. ebenum* and *O. umbellata* on FAS activity is vital for assessing their role as anti-obesity agents. Understanding their impact on FAS activity through docking and simulation is crucial for devising innovative therapeutic strategies against obesity-related conditions. The study aims to employ computational methods to assess these phytochemicals, comparing their efficacy with orlistat. The hypothesis suggests that these natural compounds may offer safer and more effective options for obesity management. Additionally, the research seeks to identify bioactive compounds from these plants for potential development into anti-obesity drugs. Through computational approaches, this research helps to gain valuable insights into the effectiveness of *D. ebenum* and *O. umbellata* phytochemicals in binding to FAS to prevent fat accumulation and manage obesity. This exploration aims to contribute significantly to the understanding and potential treatment of obesity-related disorders, leveraging the therapeutic potential of natural compounds.

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Materials and Methods

Target and Ligand Identification

The enzyme FAS plays a pivotal role as the limiting factor in triglyceride biosynthesis, establishing it as a significant therapeutic target endorsed by the World Health Organization (WHO). This research leveraged the Protein Data Bank (PDB), an openly accessible online structure database, to obtain both the sequence and structural details of the protein (11). After this, a comprehensive exploration of literature and the natural phytocompounds database led to the identification of various bioactive and phytochemical compounds present in the medicinal plants, *D. ebenum* and *O. umbellata*. Essential information such as monoisotopic mass, molecular formula, and other relevant details concerning these compounds was sourced from PubChem (12).

Analysis of ADMET descriptors

To evaluate the druggability of the compounds, it is essential to study their absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. The ADMET descriptors algorithm in Discovery Studio software was used to analyze the ADMET properties of the phytocompounds. This includes models to predict human intestinal absorption based on polar surface area and partition coefficient values, aqueous solubility levels based on a regression model, and blood-brain barrier penetration with prediction levels defining logBB ranges. Additionally, the software incorporates confidence ellipses to model intestinal absorption relative to physicochemical parameters. This comprehensive approach aids in evaluating the drug's suitability for clinical trials by analyzing its pharmacokinetic characteristics (13). Predicting these factors helps determine whether the molecule interacts with the receptor flexibly or rigidly (14).

In silico predictive toxicology assessments

The TOPKAT (Toxicity Prediction Komputer Assisted Technology version) module within Discovery Studio was utilized to assess various properties, including rodent carcinogenicity, Ames mutagenicity, developmental toxicity potential, rat oral median lethal dose (LD_{50}), biodegradability, and skin sensitization, for all potential compounds. The model selected compounds that met the validation criteria for the query, and the results were documented. These pharmacological properties played a crucial role in the selection of appropriate drug candidates aimed at reducing the activity of FAS(15).

Pharmacophore analysis for drug discovery

A pharmacophore model was generated for the selected compounds. The pharmacophore mapping module of the Discovery Studio 2016 software was employed to analyze a chemical library consisting of bioactive compounds from plants and synthetic drugs. (16). This model, derived from the structural features of an active compound, guided the filtration and screening of molecular databases. The effective pharmacophore incorporated crucial ligand properties, including hydrogen bond acceptors, hydrogen bond donors, hydrophobicity, and aromaticity (17).

Molecular docking

Molecular docking and visualization studies were conducted using the LibDock program in Discovery Studio. LibDock, a high-throughput algorithm by Diller and Merz, employs polar and apolar features as "Hotspots" for flexible docking. The protocol, utilizing the CHARMm force field, allowed ligands to be docked and scored with default parameters. A 2D diagram was generated to identify interacting residues. Ligands were prepared with three-dimensional coordinates for docking analysis (18,19).

Molecular simulation

The ligand-protein interaction dynamics were assessed to identify active amino acid residues at the FAS binding site. Using the CABS Flex 2.0 server, coarse-

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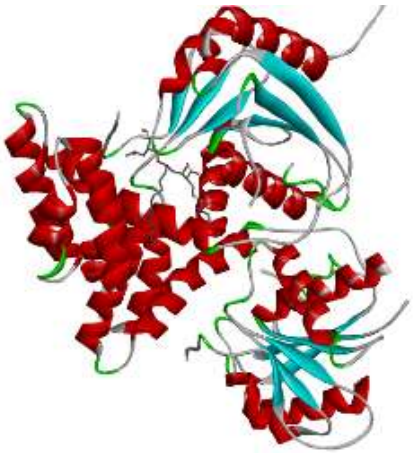

Figure 1: The complex of Drug orlistat and the catalytic domain (thioesterase) of human FAS

grained simulations were performed to analyse the protein's stable structure. Parameters included 50 cycles, a global weight of 1.0, and a temperature of 1.4. RMSF values, reflective of protein flexibility, were recorded over a 10 ns simulation to evaluate the conformational stability of the protein and the protein-ligand complex (20,21).

