A | R | J
Amrit Research Journal December 2024, Vol. 5, Issue 1, 49-58
ISSN: 2738-9553
RESEARCH ARTICLE

GC-MS Analysis, Oral Toxicity Evaluation, and Molecular Docking Studies of *Ocimum tenuiflorum* L. Essential Oil: Exploring its Anti-Diabetic Potential through Peroxisome Proliferator-Activated Receptor-Delta (PPARδ) Activation

Ashika Tamang[1,2], Nirmal Parajuli[1,2], Manila Poudel[2], Shiva M.C.[1,2], Aakar Shrestha[2], Timila Shrestha[1,2], Samjhana Bharati[1,2], Binita Maharjan[1,2], Deval Prasad Bhattarai[1], Bishnu P. Marasini[2,3,4], Jhashanath Adhikari Subin[2,5,*], Ram Lal (Swagat) Shrestha[1,2,3,*]

[1]Department of Chemistry, Amrit Campus, Tribhuvan University, Lainchaur, Kathmandu 44600, Nepal

[2]Kathmandu Valley College, Syuchatar Bridge, Kalanki, Kathmandu 44600, Nepal

[3]Institute of Natural Resources Innovation, Kalimati, Kathmandu 44600, Nepal

[4]Nepal Health Research Council, Ministry of Health and Population, Ramshah Path, Kathmandu 44600, Nepal

[5]Bioinformatics and Cheminformatics Division, Scientific Research and Training Nepal P. Ltd., Bhaktapur 44800, Nepal

**E-mail: subinadhikari2018@gmail.com, swagatstha@gmail.com*

Received: August 7, 2024; Received in Revised form: October 6, 2024; Accepted: October 22, 2024; Available online)

DOI: https://doi.org/10.3126/arj.v5i1.73524

Highlights

- The essential oil of leaf part from *Ocimum tenuiflorum* was extracted using hydro-distillation method.
- GC-MS analysis of the essential oil showed the presence of 28 different compounds.
- The acute oral toxicity of essential oil showed LD_{50} value of > 2000 mg/kg BW on the tested mice.
- Molecular docking calculations of GC-MS identified compounds with PPARδ protein was studied to evaluate the anti-diabetic potential

Abstract

Ocimum tenuiflorum (O. tenuiflorum), commonly known as Tulsi, is an aromatic herb with significant medicinal properties, particularly in the management of diabetes. This study aimed to extract essential oil from the leaves of O. tenuiflorum, perform a comprehensive GC-MS analysis, evaluate acute oral toxicity, and assess its anti-diabetic potential through computational molecular docking with the peroxisome proliferator-activated receptor-delta (PPARδ) protein. The essential oil was extracted using hydro-distillation, and GC-MS identified 28 compounds, with (E)-caryophyllene, β-elemene, and trans-isoeugenol being the most abundant. Molecular docking against PPARδ (PDB ID: 5U3R) highlighted rimuene, himachalane, valencene, and nootkatene as top candidates, exhibiting strong binding affinities comparable to the reference drug seladelpar. Predominantly hydrophobic interactions were observed due to the volatile, non-polar nature of the compounds in the oil. Acute oral toxicity tests showed an LD50 > 2000 mg/kg body weight which confirmed the safety of the essential oil, with low toxicity profile. This study identifies rimuene and himachalane as promising PPARδ modulators, suggesting further in vitro, in vivo, and computational evaluations to validate their therapeutic potential in diabetes management.

Keywords: *Binding affinity, computational approach, docking score, molecular interactions, nuclear hormone receptor.*

**Corresponding author*

Introduction

Medicinal plants have been the cornerstone of medical science since the Vedic period (3500–16 BC), as documented in Ayurvedic and Unani literature [1]. In modern complementary and alternative medicine, they remain a primary source of therapeutics due to their rich bioactive compounds, primarily secondary metabolites [2]. Among these, aromatic herbs have gained considerable attention due to their potent bioactive compounds that serve not only in therapeutic applications but also in agriculture as natural pesticides and insect repellents [3].

