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Molecular insights into volatile compounds from cocoa (*Theobroma cacao* L.) as potential multi-target therapeutic candidates for insulin resistance

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A B S T R A C T

Volatile compounds from cocoa (*Theobroma cacao* L.) represent a relatively unexplored source of potential multi-target agents for insulin resistance. This study aimed to evaluate volatile compounds from various cocoa tissues as potential multi-target interventions in insulin resistance using an integrative in silico pharmacology approach. The study involved screening compounds based on drug-likeness, predicted bioactivity, toxicity, and membrane permeability, followed by network pharmacology, gene ontology and functional annotation, molecular docking, and molecular dynamics simulations. In this study, three key target proteins related to glucose regulation, lipid metabolism, and insulin signaling were used. From an initial set of 87 volatile compounds in cocoa leaves, pods, and seeds, 48 unique structures were curated for in silico evaluation. Stepwise screening identified 12 compounds meeting drug-likeness criteria, and four compounds were ultimately selected as the most promising candidates for further structural analysis: methyl decanoate, methyl octanoate, methyl 10-methylundecanoate, and lauric acid. Molecular docking analysis revealed target-dependent ligand-protein interactions, with methyl 10-methylundecanoate showing the most favorable predicted affinity for PTP1B and PPARA, with binding energies of -5.72 and -4.68 kcal/mol, respectively, while lauric acid showed the strongest affinity for HSD11B1, with a binding energy of -4.70 kcal/mol. Further molecular dynamics simulations demonstrated that the predicted protein-ligand complexes maintained structural stability. Overall, these findings suggest that cocoa-derived volatile compounds may serve as promising lead candidates for multi-target interventions in insulin resistance. However, further in vitro, in vivo, and target-specific validation studies are needed to confirm the biological relevance of these computational findings.

Keywords: Bioactive; drug design; metabolite; phytochemical; docking

1. INTRODUCTION

Insulin resistance (IR) is a physiological state where target tissues show a reduced response to insulin at normal levels. As a result, tissues that usually respond to insulin need more of it to work properly (Lee et al, 2022). IR is a key factor in the development and progression of many metabolic disorders. These include dyslipidemia, type 2 diabetes mellitus (T2DM), hypertension, non-alcoholic fatty liver disease, cardiovascular and cerebrovascular diseases, and cancer (Mir et al., 2025). At the molecular level, the pathogenesis of insulin resistance is strongly driven by systemic inflammation, lipotoxicity, and oxidative stress, which collectively disrupt intracellular insulin signaling cascades (Mir et al., 2025; Zhao et al., 2023).

Furthermore, insulin resistance lies at the core of metabolic syndrome, whose global prevalence ranges from approximately 14–39% and is projected to continue rising as Western diets and sedentary lifestyles become more widespread (Islam et al., 2024; Obeidat et al., 2025). The global surge in diabetes and obesity also reflects the

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magnitude of the insulin resistance problem. In line with this trend, the number of diabetes cases is projected to continue rising, reaching approximately 700 million by 2045 (Klein et al., 2022; Mir et al., 2025). The considerable impact of this disease highlights the importance of understanding insulin resistance, not only from an epidemiological perspective but also by studying its underlying molecular processes.

Dysregulation of cellular signaling pathways, which are crucial for glucose metabolism, insulin homeostasis, and pancreatic cell function, is a significant factor in the pathogenesis of diabetes. These disruptions impede glucose uptake, compromise GLUT-2 functionality, and inflict damage upon β-cells, ultimately resulting in diminished insulin production and secretion. As a result, blood sugar levels increase, which disrupts the body's metabolic balance (Teodhora et al., 2021). Furthermore, key proteins and enzymes, including HSD11B1, PPARA, and PTP1B, contribute to insulin resistance via interconnected mechanisms. Specifically, HSD11B1 augments glucocorticoid activation within the liver and adipose tissue, thereby intensifying gluconeogenesis, lipotoxicity, and systemic insulin resistance (Zhao et al., 2023). Peroxisome proliferator-activated receptor alpha (PPARA) is a crucial transcriptional regulator of lipid metabolism and fatty acid oxidation; consequently, its targeted activation significantly alleviates lipotoxicity and improves systemic insulin sensitivity (Souza-Tavares et al., 2023; Sun et al., 2021). Simultaneously, PTP1B diminishes insulin and leptin signaling by dephosphorylating the insulin receptor and its associated elements, thus contributing to the development of obesity, type 2 diabetes, and related cardiometabolic disorders, while also representing a potential target for therapeutic intervention (Coronell-Tovar et al., 2024).

To manage insulin resistance, standard pharmacological therapy generally begins with metformin. Given its pleiotropic nature, metformin improves insulin sensitivity not only through canonical pathways like AMPK activation but also by interacting with various metabolic protein targets. This multi-target characteristic makes it a standard reference compound in computational pharmacological studies to establish baseline binding affinities. It may be combined with a sulfonylurea, a DPP-4 inhibitor, or an SGLT2 inhibitor (Weinberg Sibony et al., 2023; Yi et al., 2024). Conversely, prolonged administration presents potential adverse effects, specifically gastrointestinal issues, vitamin B12 deficiency, and, in specific scenarios, the possibility of lactic acidosis, thereby mandating the assessment of renal function (Drzewoski and Hanefeld, 2021; Froldi, 2024; Adithya B et al., 2025). These constraints highlight the imperative for the creation of safer therapeutic options, encompassing those derived from natural origins.

Phytochemical investigations utilizing GC–MS have revealed that cocoa tissues harbor diverse bioactive compounds with specific pharmacological potentials, including potent antioxidant, anti-inflammatory, and metabolic-regulating activities that are highly relevant for mitigating insulin resistance (Savira et al., 2024). Furthermore experimental studies have also demonstrated that cocoa ethanol extract can reduce blood glucose levels in experimental animals (Gunawan et al., 2024). Nevertheless, the available data directly demonstrating the capacity of cocoa volatile compounds to combat insulin resistance is scarce, especially concerning their molecular targets and multi-target action mechanisms. Indeed, plant-derived bioactive compounds are recognized for their ability to influence pathways implicated in glucose regulation, insulin sensitivity, oxidative stress, and inflammation (Ononamadu et al., 2025). Therefore, *in silico* methods, such as network pharmacology and molecular docking, provide a way to predict how compounds interact with important molecular targets and to better understand how they work on multiple targets (Niu et al., 2025). As a result, more research is needed to find bioactive compounds that can target multiple proteins and pathways involved in insulin resistance.

This study, therefore, seeks to assess the therapeutic potential of volatile compounds derived from cocoa as multi-target candidates. This study will use an integrative approach, combining network pharmacology and molecular docking methods, to help develop new treatments. The main goal is to develop treatments that are both more effective and safer, thus reducing the chance of unwanted side effects.

2. MATERIAL AND METHODS

2.1 Compilation and curation of cocoa volatile compounds

To construct a comprehensive library of *Theobroma cacao* L. volatile compounds, data were initially compiled from multiple GC-MS identification studies across different plant tissues. Specifically, a total of 20 volatile compounds were recorded from cocoa leaves, as reported by Savira et al. (2024). To broaden the therapeutic evaluation, this dataset was expanded by incorporating 48 compounds from cocoa pods (Chusniasih and Tutik, 2021) and 19 compounds from cocoa beans (Hala et al., 2023), resulting in an initial pool of 87 compounds. To ensure the reliability of the *in silico* screening, this combined dataset was subjected to a strict data curation process by removing duplicate structures across tissues, unresolved isomers, and compounds lacking canonical SMILES identifiers. Following this curation, a finalized library of 48 unique volatile compounds was established. Subsequently, a collection of samples in the form of canonical SMILES codes was gathered via the PubChem (https://pubchem.ncbi.nlm.nih.gov/) web server for further screening (Kim et al., 2025).