Result and discussion

Target Selectionand Ligand Identification

Fatty acid synthase (FAS), a key enzyme in lipogenesis, was investigated using UniProt for basic information. The crystal structure (PDB ID: 2PX6) of the thioesterase domain of human FAS, inhibited by Orlistat, was obtained from the PDB, offering valuable structural insights shown in Figure 1 (22).

A total of 30 bioactive compounds were sourced, with 14 from *D. ebenum* and 16 from *O. umbellata*, obtained from the IMPPAT database and extensive literature review (Table 1). These compounds exhibited diverse bioactivities, encompassing proteins, lipids, carbohydrates, alkaloids, sterols, terpenoids, polypeptides, flavonoids, and saponins. Previous research confirms the bioactivity of these phytochemicals, underscoring their potential medicinal and therapeutic applications (23, 24). The drug Orlistat is also chosen for the comparative study. The identified bioactive components were assessed for drug development cues. Further evaluation included ADMET properties, toxicity analysis, and pharmacophore examination to determine the most promising candidates for drug development to manage obesity.

Integrating ADMET in Drug Discovery and Development

In drug discovery, assessing the compound's absorption, distribution, metabolism, excretion, and toxicity (ADMET) is pivotal. An ideal drug candidate should not only exhibit efficacy against the therapeutic target but also possess favourable ADMET properties at a therapeutic dose. Table 1 details the ADMET results for the bioactive compound and the drug Orlistat, while Figure 2 depicts the polar surface area (2D PSA) and AlogP plots. Predictions based on 2D PSA and AlogP, with 95% and 99% confidence ellipses, determine intestinal absorption and blood-brain barrier penetration in the ADMET study. All molecules demonstrate excellent human intestinal absorption (HIA) and aqueous solubility, emphasizing their potential as well-absorbed compounds with good absorption characteristics in aqueous media (25). The pharmacokinetic properties of the studied molecules were assessed using six predetermined ADMET models in Discovery Studio. Optimal cell permeability criteria included PSA < 140 Å2 and AlogP98 < 5, as per the model (11). Prior research on ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) studies has disclosed valuable information about ADMET descriptors along with their corresponding rules and keys. When assessing the druggability of compounds, an in-depth evaluation of their ADMET properties (absorption, distribution, metabolism, excretion, and toxicity) is essential. These

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Table 1: ADMET profiling of the compounds present in *D. ebenum*, *O. umbellata,* and the drug orlistat

D. ebenum								
Compounds	ADMET solubility	ADMET solubility level	ADMET absorption level	ADMET AlogP98	ADMET BBB level	ADMET PPB	ADMET PSA 2D	Molecular weight (g/mol)
Ellagic acid	-3.452	3	1	1.584	4	<90%	135.723	302.19
1,4-Naphthoquinone	-2.748	3	0	1.757	2	<90%	34.601	302.19
Elliptinone	-4.987	2	0	3.612	4	<90%	110.834	158.15
Ebenone	-5.194	2	0	4.391	4	<90%	97.048	442.72
Betulin	-7.151	1	1	6.312	0	<90%	41.631	442.72
Ursolic acid	-7.617	1	1	6.492	4	≥90%	58.931	456.7
Betulinic acid	-7.567	1	2	6.546	4	<90%	58.931	412.69
Alpha-Amyrin	-8.808	0	3	7.349	4	≥90%	20.815	426.72
Bauerenol	-8.807	0	3	7.349	4	≥90%	20.815	360.36
Lupeol	-8.757	0	3	7.403	4	<90%	20.815	426.72
Alpha-Amyrenone	-9.625	0	3	7.449	4	≥90%	17.3	424.7
Stigmasterol	-7.963	1	3	7.639	4	≥90%	20.815	382.71
Beta-Sitosterol	-8.256	0	3	8.084	4	≥90%	20.815	426.72
1-Hexacosanol	-7.284	1	3	11.007	4	≥95%	20.815	374.34
O.umbellata								
Scan-doside	-0.096	4	3	-3.326	4	<90%	189.799	594.52
scandoside methyl ester	0.013	5	3	-3.1	4	<90%	177.914	390.34
Asperulosidic acid	-0.551	4	3	-2.947	4	<90%	195.214	123.11
Deacetylasperuloside	-0.066	4	3	-2.742	4	<90%	157.098	432.38
Nicotiflorin	-5.098	2	3	-0.916	4	<90%	249.29	208.21
Ruberythric acid	-3.244	3	3	-0.842	4	<90%	216.03	240.21
Nicotinic acid	-0.494	4	0	0.309	3	<90%	49.377	208.21
Purpurin	-2.812	3	0	2.082	3	<90%	97.048	534.47
Alizarin	-3.096	3	0	2.324	3	≥90%	76.232	284.26
1,3-Dimethoxy-2-hydroxy-9,10-anthraquinone	-3.743	3	0	2.533	3	<90%	73.277	254.24
Alizarin 1-methyl ether	-3.654	3	0	2.55	2	≥90%	64.347	260.29
Anthraquinone	-4.15	2	0	2.808	1	≥90%	34.601	256.21

