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Original Research Article

GC–MS PROFILING AND MOLECULAR DOCKING REVEAL MULTITARGET ANTIDIABETIC PHYTOCONSTITUENTS FROM *MUCUNA PRURIENS* (L.) DC. LEAVES

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ABSTRACT

Mucuna pruriens leaf has attracted increasing attention as a potential therapeutic agent for the management of type 2 diabetes mellitus (T2DM), particularly in low-resource settings where affordable treatment options are needed. This study investigated the phytochemical composition and in silico antidiabetic potential of *M. pruriens* leaf extract. Gas chromatography–mass spectrometry (GC–MS) analysis identified thirty-three bioactive compounds, predominantly unsaturated fatty acids, terpenoids, alcohols, and cyclic hydrocarbons. The major constituents were oleic acid (12.78%), 1,3-dioxolane (9.31%), trans-13-octadecenoic acid (8.69%), oleic acid derivatives (8.54%), and *n*-hexadecanoic acid (6.43%). Molecular docking against α-amylase, α-glucosidase, and dipeptidyl peptidase-IV (DPP-IV) revealed several compounds with binding affinities comparable to or greater than those of metformin. Trans-13-octadecenoic acid and 11,13-dimethyl-12-tetradecen-1-ol exhibited the strongest interactions with α-glucosidase (−6.4 kcal/mol), while 3,7,11-tridecatrienoic acid demonstrated high affinity for α-amylase (−6.4 kcal/mol) and DPP-IV (−5.6 kcal/mol). Oleic acid derivatives consistently showed favorable binding across all target enzymes. Ligand–protein interactions were stabilized by hydrogen bonding, hydrophobic interactions, and van der Waals forces within the enzyme active sites. These findings suggest that *M. pruriens* leaf contains bioactive phytochemicals with multitarget inhibitory potential against key enzymes involved in glucose metabolism, supporting its promise as a source of affordable plant-derived therapeutics for the management of T2DM.

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KEYWORDS

Mucuna pruriens; Type 2 diabetes mellitus; Bioactive Compounds; molecular docking; antidiabetic activity

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which occurs when there is insufficient or no insulin secretion by β-cells of the pancreas (Type 1) or when the body is resistant to insulin or does not make enough insulin (Type 2). On the other hand, Gestational diabetes mellitus (GDM) is caused by hormone of pregnancy or shortage of insulin characterized by a degree of glucose intolerance with first recognition in the second or third trimester of pregnancy [1]. According to International Diabetes Federation (IDF), Type 2 DM is the most common type of diabetes, accounting for about 90% of all diabetic cases especially in adults [2]. In the past 3 decades, the number of people living with type 2 diabetes has risen dramatically in countries of all income levels [3]. World Health Organization (WHO) indicated that

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type 2 DM is characterized by high level of blood glucose which over time leads to serious damage to the heart, blood vessels, eyes, kidneys and nerves [4]. Additionally, Reactive oxygen species (ROS) plays a key role in progression of type 2 diabetes by insulin suppression and beta-pancreatic cell dysfunction [5]. According to WHO, about 422 million people worldwide have DM, the majority living in low-and middle-income countries, and 1.5 million deaths are directly attributed to diabetes each year. There is a globally agreed target to halt the rise in diabetes and obesity by 2025.

Healthy nutrition coupled with sufficient physical activity is crucial for diabetes management [6]. Proper administration of hypoglycemic medications and insulin has so far proven to be effective and safe in older patients [7]. However, studies by [3] have shown that despite the availability and effectiveness of oral hypoglycemic medications, 5–10% of treatment develops secondary failure which occurs when beta-cell activity deteriorates, medication adherence is poor coupled with weight gain, less exercise, dietary changes, or illness [8]. In low-income countries, the major drawback in management of DM is scarcity and high cost of pharmaceutical medications and treatment as well as the negative side effects associated with them [3]. Moreover, some of the drugs for management of type 2 diabetes have major side effects. For instance, metformin possesses serious side effect such as lactic acidosis [9] while glipizide has been implicated in digestive problems such as nausea, diarrhea or constipation [10].