2.2 Screening of compounds based on drug likeness, probable bioactivity, toxicity, and compound permeability

The volatile compounds obtained from *Theobroma cacao* L. underwent a secondary selection phase based on their drug-likeness, potential biological activity, toxicity, and compound permeability. The SwissADME web server (https://swissadme.ch/index.php) was used to assess the drug-likeness of each compound. It looked at their physicochemical and pharmacokinetic properties and followed rules set by Lipinski, Veber, and Egan (Anggarani et al., 2025; Daina et al., 2017). Additionally, a bioavailability (BA) score of no less than 0.50, along with favorable gastrointestinal absorption (GIA) outcomes, were incorporated as additional selection criteria (Widyananda et al., 2022). After that, the PASS Online web server (http://www.way2drug.com/passonline/) was used to do probable bioactivity screening to find out what biological activities compounds that might be linked to insulin resistance and metabolic disorders might have (Druzhilovskiy et al., 2017). Following this, compounds that met the drug-likeness criteria and Probable bioactivity were tested for toxicity using the ProTox 3 web server (https://tox.charite.de/protox3/). This made it easier to guess what toxic effects might happen and identify compounds that are safe to use (Banerjee et al., 2024). We also used the PerMM web server (https://permm.phar.umich.edu/) to test the compounds' ability to cross the lipid bilayer (Lomize et al., 2019). Only compounds that met the requirements at all stages of screening were chosen for more study (Setiawan *et al*., 2025).

2.3 Network pharmacology

Network pharmacology was employed to investigate the interactions between multiple proteins and their related biological networks, rather than focusing on individual proteins. This approach allows for a more complete understanding of complex diseases and helps in finding drugs that can target multiple proteins (Zhai et al., 2025). To identify potential protein targets of volatile compounds originating from *Theobroma cacao* L. tissues, Swiss Target Prediction (https://swisstargetprediction.ch/) was utilized to identify direct protein targets based on the structural similarity of the compounds to known compounds (Daina et al., 2019). Furthermore, targets protein to insulin resistance and other metabolic disorders were identified by querying related keywords across diverse public databases, including DisGeNet (https://disgenet.com/), GeneCards (https://www.genecards.org/), and CTD (https://ctdbase.org/), to gather supplementary data concerning the association between protein targets and metabolic conditions (Davis et al., 2023; Hu et al., 2025; Stelzer et al., 2016). Following this, Venn diagrams were employed to identify common targets by mapping the predicted relevant ones, using the Venny 2.1.0 web server (https://bioinfogp.cnb.csic.es/tools/venny/) (Jia et al., 2021). To explore the interactions among the identified targets, we used the STITCH database to find additional protein-protein interactions (Afnani et al., 2026). These interactions were then constructed and analyzed with Cytoscape 3.10.3, facilitating a thorough network mapping and offering insights into the potential therapeutic pathways.

2.4 Gene ontology and functional annotation

Gene ontology (GO) enrichment and functional annotation analyses were performed to elucidate the biological roles and functional characteristics of the identified target proteins associated with insulin resistance. The

overlapping target genes derived from network pharmacology analysis were analyzed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources (https://davidbioinformatics.nih.gov/), with Homo sapiens selected as the reference organism (Huang et al., 2009; Sherman et al., 2022). GO enrichment was conducted across three main categories, namely biological process (BP), molecular function (MF), and cellular component (CC), to provide a comprehensive overview of gene function. In addition, pathway enrichment analysis was carried out to identify key biological pathways potentially involved in the therapeutic effects of *Theobroma cacao* L. volatile compounds. Enrichment results were considered statistically significant at a threshold of $p < 0.05$ (Wang et al., 2022). The resulting GO terms and enriched pathways were subsequently visualized using the SRplot web platform (https://www.bioinformatics.com.cn/en), enabling the generation of clear and informative graphical representations that facilitate the interpretation of the underlying biological mechanisms (Tang et al., 2023).

2.5 Molecular docking

Molecular docking simulations were conducted to assess interactions between chosen compounds and target proteins, specifically focusing on the active sites of each receptor. The three-dimensional protein structures were sourced from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/), and ligand structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Al Attarsyach et al., 2026; Burley et al., 2018). Protein preparation entailed the removal of water molecules and co-crystal ligands, while ligands were formatted for docking compatibility. The docking procedure was executed using AutoDock and AutoDock Vina, with grid box parameters optimized for the protein's active site (Afnani et al., 2024; Ivanova & Karelson, 2022). Binding affinity values were utilized to assess the strength of ligand–protein interactions, and the complex exhibiting the lowest affinity was designated as the most favorable outcome. Subsequently, the docking results were visualized using BIOVIA Discovery Studio to identify binding poses and amino acid residue interactions in both two-dimensional and three-dimensional representations (Ayodele et al., 2023).

2.6 Molecular dynamic simulation

Molecular dynamics (MD) simulation was performed to evaluate the structural flexibility and dynamic stability of the protein–ligand complexes obtained from molecular docking. The simulation was conducted using the CABS-flex 3.0 server (https://lcbio.pl/cabsflex3/), a coarse-grained modeling tool designed to efficiently analyze protein flexibility under near-physiological conditions (Nithin et al., 2024; wroblewski et al., 2025). The docked protein–ligand complexes were submitted as input structures, and simulations were carried out using default parameters. The analysis focused on the root mean square fluctuation (RMSF) values of amino acid residues to assess local flexibility and conformational changes within the protein structure upon ligand binding. Lower RMSF values indicate greater structural stability and reduced residue mobility, whereas higher values reflect increased flexibility in specific regions of the protein (da Fonseca et al., 2024).

3. Results and Discussion

3.1 Volatil compounds based on the data sampling

Based on the comprehensive compilation of previous GC-MS studies across multiple cocoa tissues (leaves, pods, and beans), an initial total of 87 volatile compounds were recorded. Following a strict data curation process to eliminate duplicate molecules and unannotated structures, a final library of 48 unique volatile compounds from *Theobroma cacao* L. was established. Each of these 48 curated compounds was assigned a canonical SMILES structure for the subsequent drug-likeness and biological activity screening. To further characterize the curated compound library, the 48 compounds were classified into major chemical groups based on their structural features and commonly reported phytochemical categories. The largest group consisted of lipids, fatty acids, and their derivatives, comprising 22 compounds. This was followed by phenolic and phenylpropanoid compounds with 11 compounds, terpenoids with 4 compounds, carbohydrate and sugar derivatives with 2 compounds, oxygen-containing heterocyclic compounds with 4 compounds, and non-lipid aliphatic or cyclic compounds with 5 compounds. This classification indicates that the curated cocoa compound library is dominated by lipid-derived metabolites and phenolic-related compounds, while smaller proportions are represented by terpenoids, carbohydrate derivatives, oxygenated heterocycles, and simple aliphatic or cyclic compounds.

3.2 Screening based on drug-likeness, probable bioactivity, toxicity and compound permeability

This screening identified compounds with the greatest potential as agents against insulin resistance. The drug-likeness screening results indicated that twelve compounds possessed suitable physicochemical properties and met the required parameters for drug candidates. Therefore, the twelve compounds successfully passed the drug-likeness selection phase (Table 1).