(*Contd.*)

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Table 1: ADMET profiling of the compounds present in *D. ebenum*, *O. umbellata,* and the drug orlistat (*Contd*.)

D. ebenum								
Cedrelopsin	-4.058	2	0	3.497	1	<90%	55.976	372.32
Pheophorbide A methyl ester	-5.564	2	1	4.023	4	<90%	120.057	404.37
Ursolic acid	-7.617	1	1	6.492	4	≥90%	58.931	594.52
Beta-Sitosterol	-8.256	0	3	8.084	4	≥90%	20.815	606.71
Drug								
Orlistat	-6.299	1	3	8.333	4	≥95%	82.572	495.7

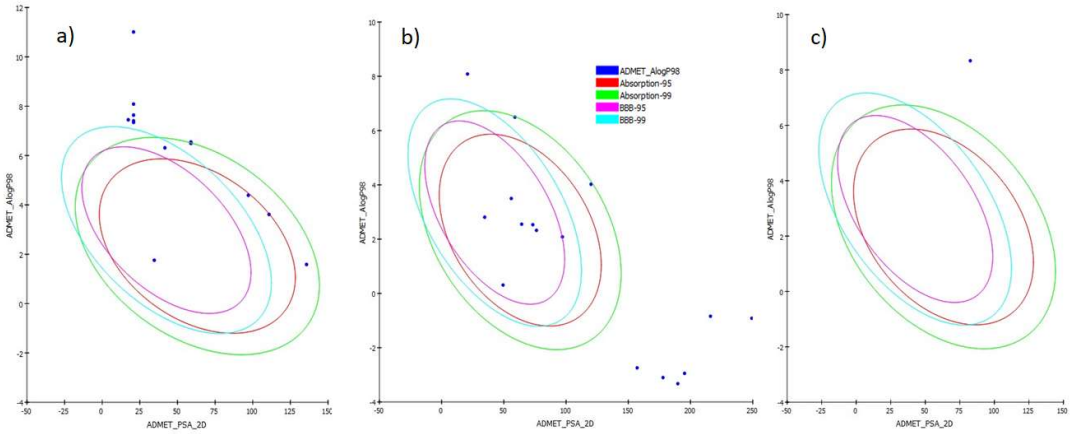

Figure 2: ADMET Description plot a) *D.ebenum* compounds b) *O.umbellata* compounds c) Drug Orlistat

properties profoundly impact the efficacy, safety, and pharmacokinetics of a drug, playing a crucial role in determining its clinical success. Selecting specific ADMET parameters and thresholds for compound evaluation requires careful consideration of several factors.

Absorption, the first consideration, predicts a drug's ability to enter systemic circulation post-administration. Parameters like human intestinal absorption (HIA), based on factors such as polar surface area and partition coefficient values, provide insights into a compound's ability to traverse biological membranes. Determining appropriate thresholds for HIA levels aids in identifying compounds with optimal absorption potential, thus enhancing the likelihood of achieving therapeutic concentrations in systemic circulation.

Aqueous solubility, the second factor, is vital for drug formulation and bioavailability. A compound's solubility level, determined through regression models, dictates its dissolution behavior and suitability for oral administration. Setting thresholds for solubility levels ensures that chosen compounds possess adequate aqueous solubility for effective drug delivery.

The ability to penetrate the blood-brain barrier (BBB) is crucial in central nervous system (CNS) drug development. Predictions of BBB permeability, typically based on logBB values, assess a

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compound's capability to penetrate the BBB and reach CNS targets. Establishing thresholds for logBB ranges assists in selecting compounds with desired CNS activity while minimizing the risk of adverse effects.

Protein binding (PPB) predictions estimate the fraction of a compound bound to plasma proteins, impacting its distribution and pharmacokinetics. Compounds with moderate PPB levels are preferred to ensure sufficient free drug concentrations for therapeutic effects.

Moreover, molecular properties such as polar surface area (PSA) and molecular weight also influence a compound's ADMET profile. PSA descriptors reflect a compound's potential for hydrogen bonding and membrane permeability, while molecular weight affects absorption, distribution, and metabolism. In conclusion, selecting specific ADMET parameters and thresholds requires balancing desirable pharmacokinetic properties with safety considerations. These criteria play a crucial role in prioritizing compounds with optimal ADMET profiles for further drug development and clinical evaluation(26,27).