The use of medicinal plants for management of disease conditions has increased throughout the world and a lot of studies showed extreme potential in the treatment of various diseases [11]. Herbal medicines are gaining popularity in the management of diabetes mellitus owing to popular belief in their safety and no side effect [12]. Specifically, *Mucuna pruriens* (MP) is well known medicinal plant for treatment of various ailment in folkloric medicine especially liver and kidney diseases [12]. Previous studies have demonstrated that the plant contains various phytochemicals with inherent efficacy in treatment of many degenerative diseases [13, 14, 15]. However, despite existing research findings supporting MP leaf and seed extracts effectiveness in treating anemia, liver diseases, kidney diseases and DM type 1, the actual mechanism of action of MP leaves extracts for type 2 diabetes remains pending. This draws special attention to the necessity for additional research to meticulously elucidate antidiabetic efficacy of MP leaf extracts against type 2 diabetes which accounts for 90% of all cases of diabetes. The aim of this work is to identify the bioactive compounds in leaf extract of *Mucuna pruriens* and carry out *in silico* molecular docking of identified compounds against enzymes implicated in diabetes mellitus type 2.

MATERIALS AND METHODS

Reagents. Ethanol, hexane, anhydrous sodium sulfate, petroleum ether and pure helium gas (99.995%)

Extraction of phytochemicals

The fresh leaves of *Mucuna pruriens* were obtained from Nnewi, Anambra State, Nigeria and was confirmed by a plant taxonomist, in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The plant leaves specimen was deposited at the departmental herbarium and a voucher number (257A) was obtained. The plant leaves specimen was deposited at the departmental herbarium and a voucher number (257A) was obtained. The leaves were cleaned and air dried for eight days and then pulverized into powder using a grinder. Briefly, 0.2g of sample was weighed and transferred in a test tube and 15ml of ethanol was added. The test tube was allowed to react in a water bath at 60^0C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution as dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of petroleum ether of which 200ul was transferred to a vial for analysis.

Identification of Bioactive compounds by GC-MS

Phytochemical analysis of the *Mucuna pruriens* leaf extract was performed using a BUCK Scientific M910 gas chromatograph coupled to a mass spectrometer (GC–MS), fitted with an HP-5MS capillary column (30 m × 250 µm × 0.25 µm film thickness). Mass spectrometric detection was performed using an electron ionization (EI) source with an electron energy of 70 eV. The instrument was manufactured by Buck Scientific, USA. Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the

extracts diluted with respective solvents was injected in an splitless mode. Relative quantity of the chemical compounds present in each of the extracts was expressed as percentage based on peak area produced in the chromatogram. Bioactive compounds extracted from different extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

Molecular docking studies

The 3D structures of the enzymes (Alpha amylase, Alpha glucosidase and Dipeptidyl Peptidase-IV) were retrieved from Protein Data Bank. The crystal structure of the enzymes were prepared by removing solvent molecules, co-crystallized ligands and optimized to simulate physiological conditions using Chimera v 1.11 [16]. Polar hydrogen were added and partial charges were assigned to the standard residue using Gasteiger partial charge, which assumes all hydrogen atoms are represented explicitly. The compounds were obtained from PubChem and the geometry were optimized using Avogadro (V 1.2.0). The optimized compounds were also prepared for docking studies using same protocol and the optimized structure obtained. The most favorable binding interactions of the compounds with the various enzymes were determined by molecular docking studies using AutoDock vina v.1.1.2. The interaction of the docked complexes were studied visually with the help of Discovery Studio 2017 R2 Client (v17.2.0.16349) and UCSF Chimera (V 1.17.3). AutoDock Vina uses conformation-dependent algorithm to score ligands binding interactions.