One notable example of aromatic plant is *Ocimum tenuiflorum* (*O. tenuiflorum*) (also known as *Ocimum sanctum*, Tulsi, or Holy Basil), a member of the Lamiaceae family[4]. Native to the Indian subcontinent and widely cultivated throughout Southeast Asia, this 30–60 cm tall aromatic herb is characterized by its strongly scented green or purple leaves [5]. It is often referred to as the "Queen of Plants" and the "Mother Medicine of Nature" for its exceptional medicinal properties. It has long been recognized as a valuable source of essential oil, rich in bioactive compounds such as eugenol, carvacrol, and ursolic acid, making it a key aromatic ingredient in the food, pharmaceutical, cosmetic, and aromatherapy industries [4], [6]. Extensive research has demonstrated that the essential oil of *O. tenuiflorume xhibits* a broad spectrum of pharmacological activities, including antibacterial, antifungal, antiviral, and antioxidant effects, primarily attributed to its rich profile of secondary metabolites [7]. Moreover, many studies have shown that *O. tenuiflorum* extracts effectively regulate blood glucose levels in diabetic rats [8].

According to the International Diabetes Federation, type 2 diabetes, caused by insulin resistance and disrupted glucose regulation due to dysfunction of receptors or disruption of signaling pathways, is a growing global health issue [9], [10]. Peroxisome proliferator-activated receptor-delta (PPARδ), a nuclear hormone receptor, plays a crucial role in regulating blood glucose by enhancing glucose transporter type 2 (GLUT2) expression and inhibiting genes involved in gluconeogenesis [11], [12]. This makes PPARδ a promising target for managing type 2 diabetes. As computational screening method is one of the reliable and cost-effective technique for the pharmacophore modelling of a compound, PPARδ receptor was chosen as the therapeutic target for the study.

This research aims to evaluate the anti-diabetic potential of *O. tenuiflorum* essential oil by analyzing its chemical composition through GC-MS, assessing acute oral toxicity, and exploring its interaction with PPARδ using molecular dockingcalculations. Identifying key bioactive compounds as potential PPARδ agonists will contribute to understanding the plant's role in diabetes management and provide guide for future drug discovery process.

Materials and Methods

Plant Sample Collection and Essential Oil Extraction

The leaves of the plant *O. tenuiflorum* were collected from Kathmandu, Nepal. The essential oil was extracted from the shaded dried leaves of the plant using hydro-distillation method with Clevenger apparatus.

Gas Chromatography-Mass Spectroscopy (GC-MS)

The extracted essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS) with the GCMS-QP 2010 instrument, following the method outlined by Tamang et al [13]. The compounds obtained were identified through MS comparison.

Computational Methodology

Selection and Preparation of Ligands and Target Protein

The compounds identified through GC-MS from *O. tenuiflorum* essential oil, were selected as ligands. Their 3D structures were obtained from the PubChem database [14] and optimized using the Avogadro program (version 1.2.0) [15] with the Universal Force Field with 5000 steps, conjugate gradients, and convergence threshold of 10^{-8} kcal/mol set up. The ligands were then converted to PDBQT format with Gasteiger charges using AutoDock Tools [16].

The PPARδ protein crystal structure (PDB ID: 5U3R) with 1.95 Å resolution was retrieved from the RCSB database [17] for computational study. Visualization and preparation were done using the PyMOL program [18], selecting chain A and removing ions, water molecules, and co-crystallized ligands. Polar hydrogens and Kollman charges were added to the protein structure

using theAutoDock Tools. Then the apo form of the protein was saved in PDBQT format which is used in molecular docking calculation.

Molecular Docking Calculations

Molecular docking was performed using the AutoDock Vina [16] to identify the optimal ligand binding pose within the receptor's orthosteric site. The method was chosen for its ease of use and ability to reproduce standard poses reliably with consistent results [19]. Binding affinities were calculated with a specialized scoring function. The docking grid was centered at (x: −22.677, y: 6.751, z: 133.969) with box sizedimensions of 40 × 40 × 40 Å3, using parameters including exhaustiveness of 16, energy range 4, and 20 docking modes. The docking results were visualized with PyMOL and Biovia Discovery Studio programs [20].