Table 1. Drug-likeness screening using Lipinski, Veber, Egan rules, GIA and BS parameter

Compound	Lipinski	Veber	Egan	GIA	BS
Methyl Decanoate	Yes	Yes	Yes	High	0.55
Methyl Octanoate	Yes	Yes	Yes	High	0.55
Methyl 10-methylundecanoate	Yes	Yes	Yes	High	0.55
Beta-Hydroxypropiovanillone	Yes	Yes	Yes	High	0.55
Dihydroocimen	Yes	Yes	Yes	High	0.55
Chavibetol	Yes	Yes	Yes	High	0.55
Methyl isovanillate	Yes	Yes	Yes	High	0.55
4-Acetyl-3-methylphenol	Yes	Yes	Yes	High	0.55
Cyclodecanol	Yes	Yes	Yes	High	0.55
Lauric acid	Yes	Yes	Yes	High	0.85
Methoxyeugenol	Yes	Yes	Yes	High	0.55
O-Eugenol	Yes	Yes	Yes	High	0.55

Lipinski's rule evaluates compounds based on four critical characteristics molecular weight, logP partition coefficient, and the count of hydrogen-bonding donors and acceptors (Lipinski et al., 2012). The compounds listed in this table all conformed to these specifications, implying their potential suitability for oral delivery. Furthermore, Veber's rule, which assesses a compound's solubility and permeability, considers the number of hydrogen-bonding donors and acceptors, as well as bond rotation; these criteria were also fulfilled by all the compounds under investigation (Veber et al., 2002). Furthermore, Egan's Rule, which focuses on solubility and permeability, produced a positive result for all the compounds, indicating they have favorable permeability characteristics (Egan et al., 2000). Gastrointestinal Absorptions (GIA) suggests that all tested compounds exhibit a favorable physicochemical profile for drug development, as evidenced by a "High" rating in this category. The Bioavailability Score (BS) assesses the compounds' potential for bioavailability; Lauric acid, in particular, achieved the highest score of 0.85, thereby indicating superior bioavailability relative to the other compounds. Therefore, the combined properties of these compounds meet the drug-likeness criteria, which suggests they should continue to be studied as potential drug candidates.

After evaluating drug-likeness, the biological activity of twelve compounds was then tested. These included insulin promoters, insulysin inhibitors, and lipid metabolism regulators, all of which are related to insulin resistance. Insulin promoters enhance insulin gene expression in pancreatic β-cells (Thakur et al., 2020). Insulysin inhibitors block the insulin-degrading enzyme (IDE), increasing insulin levels and potentially improving glucose control, offering a possible treatment for type 2 diabetes (insulin resistance) (Leissring et al., 2021). Lipid metabolism regulators control lipid synthesis, breakdown, and distribution, which are vital for energy balance and cell function. Insulin, thyroid hormones, and adrenaline regulate lipid metabolism via the PI3K-AKT pathway, influencing transcription factors that control fat synthesis (D. Zhang et al., 2022). The assessment employed two metrics: the Pa score, which reflects a compound's capacity to influence biological functions, with elevated scores implying a greater potential for effect; and the Pi score, which gauges the intensity of a compound's biological impact, where diminished values denote a more potent effect. All compounds satisfied the Pi score requirements, thereby demonstrating their capacity to elicit substantial biological effects. Consequently, the Pa score serves as the primary criterion for assessing a compound's potential to modulate insulin resistance. Compounds with higher Pa scores are considered better candidates for developing treatments for disorders related to insulin metabolism.

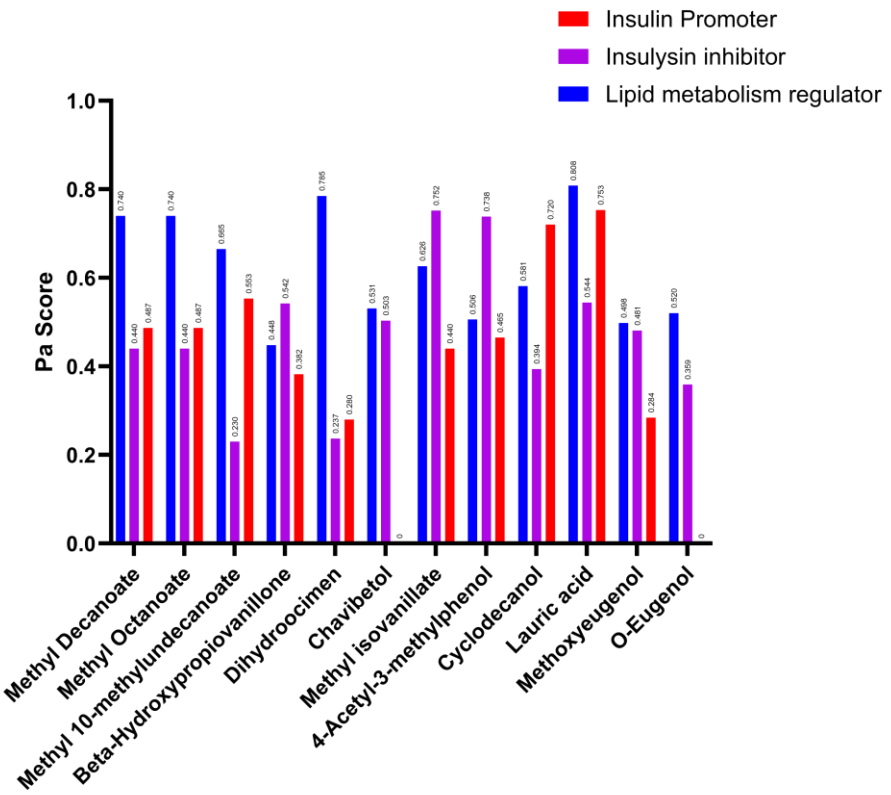

Figure 1. Predicted biological activity (Pa score) of the identified compounds, showing their potential roles as insulin promoter, insulysin inhibitor, and lipid metabolism regulator

Based on the Pa score assessment shown in the graph, the selection of candidate compounds for further testing focused on those with higher average Pa scores. A higher Pa score suggests a compound's increased capacity to affect biological processes associated with insulin resistance. Compounds like Chavibetol and O-Eugenol were excluded from consideration due to undetectable Pa scores, which precluded the demonstration of pertinent biological activity. Furthermore, Beta-Hydroxypropiovanillone, Dihydroocimen, and Methoxyeugenol were also excluded, given their low Pa scores. For instance, Beta-Hydroxypropiovanillone (Pa score 0.382–0.448) and Dihydroocimen (Pa score 0.280 for the insulin promoter and 0.237 for the insulysin inhibitor) suggested a limited ability to affect biological activity. Conversely, substances such as Methyl Decanoate, Methyl Octanoate, Methyl 10-methylundecanoate, Methyl Isovanillate, 4-Acetyl-3-methylphenol, Cyclodecanol, and Lauric Acid consistently demonstrated higher Pa scores across all three evaluated biological activity domains. For example, Lauric Acid, which exhibited a Pa score of 0.753 for the insulin promoter, and Methyl Isovanillate, with a Pa score of 0.752 for the insulin inhibitor, show significant potential. Given that these compounds meet the established criteria, they are ready for toxicity testing. This evaluation will help determine the safety and potential therapeutic uses of these substances.

According to the toxicity test outcomes presented in Table 2, the seven compounds under examination demonstrated a comparatively low potential for toxicity, as evidenced by their elevated LD50 values. These values suggest that substantial doses are necessary to elicit toxic effects in half of the test subjects (Li et al., 2024). Methyl Decanoate, Methyl Octanoate, Methyl 10-methylundecanoate, and Methyl Isovanillate, each possessing an LD50 of 5000, are categorized within Class 5, which denotes low toxicity. Furthermore, 4-Acetyl-3-methylphenol, with an LD50 of 3724, is also classified in Class 5, thereby indicating low toxicity. In contrast, Cyclodecanol, which has a lower LD50 of 1000 and is classified as Class 4, shows a higher level of toxicity compared to the other compounds. Lauric acid, classified as Class 4, has an LD50 of 900, indicating the highest predicted acute toxicity among the evaluated compounds. Nevertheless, endpoint-specific toxicity prediction showed that all seven compounds were inactive for carcinogenicity, immunotoxicity, mutagenicity, and

cytotoxicity, suggesting that none of the evaluated compounds exhibited major predicted toxicological alerts for these endpoints under the ProTox 3.0 prediction framework (Banerjee et al., 2024).