RESULTS

The GC–MS analysis of the *Mucuna pruriens* ethanol leaf extract putatively identified several phytochemical constituents. The compounds were characterized based on their mass-to-charge ratios (*m/z*) and fragmentation patterns, with each peak in the chromatogram corresponding to a distinct compound (Fig. 1). The GC–MS analysis of the ethanol extract revealed the presence of diverse bioactive constituents, predominantly unsaturated fatty acids, alcohols, and cyclic hydrocarbons (Table 1) with oleic acid (12.78%), trans-13-octadecenoic acid (8.69%), and n-hexadecanoic acid (6.43%) the most dominant. The result (Table 2) presented molecular docking of several natural compounds against three key enzymes involved in carbohydrate metabolism: α-amylase, α-glucosidase, and Dipeptidyl Peptidase-IV. These enzymes are important in glucose regulation, so inhibitors can have potential antidiabetic effects. The binding energies (in kcal/mol) which indicate the strength of interaction: more negative values suggest stronger binding affinity. Several compounds showed notable binding affinities, comparable or even stronger than the reference drug Metformin. Strongest α-amylase binders were 3,7,11-Tridecatrienoic acid (Peak 20, -6.4 kcal/mol), 6-Octadecenoic acid (Peak 10, -5.8 kcal/mol), p-Menth-8(10)-en-9-ol (Peak 11, -5.7 kcal/mol). These values are stronger than Metformin (-4.5 kcal/mol). Strongest α-glucosidase binders were Hexadecenoic acid (Peak,-6.3 kcal/mol) Trans-13-Octadecenoic acid (Peak 6, -6.4 kcal/mol), 11,13-Dimethyl-12-tetradecen-1-ol (Peak 8, -6.4 kcal/mol). Metformin scored -5.4 kcal/mol, so these natural compounds show better affinity. Strongest Dipeptidyl Peptidase-IV binders were 3,7,11-Tridecatrienoic acid (Peak 29, -5.6 kcal/mol), Oleic acid variants (Peaks 9, 12, 14, 18, 24) consistently showed -5.1 to -5.5 kcal/mol. Metformin scored -4.2 kcal/mol, again weaker than several natural compounds.

The results in Table 3 showed the top five unsaturated fatty acids and related derivatives which exhibited notable inhibitory potential against carbohydrate-metabolizing enzymes, with *Trans-13-Octadecenoic acid*, *11,13-Dimethyl-12-tetradecen-1-ol*, and *Hexadecenoic acid* demonstrating strong binding to α-glucosidase (−6.4 to −6.3 kcal/mol). *3,7,11-Tridecatrienoic acid* displayed multi-target affinity toward α-amylase and Dipeptidyl Peptidase-IV (−6.4 to −6.0 kcal/mol), while *6-Octadecenoic acid* showed moderate binding to α-amylase (−5.8 kcal/mol). Additionally, the docking Figures (Fig. 2-5) illustrated hydrogen bonding, hydrophobic interactions, and van der Waals forces that stabilize these complexes.