Acute Oral Toxicity Test

The acute oral toxicity study of the essential oil from plant *O. tenuiflorum* was conducted by following the Organization for Economic Cooperation and Development (OECD) guidelines, specifically Method 423 [21]. The experimental procedure followed the method described by Ranjitkar R et al., using the essential oil at a dose of 2000 mg/kg body weight (BW) [22].

Results and Discussions

GC-MS Spectra Analysis

The GC-MS analysis of the essential oil of *O. tenuiflorum* revealed a chromatogram with 28 distinct peaks, as shown in Fig 1. These peaks correspond to 28 different compounds, which are detailed in Table 1 along with their PubChem CID, retention time (RT), and peak area (%). Among these, the most abundant compounds were (E)-caryophyllene (27.71%), β-elemene (11.99%), and trans-isoeugenol (10.96%) which are highlighted in bold in Table 1.

Table 1: Components identified in the essential oil of *O. tenuiflorum* based on GC-MS analysis

Peak No.	Name of compounds	PubChemCID	RT	Peak Area (%)
1	α-Pinene	6654	9.435	2.30
2	Rimuene	12314971	9.585	1.36
3	2-Tridecenenitrile	90818	10.955	1.29
4	Isocedranol	6713078	17.303	1.08
5	Valencene	9855795	21.060	1.13
6	Vanillyl alcohol	62348	28.120	0.99
7	Trans-isoeugenol	853433	29.090	**10.96**
8	Nootkatene	25200342	29.415	1.09
9	α-ylangene	19725	29.941	1.53
10	β-elemene	6918391	30.645	**11.99**
11	Methyl-isoeugenol	1549045	31.194	4.13
12	(E)-Caryophyllene	5281515	31.925	**27.71**
13	α-Humulene	5281520	33.416	1.38
14	β-Selinene	442393	34.852	5.76
15	α-Selinene	10856614	35.221	5.09
16	Spathulenol	92231	36.072	7.68
17	δ-Cadinene	441005	36.360	2.11
18	Ethyl 3-hydroxybutyrate	62572	41.565	1.10
19	β-Cyclohomocitral	61124	42.441	1.14
20	Phenylbutyrate-4-, ethyl-	NR	43.785	1.25
21	Furopelargone A	6451194	44.391	1.11
22	γ- Dehydro-ar-himachalene	6428455	48.335	1.07

23	Eremophilone	21591457	48.696	1.08
24	Theaspirane	61953	48.855	0.99
25	(-)-Nopsan-4-ol	91748572	49.061	1.39
26	β-Resorcinolic acid	1491	51.061	1.03
27	γ-Costol	91730038	52.175	1.14
28	α -Butylcinnamaldehyde	5385520	52.848	1.05

Fig 1. Chromatogram of essential oil of *O. tenuiflorum* obtained from GC-MS analysis

Analysis of Computational Outputs

Molecular Docking Calculation Scores

Molecular docking was used to evaluate ligand-protein interactions and to determine the ligand's optimal binding pose within the protein's active site based on binding affinity [23].

Docking of the native ligand 7V1 (PubChem CID: 117629789) revealed an overlap between the docked structure and its arrangement in the modeled structure, as shown in Fig 2.

Fig 2. Superimposed structures of the native ligand (yellow) from the crystal structure and docked ligand (red) in PPARδ (heavy atom RMSD of 0.801 Å

A heavy-atom RMSD of 0.801 Å was recorded between the two conformations of the same molecule, validating the accuracy of the molecular docking algorithm and its selected parameters.

All the compounds obtained from GC-MS analysis of the essential oil of *O. tenuiflorumavailable* in PubChem database in 3D structure were advanced to molecular docking study. Ligands rimuene, himachalene (Gamma-dehydro-ar), valencene and nootkatene were found to be top four candidate with docking scores −9.3, −9.0, −8.8 and −8.6 kcal/mol, respectively. A PPARδ agonist, Seladelpar was taken as reference drugs which scored −9.8 kcal/mol meanwhile ligand in the crystal structure of protein (native) interacted with docking score −10.4 kcal/mol with the receptor. The docking results of the top four ligands with the receptor PPARδ (PDB ID: 5U3R) are listed in the Table 2 with their 2Dmolecular structures in Fig 3.