Table 2. Toxicity examination based on LD50, prediction accuracy and the probability of inducing toxicity.

Compound	LD_{50}	Class	Prediction accuracy	Carcino genicity	Immuno toxicity	Mutagenicity	Cytotoxicity
Methyl Decanoate	5000	5	100	Inactive	Inactive	Inactive	Inactive
Methyl Octanoate	5000	5	100	Inactive	Inactive	Inactive	Inactive
Methyl 10-methylundecanoate	5000	5	100	Inactive	Inactive	Inactive	Inactive
Methyl isovanillate	5000	5	70.97	Inactive	Inactive	Inactive	Inactive
4-Acetyl-3-methylphenol	3724	5	70.97	Inactive	Inactive	Inactive	Inactive
Cyclodecanol	1000	4	70.97	Inactive	Inactive	Inactive	Inactive
Lauric acid	900	4	100	Inactive	Inactive	Inactive	Inactive

In contrast, the predictive accuracy results showed differences among the specific compounds. Therefore, the best compounds were chosen based on those with a 100% accuracy score. This score indicated that these compounds were accurately predicted to be non-toxic, meaning they were not expected to cause cancer, mutations, immune system damage, or cell death. Of the seven compounds evaluated, four—Methyl Decanoate, Methyl Octanoate, Methyl 10-methylundecanoate, and Lauric Acid—demonstrated a 100% prediction accuracy, thereby validating the data and suggesting these compounds are safe, with no significant toxic potential. In contrast, compounds such as Methyl Isovanillate, 4-Acetyl-3-methylphenol, and Cyclodecanol, notwithstanding their elevated LD50 values, failed to satisfy the established prediction accuracy criteria (approximately 70.97%), which indicated less reliable and potentially hazardous toxicity prediction outcomes. Consequently, the four compounds exhibiting 100% prediction accuracy were selected for advancement to the subsequent testing phase.

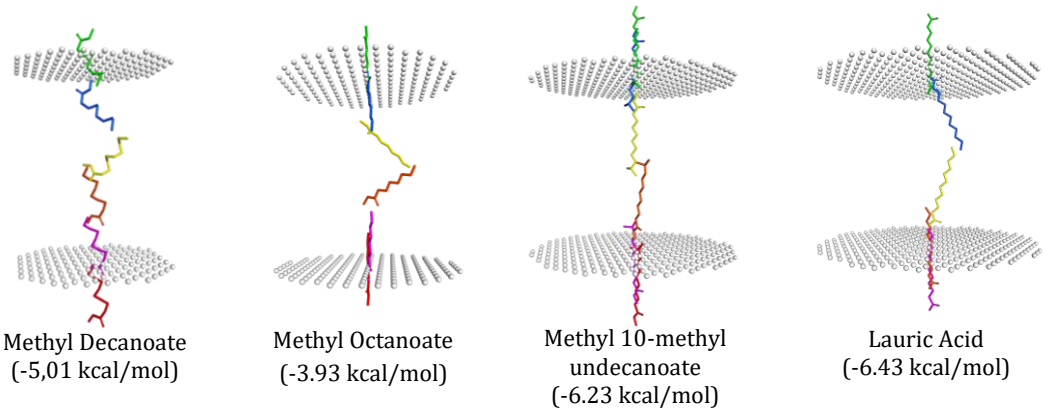

Figure 2. Simulation of the methyl decanoate, methyl octanoate, methyl 10-methylundecanoate and lauric acid penetrating the lipid bilayer.

Four selected compounds showed negative membrane interaction energy values in the DOPC lipid bilayer model, namely -5.01, -3.93, -6.23, and -6.43 kcal/mol for methyl decanoate, methyl octanoate, methyl 10-methylundecanoate, and lauric acid, respectively (Figure 2). In PerMM-based analysis, membrane permeability is evaluated using a physics-based framework that considers membrane binding energy, translocation energetics, and permeability-related parameters across lipid bilayers. Therefore, the negative energy values observed in this study indicate favorable membrane association or partitioning of the compounds into the lipid bilayer, which may support their passive membrane interaction potential. However, these values should be interpreted as predictive permeability-related indicators rather than definitive experimental evidence of membrane penetration or bioavailability (Lomize et al., 2019).

3.3 Network pharmacology

SwissTarget Prediction was used to identify potential protein targets for the compounds being studied. Target prediction analysis identified 105, 103, 103, and 107 potential protein targets for methyl decanoate, methyl octanoate, methyl 10-methylundecanoate, and lauric acid, respectively. Subsequently, a Venn diagram analysis of these compound-specific targets revealed 24 shared proteins (Figure 3A). The overlapping targets suggest the main proteins that could be affected by the combined action of all four compounds. Insulin resistance-related disease protein targets were gathered from several databases: DisGeNet (160 targets), CTD (150 targets), and GeneCards (250 targets). A Venn diagram analysis revealed 21 protein targets common to these datasets (Figure 3B), thereby highlighting the key proteins associated with the disease. Subsequently, the compound-related targets and the disease-associated targets were combined to identify common targets. The intersection analysis revealed three proteins that were found together: HSD11B1, PPARA, and PTPN1 (PTP1B) (Figure 3C). These proteins were then chosen as the main targets for further mechanistic studies

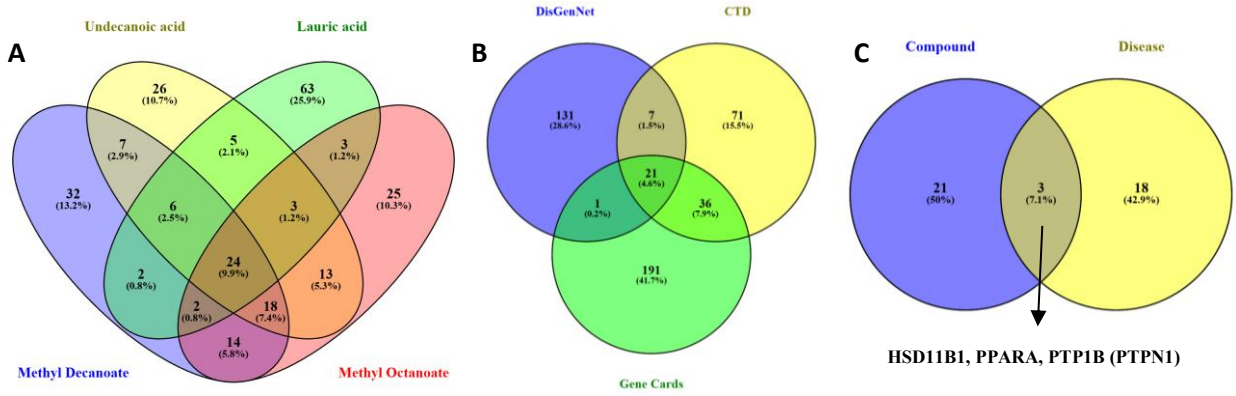

Figure 3. Venn diagram analysis showing the intersection of predicted targets:
A. Compound-associated protein targets, **B**. Disease-associated protein targets, **C.** Intersection of compound and disease associated targets