Out of the 30 examined compounds from *D. ebenum* and *O. umbellata*, Notably, 1,4-naphthoquinone from *D. ebenum* and compounds such as Nicotinic acid, Alizarin,Alizarin 1-methyl ether, 1,3-Dimethoxy-2-hydroxy-9,10-anthraquinone, Anthraquinone, Dasycarpidan-1-methanol, acetate, Cedrelopsin, E-11-Methyl-12-tetradecen-1-ol acetate from *O. umbellata* exhibited satisfactory ADMET properties. Notably, the commonly used drug Orlistat demonstrated suboptimal ADMET characteristics. Consequently, only the nine bioactive compounds, along with Orlistat, were selected for toxicity evaluation. These compounds adhered to drug-likeness guidelines, underscoring their potential suitability for oral medication.

Evaluating TOPKAT profiling

The safety efficacy of the selected bioactive compounds was comprehensively examined in this study through various toxicity indicators, including Ames mutagenicity (AMES), rodent carcinogenicity (based on the Food and Drug Administration (FDA) dataset),biodegradability, skin sensitization and developmental toxicity potential properties, were assessed using the TOPKAT module of Discovery Studio. The TOPKAT carcinogenicity predictor estimated the probability of carcinogenicity in the range of 0.000 – 0.006. Particularly Cedrelopsin and Alizarin 1-methyl ether exhibited a lack of carcinogenicity, supported by negative discriminant scores and very low probabilities.

The TOPKAT mutagenicity predictor identified some compounds as mutagenic, with positive discriminant scores and probabilities ranging from 0.071 to 9.982 (28, 29). However, the Compounds Cedrelopsin and Alizarin 1-methyl demonstrated nonmutagenic properties. Furthermore, these compounds displayed Rat Oral LD50 values within the Optimum Prediction Space. Considering the collective findings (Table 2), Cedrelopsin and Alizarin 1-methyl ether emerged as ideal lead compounds, exhibiting lower Ames mutagenicity, rodent carcinogenicity, and developmental toxicity potential compared to the established drug Orlistat. This strongly suggests their promising application in drug development. In summary, Cedrelopsin and Alizarin 1-methyl ether were identified as safe drug candidates, and their selection for subsequent pharmacophore, docking, and simulation studies is supported by their favorable safety profiles.

This study identified two novel bioactive compounds - Cedrelopsin, a coumarin, and alizarin-1-methyl ether, an anthraquinone derivative. Previous *in silico* analyses suggested Cedrelopsin holds osteoporosis therapeutic properties, with favorable drug-like properties (30, 31). Moreover, coumarins from many edible plants demonstrate versatile bioactivities including antibacterial, antifungal, antiviral, anti-HIV, and anticancer effects (32). Alizarin-1-methyl ether also exhibited potent antifungal activity against human pathogens (33,34). Anthraquinones

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likewise demonstrate diverse bioactivities encompassing anticancer, anti-inflammatory, antimicrobial, diuretic, vasorelaxant, and phytoestrogen effects, suggesting possible clinical utility for various diseases (35). In summary, these novel compounds have an array of biological properties spanning antibacterial to cytotoxic that warrant further probing to fully determine their therapeutic potential. Elucidating the mechanisms underlying their bioactivities could uncover new treatment approaches for treating obesity. This computational study provides a robust way to explore their pharmacological activity experimentally.

Pharmacophore findings

Toxicity screening revealed two phytochemicals, Cedrelopsin, and alizarin 1-methyl ether, as promising hit compounds for further evaluation as FAS inhibitors. This study leveraged pharmacophore modeling to identify compounds with molecular features conferring anti-obesity bioactivity through FAS binding. The pharmacophore features of a compound play a crucial role in predicting its interactions with the target. Heterocyclic compounds, integral in drug design, are found in over 90% of commercially available drugs, showcasing their broad therapeutic applications. The presence of specific