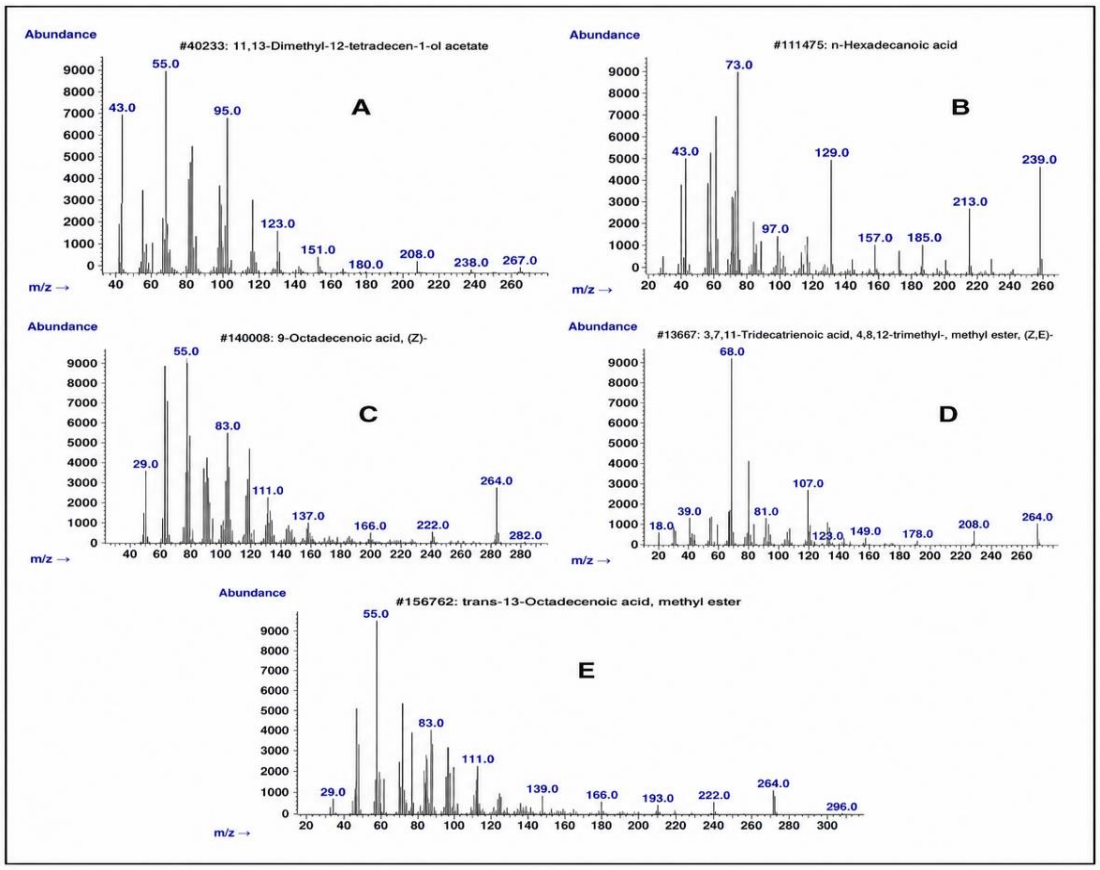
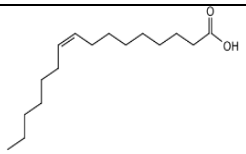
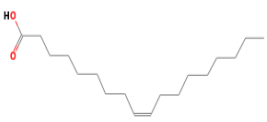
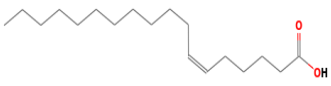
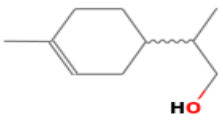


Fig. 1 Mass spectra of selected bioactive constituents from *M. pruriens* leaf extract: 11, 13-Dimethyl-12-tetradecen-1-ol (A); Hexadecenoic acid (B); 6-Octadecenoic acid (C); 3, 7, 11-Tridecatrienoic acid (D) and Trans-13-Octadecenoic acid (E).

Table 1 Selected bioactive compounds identified in *Mucuna pruriens* leaf ethanol extract via GC-MS analysis

PEAK	RT	COMPOUND NAME	STRUCTURE	% COMPOSITION
3	16.99	Hexadecenoic acid		3.67
6	18.804	Trans-13-Octadecenoic acid		8.69
8	19.217	11,13-Dimethyl-12-tetradecen-1-ol		0.43
9	19.434	Oleic acid		12.78
10	19.601	6-Octadecenoic acid		7.17
11	19.795	p-Menth-8(10)-en-9-ol		0.67

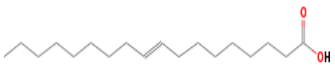
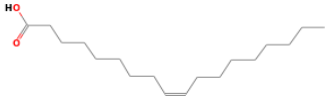
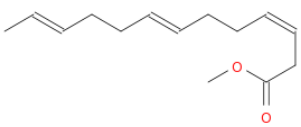
13	19.981	9-octadecenoic acid		4.50
14	20.228	Oleic acid		8.54
15	20.328	9-Octadecenoic acid		1.65
20	21.044	3,7,11-Tridecatrienoic acid		2.35