Network pharmacology analysis was performed to elucidate the multi-target interactions between selected volatile compounds from *Theobroma cacao* L. and proteins associated with insulin resistance. The constructed network revealed a complex interaction landscape involving four selected compounds with multiple protein targets. Among these, three key proteins protein tyrosine phosphatase 1B (PTP1B), peroxisome proliferator-activated receptor alpha (PPARA), and hydroxysteroid 11-beta dehydrogenase 1 (HSD11B1) were identified as core or direct targets, as they were consistently predicted across compound-target and disease-target intersection analyses. These proteins occupy central positions within the network, indicating their potential role as primary mediators of the therapeutic effects. In addition to the direct targets, a broader network of indirect targets was identified, comprising proteins involved in insulin signaling, lipid metabolism, and related regulatory pathways. Figure 4 illustrates the compound–target–pathway interaction network

The network pharmacology findings demonstrate that the selected cocoa volatile compounds exert potential therapeutic effects through a multi-target mechanism rather than a single-target mode of action. The identification of PTP1B, PPARA, and HSD11B1 as central nodes is particularly significant, as these proteins are well-established regulators of glucose and lipid metabolism (Jais et al., 2014; Joshi et al., 2026). PTP1B is a negative regulator of insulin signaling, and its inhibition has been widely recognized as a promising strategy to enhance insulin sensitivity (Koren & Fantus, 2007). Meanwhile, PPARA plays a crucial role in fatty acid oxidation and lipid homeostasis, which are closely linked to metabolic balance and insulin responsiveness (Tahri-Joutey et al., 2021). HSD11B1 contributes to local glucocorticoid activation, thereby influencing gluconeogenesis and metabolic stress, both of which are key drivers of insulin resistance (Beaupere et al., 2021).

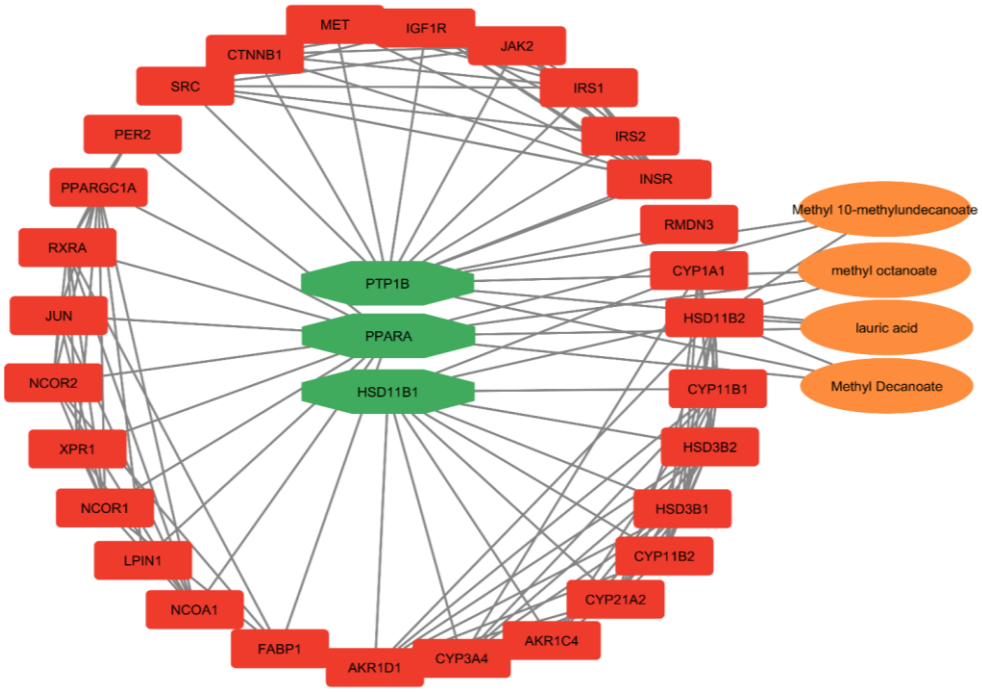

Figure 4. Network pharmacology analysis of compound–target–protein interactions. The orange nodes represent volatile compounds derived from *Theobroma cacao* L., the green nodes indicate direct protein targets and the red nodes represent indirect targets involved in protein–protein interaction networks.

Beyond these direct targets, the presence of multiple indirect targets within the network suggests that these compounds may modulate broader signaling cascades, including insulin receptor activation, downstream PI3K-AKT signaling, and transcriptional regulation of metabolic genes. Proteins such as INSR, IRS1/2, and IGF1R highlight the involvement of canonical insulin signaling pathways, whereas enzymes from the cytochrome P450 family and aldo-keto reductases indicate potential roles in metabolic detoxification and lipid processing (Li et al., 2022; X. Zhang et al., 2022). Importantly, the dense interconnectivity observed in the network supports the concept of polypharmacology, where natural compounds simultaneously influence multiple molecular targets to achieve synergistic therapeutic effects.

3.4 Gene ontology and functional annotation

GO and pathway enrichment analyses were conducted to further elucidate the functional roles of the identified target proteins involved in insulin resistance. The enrichment results revealed significant biological processes, molecular functions, cellular components, and associated pathways. As shown in Figure 5A, the BP category was predominantly enriched in metabolic-related processes, including lipid metabolic process, steroid metabolic process, hormone metabolic process, and glucose homeostasis. Additionally, processes related to insulin receptor signaling pathway and positive regulation of transcription by RNA polymerase II were also significantly represented.

In terms of MF, the enriched terms (Figure 5B) included phosphatidylinositol 3-kinase binding, protein tyrosine kinase activity, insulin receptor substrate binding, and oxidoreductase activity. These functions highlight the involvement of target proteins in enzymatic activity and signal transduction mechanisms associated with insulin signaling. For CC analysis (Figure 5C), the identified targets were mainly localized in intracellular membrane-bounded organelles, mitochondrial membranes, nucleoplasm, and insulin receptor complexes, suggesting their functional roles within subcellular structures critical for metabolic regulation (Song et al., 2024).

Furthermore, pathway enrichment analysis (Figure 5D) demonstrated that the target proteins are involved in several key signaling pathways, including the insulin signaling pathway, adipocytokine signaling pathway, chemical carcinogenesis–receptor activation, and endocrine resistance pathways. These pathways are closely associated with metabolic homeostasis and insulin sensitivity.