Table 2. Top four GC-MS docked compounds, reference drugs, their Pub Chem CID and docking scores with the receptor (PDB ID:5U3R)

S.N.	Compounds	PubChem CID	Docking score (kcal/mol)
1.	Rimuene	12314971	−9.3
2.	Himachalene (Gamma-dehydro-ar)	6428455	−9.0
3.	Valencene	9855795	−8.8
4.	Nootkatene	25200342	−8.6
Native Ligand	**7V1**	117629789	−10.4
Reference Drug	**Seladelpar***	11236126	−9.8

*Reference drug

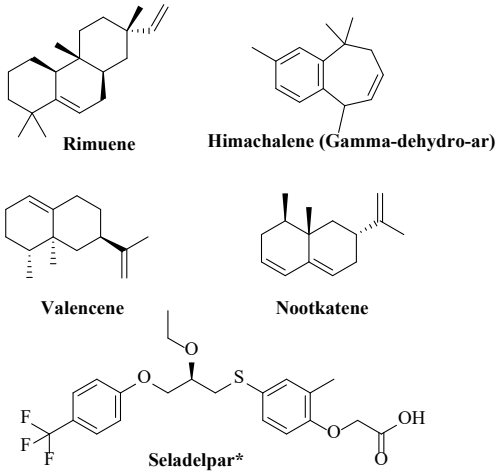


Fig 3. Molecular structures of top four candidates with reference drug

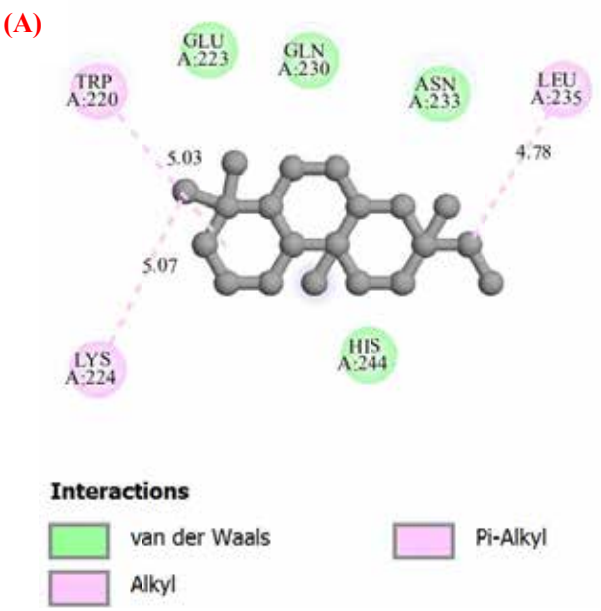
Protein-Ligand Interaction

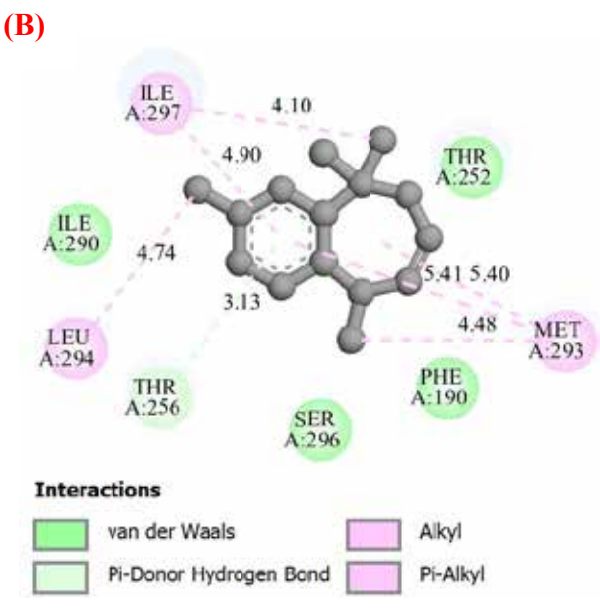
The types of interactions and associated amino acid residues during the complex formation between ligand and receptor with the interactive distance are tabulated in Table 3 along with the bond distance (Å).