Table 2 Binding energies of identified bioactive compounds and standard drug (metformin) against α-amylase, α-glucosidase and Dipeptidyl Peptidase-IV

Peak	Compound	Pubchem ID	Binding Energy (Kcal/mol) α-amylase	Binding Energy (Kcal/mol) α-glucosidase	Binding Energy (Kcal/mol) Dipeptidyl Peptidase-IV
1	3-Methyl-6-hepten-1-yn-3-ol	142821	-4.6	-5.7	-4.2
2	Citral	638011	-4.9	-6.0	-4.5
3	Hexadecenoic acid	445638	-4.1	-6.3	-5.1
4	n-Hexadecanoic acid	985	-3.9	-6.2	-4.8
5	Cyclopentaneundecanoic acid	534549	-3.9	-5.5	-4.9
6	Trans-13-Octadecenoic acid	6161490	-2.4	-6.4	-5.1
7	Methyl stearate	8201	-1.8	6.0	-4.8
8	11,13-Dimethyl-12-tetradecen-1-ol	85809149	-5.6	-6.4	-4.9
9	Oleic acid	445639	-5.2	-4.8	-5.3
10	6-Octadecenoic acid	5282754	-5.8	-4.8	-5.0
11	p-Menth-8(10)-en-9-ol	543946	-5.7	-5.4	-4.0
12	Octadic-9-enoic acid	445639	-5.4	-4.7	-5.3
13	9-octadecenoic acid	637517	-5.4	-4.9	-5.4
14	Oleic acid	445639	-5.5	-4.8	-5.2
15	9-Octadecenoic acid	637517	-5.3	-5.0	-5.2
16	1,3-Dioxolane	12586	-5.3	-2.7	-2.9
17	Cyclopropane	6351	-2.3	-2.2	-2.6
18	Octadec-9-enoic acid	965	-5.5	-5.0	-5.1
19	9,17-Octadecadienal	6431297	-4.9	-5.1	-4.6
20	3,7,11-Tridecatrienoic acid	10422241	-6.4	-4.7	-5.4
21	Tris (trimethylsiloxy) silane	6329086	-3.6	-3.5	-3.2
22	Glyceric acid	752	-4.0	-4.2	-4.0
23	2,5-furandione	7923	-4.2	-3.9	-4.3
24	Oleic acid	445639	-4.4	-4.9	-5.5
25	(1R,4R)-1-methyl-4-(6-Methlhept-5-en-2-yl) cyclohex-2-enol	13213649	-5.8	-5.9	-5.2
26	1H-Indene	7219	-4.5	-5.4	-3.8
27	1,2-hydrazinedicarbothioamide	2724564	-3.9	-4.0	-3.7
28	9-Octadecenoic acid	637517	-4.4	-4.8	-3.7
29	3,7,11-Tridecatrienoic acid	10422241	-5.2	-6.0	-5.6
30	Benzoic acid	243	-5.0	-5.1	-4.2
31	3,7,11-Tridecatrienoic acid	10422241	-5.5	-6.0	-5.1
32	6-Octadecenoic acid	5282754	-4.4	-4.6	-5.1
33	Bicyclo(4.2.0)oct-2-ene	21430317	-5.1	-4.7	-3.6

34	Metformin	4091	-4.5	-5.4	-4.2

Table 3 Selected bioactive compounds identified by GC-MS in *Mucuna pruriens* leaf extract and their predicted binding affinities to diabetes-related enzymes

Rank	Compound	Peak	Binding Energy (kcal/mol)	Target Enzyme
1	Trans-13-Octadecenoic acid	6	-6.4	α-Glucosidase
2	11,13-Dimethyl-12-tetradecen-1-ol	8	-6.4	α-Glucosidase
3	3,7,11-Tridecatrienoic acid	20/29/31	-6.4 / -6.0 / -6.0	α-Amylase & Dipeptidyl Peptidase-IV
4	Hexadecenoic acid	3	-6.3	α-Glucosidase
5	6-Octadecenoic acid	10	-5.8	α-Amylase