Table 2: The toxicological assessment of compounds using Discovery Studio

Toxicity prediction									
Source of compounds	Compounds	Ames mutagenicity	FDA carcinogenicity (female mouse)	FDA carcinogenicity (male mouse)	Biodegradability	FDA carcinogenicity female rat	FDA carcinogenicity male rat)	Rat oral LD_{50}	Skin sensitization
D. ebenum	1,4-naphthoquinone	1	1	0.393	1	0.001	1	190 mg/kg	0.998
O. umbellata	Nicotinic acid	0	0	0	1	0	0	1.8g/kg	0
	Alizarin	1	0	1	0	0	1	2.1g/kg	1
	Alizarin 1-methyl ether	0.998	0.001	0.951	0	0.001	0.951	5.7 g/kg	0.005
	1,3-Dimethoxy-2-hydroxy-9,10-anthraquinone	1	0	0.645	0.022	0	0.645	7.9 g/kg	0.008
	Anthraquinone	0.001	1	1	0.982	1	1	3.5 g/kg	1
	Dasycarpidan-1-methanol, acetate	0	1	1	0	1	1	25.9mg/kg	1
	Cedrelopsin	0	0.152	0	0.976	0.152	0	5.7 g/kg	0
	E-11-Methyl-12-tetradecen-1-ol acetate	0.993	1	0.936	1	1	0.936	10 g/kg	0
Drug	Orlistat	1	1	0	0	1	0	3.5 g/kg	0

Diospyros ebenum and *Oldenlandia umbellata* Phytochemicals

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functional groups or pharmacophores in a compound's structure often correlates with its toxicity (36).The geometric parameters within a 3D pharmacophore model delineate properties essential for target affinity. Calculated x, y, and z coordinates defining the spatial arrangement of key chemical features necessary for a molecule's interaction with a target protein (15,37). Moreover, distinguishing hydrophilic from hydrophobic points through the scrutiny of surrounding protein atoms, based on hydrogen-bond donating or accepting protein heavy atoms within a 3 Å radius, aligned with the typical distances observed in protein-ligand complexes where hydrogen bonds form between two heavy atoms (38). This approach provides valuable insights into the structural characteristics required for effective target interaction, laying the groundwork for the development of novel therapeutics in Obesity. In essence, the findings highlight the potential of Cedrelopsin and Alizarin 1-methyl ether to treat FAS-related medical conditions.

Table 3 and Figure 3 illustrate the pharmacophore features of the selected compounds, encompassing hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), aromatic rings, and hydrophobic features. These features align with observations on Orlistat, a known FAS inhibitor. Ultimately, both hit compounds underwent docking studies against the FAS active site, setting the stage for further exploration of their potential as effective FAS inhibitors.

Exploration of Molecular Binding Sites

The study investigated the inhibitory efficacy of ligands by focusing on their interaction sites with FAS. A detailed examination identified the active site of FAS, and the nature of the interaction between Orlistat and FAS was determined using Discovery Studio 2D interaction analysis and literature review. The amino acid residues in the active site of FAS contain ARG 2482, SER 2308, TYR 2343, ALA 2419, HIS 2481, LEU 2222, SER 2308, GLU 2251, THR 2255, VAL 2256, THR 2307, SER 2221, PRO 2249, ASP 2338, PHE 2370, PHE 2371, LYS 2426, SER 2422, GLY 2339, TYR 2309, THR 2342, SER 2340, ILE 2250, GLN 2374, PHE 2423, PHE 2375 (20).Noteworthy findings revealed that Orlistat exhibited a substantial interaction with the active site, forming three hydrogen bonds with amino acids ARG 2482, SER 2308, and TYR 2343. Additionally, alkyl bond interactions were discerned with LEU 2222, HIS 2481, TYR 2343, and ALA 2419. These findings help to design novel FAS inhibitors that leverage similar residue interactions to effectively bind the activation site and reduce enzymatic activity for therapeutic effect Table 4.

Molecular docking for drug discovery and design

In the molecular docking study targeting FAS, the filtered compounds, Cedrelopsin and Alizarin 1-methyl ether, demonstrated notable binding affinity as indicated by LibDock scores. The docking results exhibited favorable interactions, including hydrogen bonds and hydrophobic contacts within the binding site of FAS, indicating the potential for stable complex formation (Figure 4). Notably, multiple configurations were observed during docking, each characterized by a combined score encompassing Vander Waals forces, H-bonds, pi interactions, and other parameters in the form of LibDockscores (16).

Comparison of the docking scores of Cedrelopsin and Alizarin 1-methyl ether with the control drug Orlistat revealed promising results, suggesting their potential as FAS inhibitors. These findings offer molecular insights into the interaction mechanisms of the bioactive compounds with the target, providing a foundation for understanding their mode of action. The presence of hydrogen bonds in the docked complexes implies a role in stabilizing the interactions, contributing to conformational stability and, consequently, significant inhibitory activity against FAS. In summary, the molecular docking results suggest that Cedrelopsin and Alizarin 1-methyl ether may serve as potential inhibitors of FAS, paving the way for further exploration in drug development.