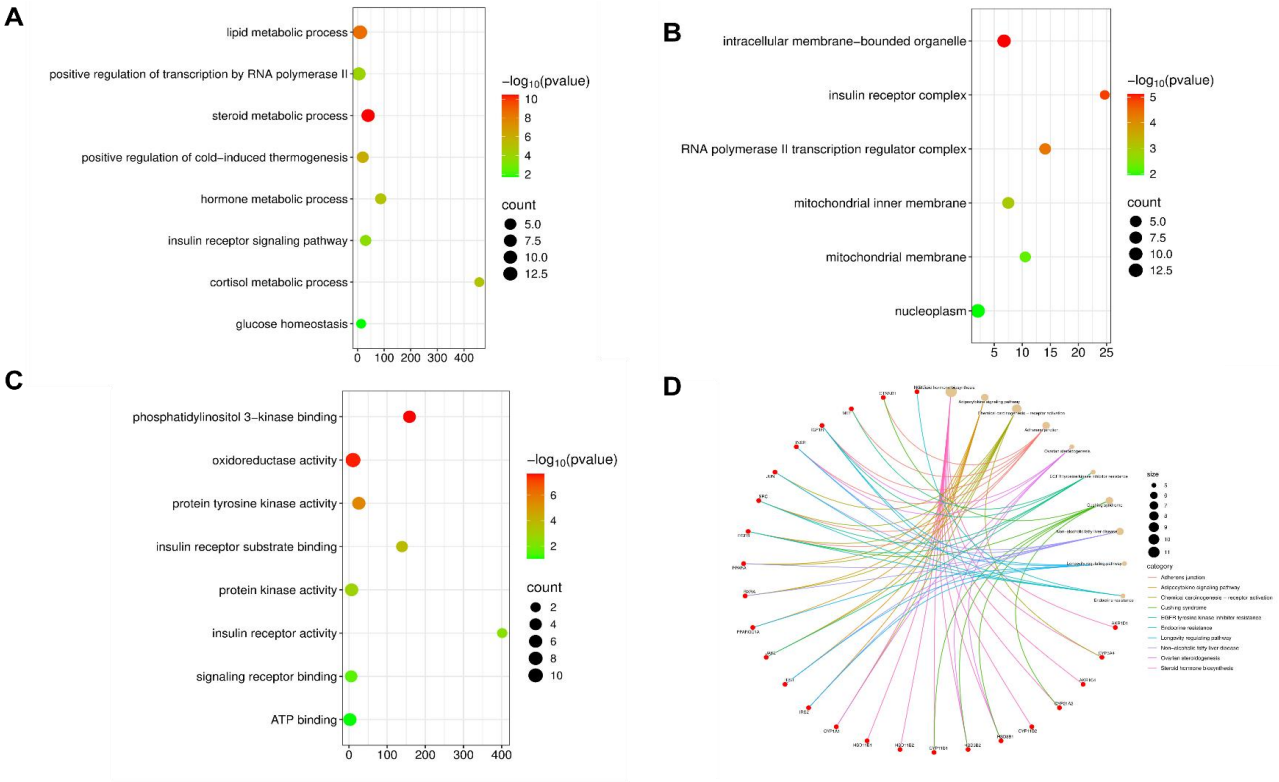

Figure 5. Enrichment analysis for gene ontology.
A. Biological process. B. Molecular Function. C. Cellular component. D. Pathway enrichment analysis

The GO enrichment analysis provides mechanistic insights into how cocoa volatile compounds may exert therapeutic effects through modulation of multiple biological processes and pathways relevant to insulin resistance. The significant enrichment of lipid, steroid, and hormone metabolic processes indicates that the identified targets play a central role in metabolic regulation, particularly in pathways that govern energy balance and glucose utilization. Dysregulation of these processes is a hallmark of insulin resistance and related metabolic disorders. The involvement of the insulin receptor signaling pathway further supports the relevance of the identified targets in maintaining glucose homeostasis. Proteins participating in this pathway, such as IRS1/2 and INSR, are critical for downstream signaling events that regulate glucose uptake and insulin sensitivity. Therefore, modulation of these targets by bioactive compounds may contribute to improved insulin responsiveness (Li et al., 2022; X. Zhang et al., 2022).

From a molecular function perspective, the enrichment of kinase activity and phosphatidylinositol 3-kinase binding suggests that the identified targets are closely linked to intracellular signaling cascades, particularly the PI3K–AKT pathway, which is essential for mediating insulin action. The presence of oxidoreductase activity also indicates a potential role in redox regulation, which is increasingly recognized as an important factor in metabolic dysfunction and insulin resistance (Le et al., 2023; Verma et al., 2023). The cellular component analysis highlights that many of the target proteins are localized in mitochondria and membrane-associated complexes, emphasizing the importance of subcellular compartmentalization in metabolic regulation. Mitochondrial dysfunction is widely implicated in insulin resistance, and the involvement of mitochondrial membranes suggests that these targets may influence energy metabolism and oxidative stress (Cojocaru et al., 2023).

Finally, pathway enrichment analysis reinforces the multi-target therapeutic potential of cocoa volatile compounds. The identification of pathways such as adipocytokine signaling and endocrine resistance suggests broader regulatory effects on hormonal signaling and metabolic adaptation. Collectively, these findings support the concept that the studied compounds may act through coordinated modulation of interconnected biological systems, thereby offering a comprehensive strategy to mitigate insulin resistance.

3.5 Molecular docking

The molecular docking analysis reveals that all tested ligands demonstrate binding affinity towards HSD11B1, PPARA, and PTP1B, albeit with differing levels of affinity for each target. According to the binding affinity data presented in Figure 6, PTP1B exhibits the greatest responsiveness to the test ligands, as evidenced by the lowest binding energy values for almost all compounds, specifically methyl 10-methylundecanoate (−5.72 kcal/mol), methyl octanoate (−5.59 kcal/mol), methyl decanoate (−5.58 kcal/mol), and lauric acid (−5.56 kcal/mol). Conversely, the binding affinity for PPARA seems to be less strong, with values ranging from −3.81 to −4.68 kcal/mol. Regarding HSD11B1, all ligands showed a relatively strong affinity, with lauric acid and metformin both yielding a value of −4.70 kcal/mol. Metformin was selected as the reference control compound. While its primary canonical mechanism involves AMPK activation, its well-documented pleiotropic nature allows it to interact with various metabolic targets. Consequently, it serves as a robust clinical baseline in this *in silico* study to benchmark the comparative binding affinities of the candidate compounds against HSD11B1, PPARA, and PTP1B (Dutta et al., 2023). In general, most of the test ligands exhibited lower binding affinities than metformin, particularly toward PTP1B, suggesting the potential for stronger interactions at that target (Agrawal et al., 2023).

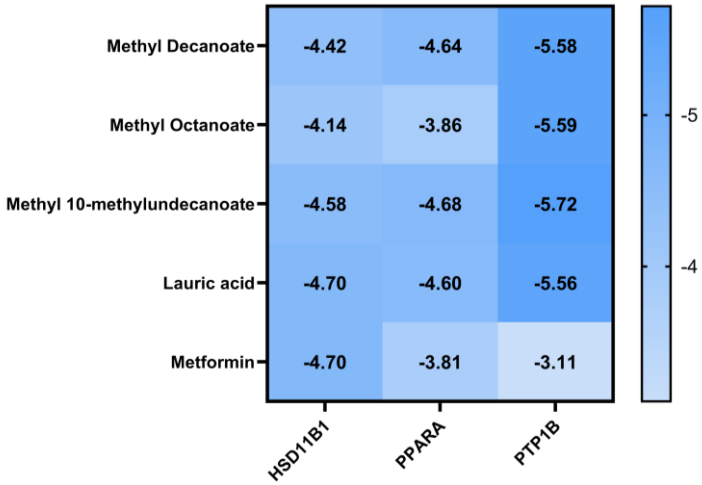

Figure 6. Result molecular docking show binding affinity

For HSD11B1, 3D and 2D interaction visualizations show that all ligands occupy the same protein binding pocket, but with different orientations and contact patterns. The relatively close affinity values indicate that this target remains relevant for all tested compounds. The closeness of the binding affinity values between lauric acid and metformin at HSD11B1 indicates that medium-chain carbon-based compounds still possess binding capabilities comparable to the control at this target. Biologically, HSD11B1 is known to be involved in tissue glucocorticoid regulation and is associated with insulin resistance; therefore, the interactions formed at this target remain important in the context of metabolic disorders (Morgan & Tomlinson, 2010).

In PPARA, all ligands were also detected in the same binding pocket, but their affinity was lower than that of HSD11B1 and PTP1B. Methyl 10-methylundecanoate demonstrated the most favorable binding affinity for the target, registering −4.68 kcal/mol; methyl decanoate and lauric acid followed, with values of −4.64 kcal/mol and −4.60 kcal/mol, respectively. Conversely, metformin exhibited the weakest binding, with a value of −3.81 kcal/mol. The data suggests that PPARA isn't the main target of the compounds tested, even though some interaction was seen. This is consistent with PPARA's known role in controlling lipid metabolism. In contrast, its effect on insulin sensitivity is usually less direct than that of PTP1B and HSD11B1(Haluzík and Haluzík, 2006).