Table 3: Types of interaction and responsible amino acid residues with bond distance (Å) in best docked position of the ligand with the receptor

Ligands	Type of interactions	Amino acid residues with bond distance (Å)
Rimuene	Pi-alkyl	TRP220 (5.03)
	Alkyl	LEU235 (4.78), LYS224 (5.07)
	van der Waals	HIS244, ASN233, GLN230, GLU223
Himachalene (Gamma-dehydro-ar)	Pi-Donor Hydrogen Bond	THR256 (3.13)
	Pi-alkyl	ILE294 (4.90), MET293 (5.41)
	Alkyl	ILE297 (4.10), MET293 (4.48, 5.40), LEU294 (4.74)
	van der Waals	PHE190, THR252, ILE290, SER296

Valencene	Pi-alkyl	HIS430 (4.21, 4.25), TYR247 (4.75)
	Alkyl	PRO236 (3.90, 4.06, 4.22), PRO431 (4.81), CYS251 (4.50, 3.95)
	van der Waals	SER428, GLN250, VAL254, GLY234, LEU223
Nootkatene	Alkyl	ILE297 (4.57, 5.37, 3.97), MET293 (5.43), LEU294 (5.28, 4.69), ILE290 (4.88)
	van der Waals	MET192, THR256
Native ligand	Conventional Hydrogen Bond	TYR437 (2.09)
	Pi-donor Hydrogen bond	CYS249 (4.13)
	Pi-Sulphur	CYS249 (5.66)
	Pi-Sigma	VAL305 (3.88)
	Pi-alkyl	ARG248 (3.89), VAL245 (4.44), VAL312 (4.93), VAL305 (5.10), LEU303 (5.26), ILE328 (4.74), LYS331 (4.94), HIS413 (5,10), CYS249 (5.10)
	Alkyl	CYS249 (4.08), ILE327 (4.17)