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Table 3: Pharmacophore features (Hydrogen donor, Hydrogen acceptor, Aromatics, and Hydrophobic) of the compounds

Compounds					
	Pharmacophore features	X	Y	Z	Radius
Cedrelopsin	HB_ACCEPTOR1	3.3	-4.56	2.72	2.2
	HB_ACCEPTOR2	5.08	-3.04	0.1	2.2
	HB_ACCEPTOR3	3.84	2	-0.06	2.2
	HB_ACCEPTOR4	-5.84	3.26	-0.16	2.2
	HB_ACCEPTOR5	-6.68	0.34	-0.08	2.2
	HB_DONOR1	5.08	-3.04	0.1	2.2
	HB_DONOR2	3.84	2	-0.06	2.2
	RING_AROMATIC1	0.307641	-1.46434	3.01502	2.2
	RING_AROMATIC2	0.355359	-1.62333	-2.98269	2.2
	Hydrophobic1	2.72	-4.04	-1.18	1.6
	Hydrophobic2	-1.08	3.36	0.9	1.6
	Hydrophobic3	0.44	-2	0.02	1.6
Alizarin 1-methyl ether	HB_ACCEPTOR1	2.92	-3.1	2.96	2.2
	HB_ACCEPTOR2	0.7	-5.26	0.48	2.2
	HB_ACCEPTOR3	-2.02	5.76	-0.42	2.2
	HB_ACCEPTOR4	6.62	2.26	0.06	2.2
	HB_ACCEPTOR5	5.26	-2.74	0.42	2.2
	HB_DONOR1	6.62	2.26	0.06	2.2
	HB_DONOR2	5.26	-2.74	0.42	2.2
	RING_AROMATIC1	1.88724	0.650384	-2.9348	2.2
	RING_AROMATIC2	-3.10253	-0.129466	2.99651	2.2
	RING_AROMATIC3	1.71709	1.08328	3.04714	2.2
	RING_AROMATIC4	-2.92913	-0.564534	-2.98518	2.2
	Hydrophobic1	2.76	-2.5	-0.96	1.6
	Hydrophobic2	-3.86	-0.58	0	1.6
	Hydrophobic3	2.56	1.58	0	1.6
Orlistat	HB_ACCEPTOR1	8.38	-1.34	2.22	2.2
	HB_ACCEPTOR2	8.92	4.5	2.46	2.2
	HB_ACCEPTOR3	3.18	-2.52	-0.92	2.2
	HB_ACCEPTOR4	5.98	-2.96	0.16	2.2
	HB_ACCEPTOR5	2.06	0.28	-1.08	2.2
	HB_ACCEPTOR6	13.16	1.26	-3.66	2.2
	HB_ACCEPTOR7	14.88	3.3	-2.26	2.2
	HB_ACCEPTOR8	18.96	3.82	-0.64	2.2
	HB_ACCEPTOR9	16.76	1.82	-0.92	2.2

(*Contd.*)

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Table 3: Pharmacophore features (Hydrogen donor, Hydrogen acceptor, Aromatics, and Hydrophobic) of the compounds (*Contd.*)					
Compounds					
	HB_ACCEPTOR10	18.36	6.66	0.24	2.2
	HB_DONOR1	14.54	1.18	-0.76	2.2
	Hydrophobic1	19.34	-8.42	0.06	1.6
	Hydrophobic2	18.04	-6.3	-0.02	1.6
	Hydrophobic3	14.92	-3.82	0.16	1.6
	Hydrophobic4	11.96	-0.86	0.02	1.6
	Hydrophobic5	-1.9	3.7	0.04	1.6
	Hydrophobic6	2.26	3.22	0.26	1.6
	Hydrophobic7	10.48	6.62	0.62	1.6

Table 4: Molecular docking of FAS with *D.ebenum,* and *O.umbellata* compounds

Molecule	Absolute energy	Relative energy	Libdock score	No. of H-bond & interaction	Other bond interaction	No. of Carbon hydrogen bond & interaction
Cedrelopsin	42.6263	12.6795	69.5082	3 OH-HIS 2481, O-TYR 2343, O-ARG 2482	6 GLU 2251, ARG 2482, TYR 2307, VAL 2256	1 H-GLU 2251
Alizarin 1-methyl ether	48.3457	3.04866	52.8854	3 O-TYR 2343, O-SER 2308, O-TYR 2307	6 PRO 2249, HIS 2481, ILE 2250, GLU 2251	1 H-GLU 2251
Orlistat	67.4006	8.26036	144.714	3 O-ARG 2482, O-SER 2308, O-TYR2343	4 LEU 2222, HIS 2481, TYR 2343, ALA 2419	2 H-2308, H-2343