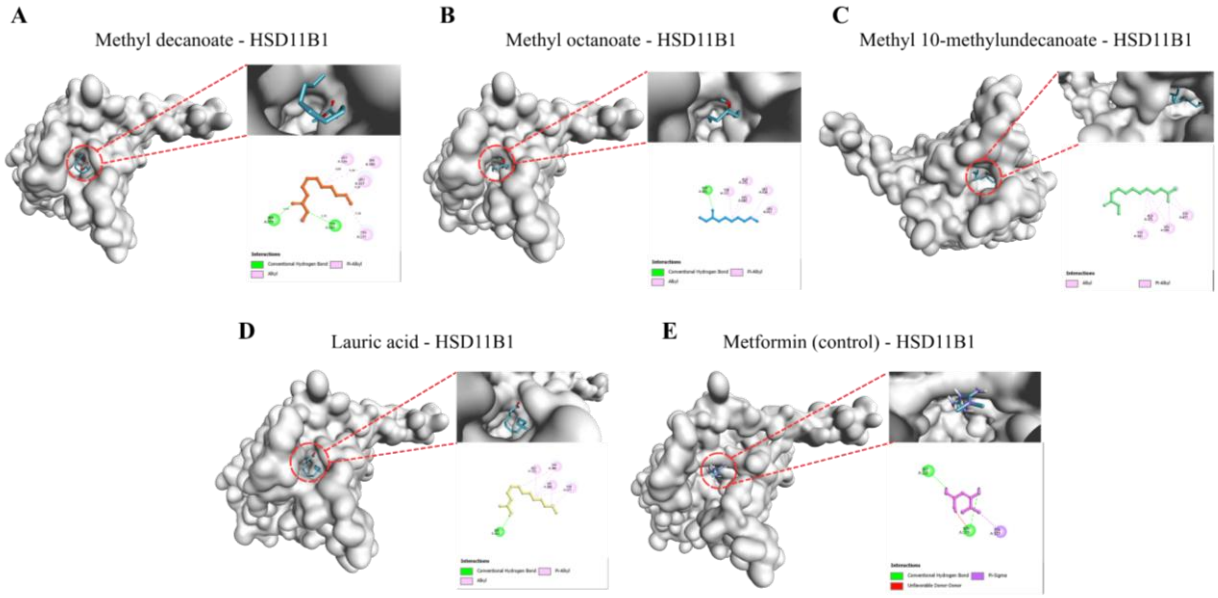

Figure 7. 3D and 2D visualization of protein HSD11B1-ligand (compounds) interactions.
A) HSD11B1- methyl decanoate. B) HSD11B1 – methyl octanoate. C) HSD11B1 – methyl 10-methylundecanoate. D) HSD11B1 – lauric acid. D) HSD11B1 – metformin (control)

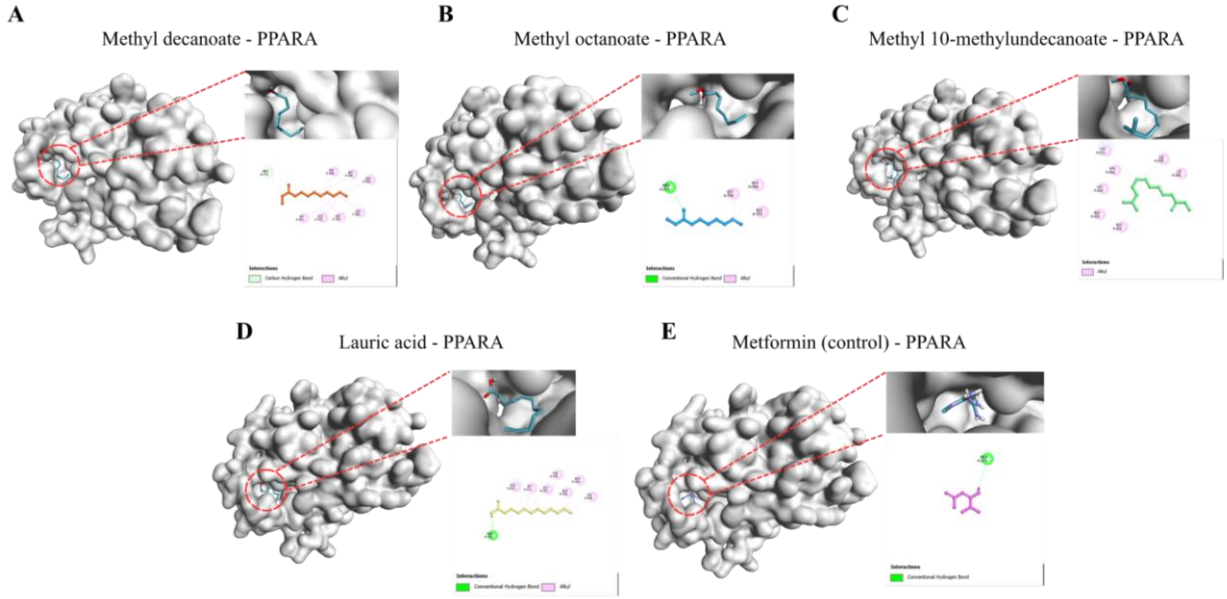

Figure 8. 3D and 2D visualization of protein PPARA-ligand (compounds) interactions.
A) PPARA – methyl decanoate. B) PPARA – methyl octanoate. C) PPARA – methyl 10-methylundecanoate. D) PPARA – lauric acid. D) PPARA – metformin (control)

Unlike the other two targets, PTP1B exhibited the highest binding affinity for nearly all test ligands. Docking visualizations revealed that all compounds bound to the protein's active site, with 10-methylundecanoate yielding the lowest value of −5.72 kcal/mol. This finding is significant because PTP1B is known as a negative regulator of the insulin signaling pathway; thus, inhibition of this target has the potential to enhance insulin sensitivity. Consequently, the dominant binding affinity observed for PTP1B indicates that this target is the most promising protein for the tested ligands. This trend is also supported by previous research identifying PTP1B as one of the key antidiabetic targets (Agrawal et al., 2023). Furthermore, support from prior studies on lauric acid and medium-chain fatty acids suggests that compounds with a medium-chain carbon backbone may be associated with improvements in metabolic parameters relevant to insulin resistance (Tham et al., 2020).