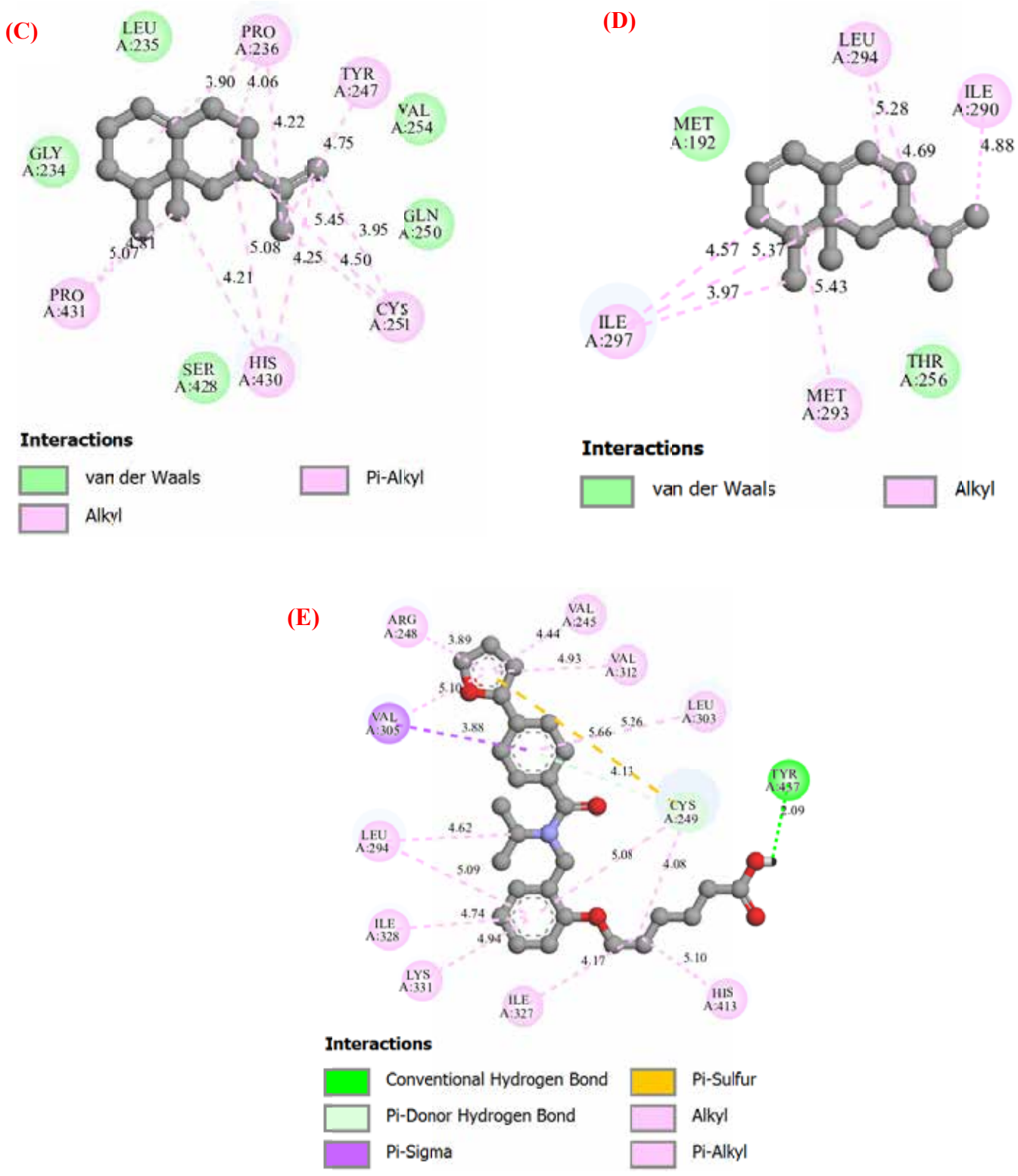


Fig 4. 2D interactions of top four ligands (A) Rimuene, (B) Gamma-dehydro-ar-himachalene, (C) Valencene, (D) Nootkatene and (E) Native ligand with the active site residues in the binding pocket of the receptor

Rimuene showed the highest docking score, interacting at the receptor's allosteric site through two hydrophobic interactions: Pi-alkyl with amino acid residue TRP220 and alkyl interactions with LYS224 and LEU235. Additionally, GLU223, GLN230,

ASN233, and HIS244 contributed *via* van der Waals interactions with reasonable bond distance (Figure 4A). Himachalane docked at the orthosteric site, interacting with amino acid residues THR256 through a Pi-donor hydrogen bond along with LEU297 and MET293 *via* alkyl interactions. Similarly, ILE297 was found to be interacted with aromatic ring through Pi-alkyl, and supportive van der Waals interactions (Figure 4B) with the structure of himachalane. Likewise, ligands valencene and nootkatene were entirely bound through hydrophobic interactions, including alkyl, Pi-alkyl, and van der Waals forces. Valencene interacted with PRO256, TYR247, PRO431, CYS430, and CYS251 at the allosteric site (Figure 4C), while nootkatene interacted with ILE297, MET293, LEU294, and ILE290 at the orthosteric site (Figure 4D). The predominance of hydrophobic interactions in the complexes could be the result from the ligands' active hydrophobic groups and absence of hydrophilic sites in the ligand.

The compounds identified from GC-MS analysis were taken as ligand in molecular docking study. Generally, GC-MS characterizes volatile and non-polar compounds which might be the major cause of the dominance of hydrophobic interactions in all ligand-receptor complexes. Two ligands, rimuene and valencene docked at the allosteric site, likely due to their structural compatibility and molecular recognition of ligands at allosteric site. Himachalane and nootkatene showed similar hydrophobic interactions to the native ligand, involving the common amino acid residue LEU294. It was found that the docking scores of these top ligands were slightly lower than that of the native ligand.The comparative study showed that rimuene and himachalane had docking scores comparable to the reference drug Seladelpar, while the other two ligands scored slightly lower (Table 2). The docking study indicated that rimuene and himachalane bound more effectively to the PPARδ receptoras compared to other selected ligands. To further assess complex stability, molecular dynamics simulations (MDS), quantitative structure-activity relationship (QSAR), and principal component analysis (PCA) are recommended. Additionally, *in vitro* and *in vivo* bio-characterization of rimuene and himachalane should be conducted to evaluate their potential in enhancing insulin sensitivity and reducing hyperglycemia through PPARδ modulation.