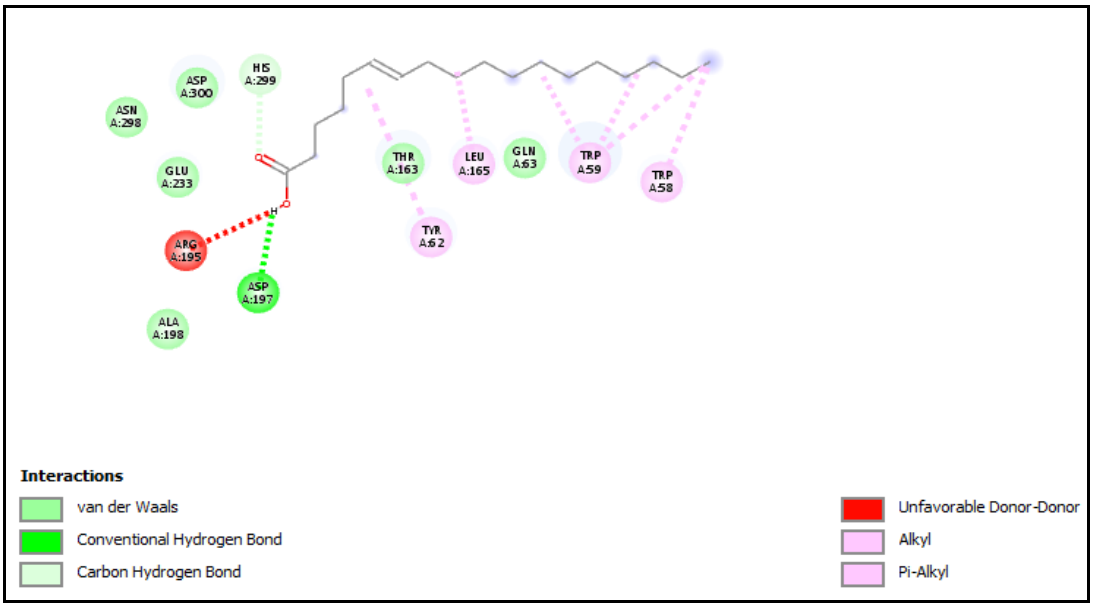


Fig. 2 6-Octadecenoic acid docked to α- amylase (2D interactions)