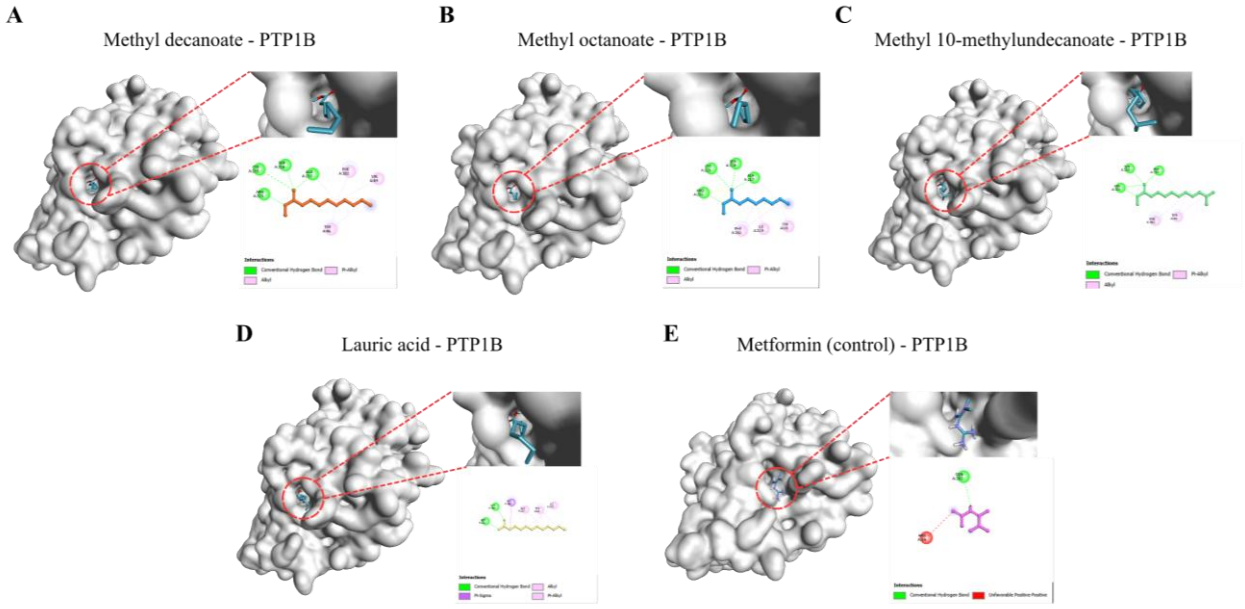

Figure 9. 3D and 2D visualization of protein PTP1B-ligand (compounds) interactions.
A) PTP1B – methyl decanoate. B) PTP1B – methyl octanoate. C) PTP1B – methyl 10-methylundecanoate. D) PTP1B – lauric acid. D) PTP1B – metformin (control)

3.6 Molecular dynamic

The stability and flexibility of the protein–ligand complexes were evaluated using RMSF parameter, where RMSF values below 4 Å are generally considered indicative of stable protein conformations with minimal structural deviation (Da Fonseca et al., 2024). As presented in Figure 8, the RMSF profiles of HSD11B1, PPARA, and PTP1B complexes demonstrate that the majority of amino acid residues exhibit fluctuation values below this threshold. Although slight increases in RMSF were observed in several regions, particularly in flexible loop segments, the overall average values remained consistently below 4 Å across all complexes. These findings indicate that the protein–ligand systems maintain structural integrity throughout the simulation.

The RMSF analysis highlights that the interaction between cocoa volatile compounds and the target proteins results in structurally stable complexes. The predominance of low fluctuation values suggests that ligand binding does not induce significant conformational perturbations in the protein backbone. This is particularly relevant for proteins such as PTP1B, PPARA, and HSD11B1, where structural stability is essential for maintaining functional activity.

The minor fluctuations observed in certain regions are likely associated with intrinsically flexible segments, such as loops and terminal residues, which commonly exhibit higher mobility. These localized dynamics are not indicative of instability but rather reflect normal protein behavior that may facilitate ligand accommodation and adaptive binding interactions (Mavani et al., 2023). Overall, the consistently low RMSF values across all complexes support the notion that the selected compounds form stable interactions with key proteins involved in insulin resistance. This dynamic stability further strengthens the potential of these compounds as multi-target therapeutic candidates, complementing the findings obtained from molecular docking analyses.

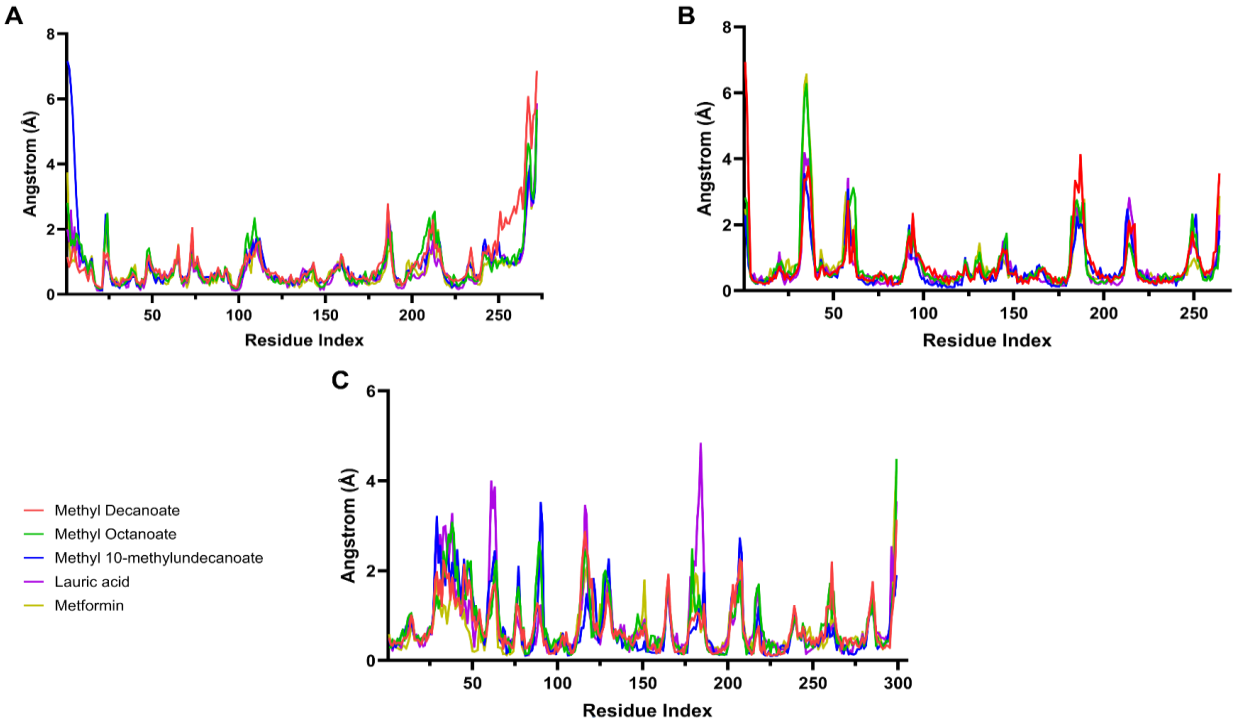

Figure 10. RMSF profiles of protein–ligand complexes obtained from molecular dynamics simulations. RMSF values of amino acid residues in **A.** HSD11B1 **B.** PPARA, and **C.** PTP1B complexes with selected compounds are shown. All complexes exhibit RMSF values predominantly below 4 Å, indicating stable structural dynamics.

4. Conclusion

In conclusion, this study demonstrates that volatile compounds derived from various tissues of *Theobroma cacao* L. have promising potential as multi-target candidates against insulin resistance. The *in silico* screening results showed that methyl decanoate, methyl octanoate, methyl 10-methylundecanoate, and lauric acid fulfilled the criteria for drug-likeness, favorable gastrointestinal absorption, acceptable bioavailability, low predicted toxicity, and good membrane permeability. Network pharmacology analysis identified HSD11B1, PPARA, and PTP1B as the main therapeutic targets. Furthermore, gene ontology and pathway enrichment analyses revealed their close association with insulin signaling, lipid metabolism, glucose homeostasis, and adipocytokine-related pathways. Molecular docking analysis further indicated target-dependent ligand–protein interactions, in which methyl 10-methylundecanoate showed the most favorable predicted affinity toward PTP1B and PPARA, with binding energies of -5.72 and -4.68 kcal/mol, respectively, whereas lauric acid demonstrated the strongest affinity toward HSD11B1, with a binding energy of -4.70 kcal/mol. Molecular dynamics simulations further supported the structural stability of the predicted protein–ligand complexes. Collectively, these findings suggest that cocoa-derived volatile compounds may serve as promising lead candidates for multi-target intervention in insulin resistance through the coordinated modulation of key metabolic targets. However, further studies are required to validate these computational findings. Future research should include in vitro assays using insulin-resistant cell models to evaluate glucose uptake, insulin signaling, lipid metabolism, and inflammatory markers, followed by *in vivo* studies to assess pharmacological efficacy, pharmacokinetics, dose-response effects, and systemic safety. Target-specific enzymatic assays against PTP1B, PPARA, and HSD11B1 are also recommended to confirm whether the predicted binding interactions are biologically relevant.

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