Acute Oral Toxicity Test

The obtained data for the acute oral toxicity test for the essential oil of plant *O. tenuiflorum* performed on under environmental conditions is presented in Table 4 along with a median lethal dose (LD_{50}). The data suggested that the essential oil of *O. tenuiflorum* is likely safe and causes little harm when taken orally, with no signs of toxicity observed in *in vivo* test [24]

Table 4. Median lethal dose (LD_{50}) of essential oil of *O. tenuiflorum*

Essential Oil of *O. tenuiflorum*	LD_{50} (mg/kg BW)	Hazard Statement	Remarks
	> 2000	May be harmful if swallowed	No death at 2000 mg/kg

Conclusions

The GC-MS analysis of the essential oil extracted from *O. tenuiflorum* identified 28 distinct compounds, with (E)-caryophyllene, β-elemene, and trans-isoeugenol being the most predominant. Acute oral toxicity testing demonstrated the oil's safety, with an LD 50 value exceeding 2000 mg/kg BW, indicating low toxicity. Computational molecular docking further highlighted rimuene and himachalane as the top candidates among the identified compounds, exhibiting strong binding affinities to PPARδ, and found comparable docking score to the reference drug Seladelpar. The ligand-receptor interactions were predominantly hydrophobic, reflecting the non-polar nature of the essential oil constituents. To validate the therapeutic potential of rimuene and himachalane as PPARδ agonists, further investigations involving advanced computational screening including molecular dynamics simulations, QSAR studies, along with bio-characterization through in *vitro/in vivo* methods are recommended. This work proposes top candidate found in *O. tenuiflorum* essential oil for the modulation of PPARδ activity which offer promising insights for diabetes management and drug discovery.

Acknowledgments

The authors acknowledge the Department of Plant Resources, Thapathali for GC-MS, and acute oral toxicity test.