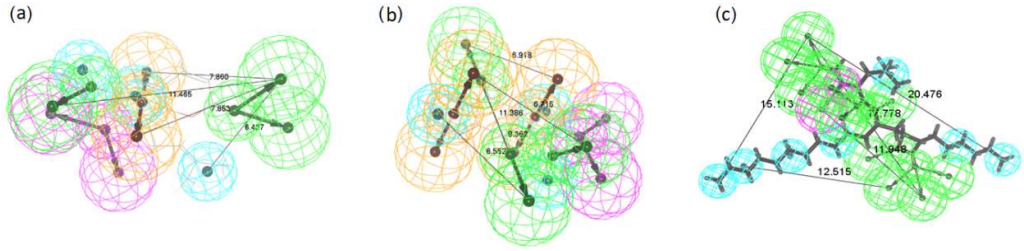

Figure 3: Pharmacophore model of FAS inhibitors (a) Cedrelopsin, (b) Alizarin 1-methyl ether, and (c) Orlistat mapping. Pharmacophoric features are represented as follows: hydrogen bond acceptor (green), hydrogen bond donor (magenta), hydrophobic (cyan), and ring aromatic (orange)

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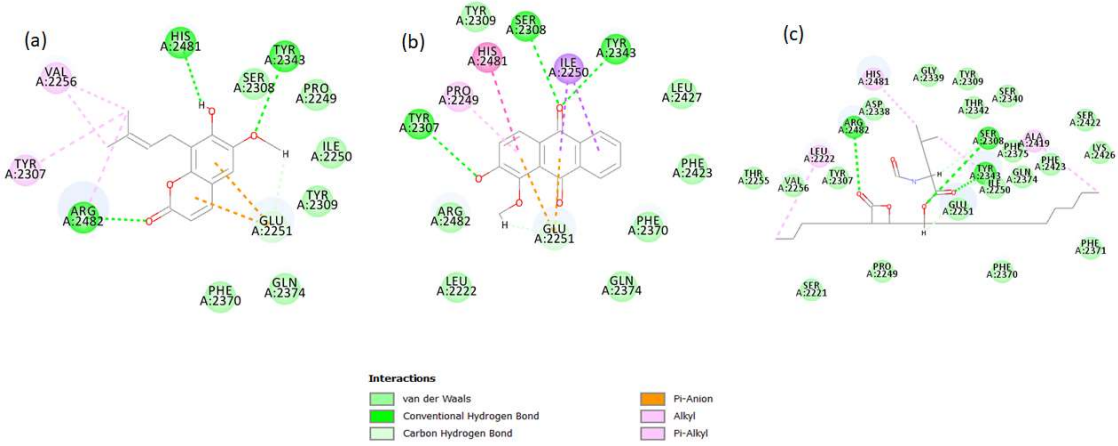

Figure 4: 2D interactions of enzyme FAS with a) Cedrelopsin b) Alizarin 1-methyl ether c) Orlistat

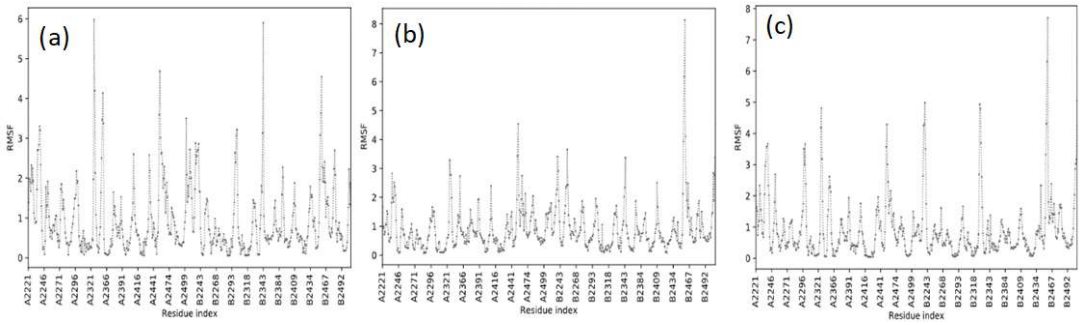

Figure 5: Value of the root mean square fluctuation (RMSF) plot of the compounds and FAS complex a) Cedrelopsin b) Alizarin 1-methyl ether c) Orlistat

Molecular dynamics analysis using cabs-flex 2.0

The conformational stability of FAS and its complexes with phytochemical ligands was assessed using CABS-flex. The dynamics of ligand-protein interactions were explored to identify key amino acid residues involved. Specifically, the analysis focused on a potent compound from previous docking studies, including Cedrelopsin, Alizarin 1-methyl ether, and the FDA-approved drug Orlistat. Results revealed that the Root Mean Square Fluctuation (RMSF) value of FAS, when bound to Alizarin 1-methyl ether and Orlistat, was notably high at 8 Å, decreasing to below 6 Å when bound to Cedrelopsin (Figure 5). The reduced RMSF values suggest stable interactions in the latter complexes, indicating favorable conformational stability and highlighting the potential of Cedrelopsin as a stabilizing agent for FAS.