References

1. A.D. Sharma, et al. Comparative phytochemistry, antioxidant, antidiabetic, and anti-inflammatory activities of traditionally used *Ocimum basilicum* L., *Ocimumgratissimum* L., and *Ocimum tenuiflorum* L., *BioTechnol*, 2022, 103(2), 131–376. https://doi.org/10.5114/bta.2022.116206
2. H.A. Yamani, et al. Antimicrobial activity of Tulsi (*Ocimum tenuiflorum*) essential oil and their major constituents against three species of bacteria, *Front in Microbiol*, 2016, 7 (MAY), 1–10. https://doi.org/10.3389/fmicb.2016.00681
3. M.N. Boukhatem, &W. N. Setzer. Aromatic herbs, medicinal plant-derived essential oils, and phytochemical extracts as potential therapies for coronaviruses: Future perspectives, *Plants*, 2020, 9(6), 800. https://doi.org/10.3390/plants9060800
4. A. Piras et al. *Ocimum tenuiflorum* L. and *Ocimum basilicum* L., two spices of Lamiaceae family with bioactive essential oils, *Ind Crops and Prod*, 2018, 113, 89–97. https://doi.org/10.1016/j.indcrop.2018.01.024
5. L.S. Naik et al. Antimicrobial activity and phytochemical analysis of *Ocimum tenuiflorum* leaf extract, *Int J PharmTech Res*, 2015, 8(1), 88–95.
6. P. Malav, et al. Morphological variability in holy basil (*Ocimum tenuiflorum* L.) from India, *Genet Resour and Crop Evol*, 2015, 62(8), 1245–1256. https://doi.org/10.1007/s10722-015-0227-5
7. S. Parasuraman, et al. Evaluation of antidiabetic and antihyperlipidemic effects of hydroalcoholic extract of leaves of *Ocimum tenuiflorum* (Lamiaceae) and prediction of biological activity of its phytoconstituents, *Pharmacognosy Res*, 2015, 7(2), 156–165. https://doi.org/10.4103/0974-8490.151457
8. L. Mousavi, et al. Phytochemical and bioactive compounds identification of *Ocimum tenuiflorum* leaves of methanol extract and its fraction with an antidiabetic potential, *Int J of Food Prop*, 2018, 21(1), 2390–2399. https://doi.org/10.1080/10942912.2018.1508161
9. [report] IDF Diabetes Atlas. International Diabetes Federation. https://diabetesatlas.org/atlas/tenth-edition/; 2021 [accessed 19 December 2003].
10. U. Galicia-Garcia, et al. *Costus ignus*: insulin plant and its preparations as remedial approach for diabetes mellitus, *Int J of Mol Sci*, 2020, 1–34.
11. M. Cao, et al. Activation of PPARδ up-regulates the expression of insulin gene transcription factor MafA and ameliorates glucose-induced insulin secretion impaired by palmitate, *Mol Cell Biochem*, 2012, 366 (1–2), 183–189. https://doi.org/10.1007/s11010-012-1296-9
12. F. Hong, et al. PPARs as nuclear receptors for nutrient and energy metabolism. *Molecules,* 2019, 24(14), 2545. https://doi.org/10.3390/molecules24142545
13. A. Tamang, et al. Chemical analysis and biological studies of leaf extracts of *Smallanthus sonchifolius* (Poepp.) H. Rob. with an *in-silico* assessment of GC-MS identified compounds, *South Asian Res J of Nat Prod*, 2024, 7(3), 279–297.
14. S. Kim, et al. PubChem 2023 Update, *Nucleic Acids Res.* 2023; 51(D1) :D1373–D1380. https://doi.org/10.1093/nar/gkac956
15. M.D. Hanwell,et al. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform *J. Cheminform*. 2012; 4:17.https://doi.org/10.1186/1758-2946-4-17
16. O. Trott ,& A.J. Olson. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, *J Comput Chem*. 2010; 31(2) :455–461. https://doi.org/10.1002/jcc.21334
17. S.K. Burley, et al. RCSB protein data bank (rcsb.org): delivery of experimentally-determined pdb structures alongside one million computed structure models of proteins from artificial intelligence/machine learning, *Nucleic Acids Res.* 2023; 51(D1) :D488–508. https://doi.org/10.1093/nar/gkac1077

18. S. Yuan, H.C.S. Chan, & Z. Hu. Using PyMOL as a platform for computational drug design, *Wiley Interdiscip Rev Comput Mol Sci.* 2017; 7(2) :e1298. https://doi.org/10.1002/wcms.1298

19. S.Y. Huang. Comprehensive assessment of flexible-ligand docking algorithms: Current effectiveness and challenges, *Briefings in Bioinformatics*, 2018, 19(5), 982–994. https://doi.org/10.1093/bib/bbx030

20. DS. Biovia Discovery studio visualizer v21. 1.0. 20298, *San Diego Dassault Systèmes.* 2021.

21. S. Sanpinit, et al. Characterization of the antioxidant activity, identified free radical-relieving components by LC/QTOF/MS and acute oral toxicity studies of Tri-Tharn-Thip tea, *J Agric Food Res, 2024*, 16, 101131.https://doi.org/10.1016/j.jafr.2024.101131

22. R. Ranjitkar, et al. Acute toxicity test of ten commercial essential oils of Nepalese origin, *J Plant Res*, 2019, 17(1), 82–85.

23. P. Thanait, et al. α-glucosidase inhibitory activity of compounds from the essential oil of *Leucas lavandulifolia* Sm.: insights from GC-MS analysis and molecular docking studies, *Asian J. Chem. Sci.* 2024; 14(4):47–66. https://doi.org/10.9734/ajocs/2024/v14i4317

24. B. Olela, et al. Acute oral toxicity and anti-inflammatory and analgesic effects of aqueous and methanolic stem bark extracts of *Piliostigma thonningii* (Schumach.),*Evid Based Complement Altern Med*, 2020, 2020(1). https://doi.org/10.1155/2020/5651390