An examination of the RMSF values reveals that the amino acid residues at the termini of the protein demonstrated high flexibility with RMSF values above the average of 3.0 Å. In contrast, the majority of the interacting residues involved in binding displayed RMSF values under 2.0 Å, underscoring their comparative stability during the 10 nanosecond molecular dynamics simulation. Not with standing, a few

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critical binding residues near positions 2467, 2343, and 2321 showed some flexibility with RMSF values over 2.0 Å. Similarly, in its unbound state, the terminal residues exhibited higher RMSF values than the central region of the protein. Meanwhile, the changing RMSF values of the ligand signify ligand continuously altered its binding pose in an attempt to find a more stable configuration (39). The MD simulations affirmed the structural flexibility observed in the NS2B/NS3-taraxerol docking complex and NS5–apigenin complex, with RMSF values consistently 5 Å. This dynamic behavior aligns with findings from prior studies, highlighting the importance of considering structural flexibility in understanding protein-ligand interactions (40). The RMSF analysis uncovered significant fluctuations in the protein/peptide complexes, with SERPINI1 (serine proteinase inhibitor 1) exhibiting the most noteworthy fluctuation at 8.807 Å, observed at residue no. 353. These findings align with earlier studies that similarly emphasized the importance of understanding protein flexibility for elucidating functional implications. The observed diverse structural dynamics in the complexes contribute valuable insights into their flexibility, potentially influencing their biological functions (41).

The findings of this study align with previous research on FAS inhibitors and anti-obesity agents derived from natural sources. Specifically, the identification of organosulfur compounds in garlic, such as Allicin, Alliin, E-Ajoene, and Z-Ajoene, as potential FAS inhibitors resonates with the broader exploration of natural compounds for their anti-obesity properties. The similarity in binding sites between these compounds and statins underscores their potential as alternative therapeutic agents for managing lipid-related disorders (42). Additionally, the study is in line with previous research on FAS inhibitors like curcumin, resveratrol, and EGCG. Moreover, the discovery of citral's anticancer properties through lipogenesis inhibition further reinforces the multifaceted potential of natural compounds in combating obesity and cancer (43). Furthermore, an earlier study introduces rosmarinic acid and its analogs, particularly ZINC85948835, as potential FAS inhibitors targeting the Thioesterase domain. Compared to previous studies, these compounds exhibit promising binding affinities and interactions with the FAS TE domain, suggesting their potential as novel therapeutic agents. In sum, this study expands the repertoire of natural compounds with anti-obesity and anticancer properties, offering new avenues for drug development and highlighting the potential of phytochemicals in combating metabolic and oncological diseases (44, 45).

Currently, no experimental validations have confirmed the predicted interactions between the lead compounds, Cedrelopsin, and Alizarin 1-methyl ether, and FAS. The study suggests further experimental validation to confirm these compounds' efficacy as anti-obesity agents. This typically involves in vitro assays to assess their inhibitory activity against FAS directly. While the earlier study proposed a novel mass spectrometry-based assay for monitoring FAS activity and product specificity, experimental validation of the lead compounds' interactions with FAS is crucial to confirm their inhibitory effects (46). Additionally, in vivo studies may be conducted to evaluate the efficacy of the lead compounds in animal models of obesity, further validating their potential as therapeutic agents.

The present study utilized several *in silico* prediction methods to analyze the oral bioavailability of compounds, which could facilitate the design of novel, safer drugs. Performing preliminary computational analyses enables the prediction of bioavailability before experimental testing, thus saving considerable time and money in drug development. In summary, *O. umbellata* phytochemicals, particularly Cedrelopsin and Alizarin 1-methyl ether, exhibit potent anti-obesity potential by targeting fatty acid synthase, offering non-toxic, efficacious alternatives for managing fatty acid accumulation and preventing obesity.

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Conclusion

In conclusion, the computational analyses into fatty acid synthase (FAS) inhibition highlighted the phytochemicals from *O.umbellata*, Cedrelopsin and Alizarin 1-methyl ether as promising lead compounds with favorable ADMET profiles, low toxicity, and significant binding affinity to FAS. Molecular docking and simulation studies underscored their potential as effective FAS inhibitors, offering insights into their mode of action and conformational stability. These initial *in silico* experiments highlight the potential of leveraging *O. umbellata* compoundsas potential candidates for further exploration in drug development to manage obesity, emphasizing their importance in advancing therapeutic strategies against lipogenesis.

Conflict of interest

The authors have declared that there are no existing conflicts of interest

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