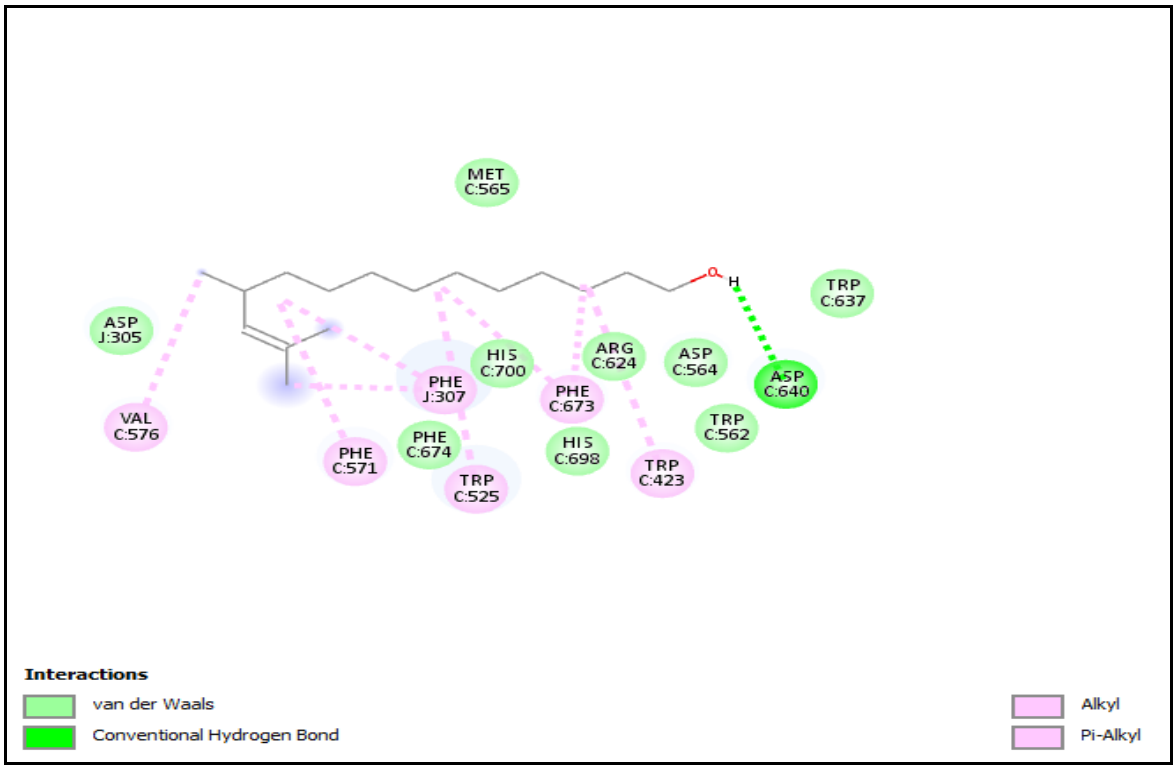


Fig. 3 11,13-Dimethyl-12-tetradecen-1-ol docked to α- glucosidase (2D interactions)

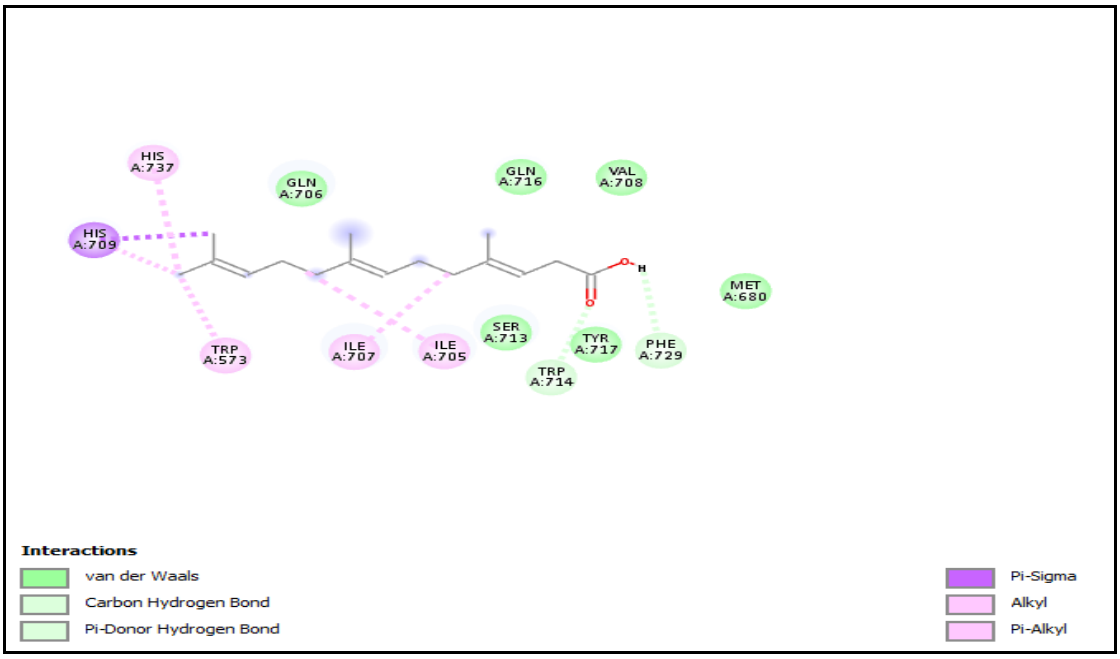


Fig. 4 3,7,11-Tridecatrienoic acid docked to Dipeptidyl Peptidase-IV (2D interactions)

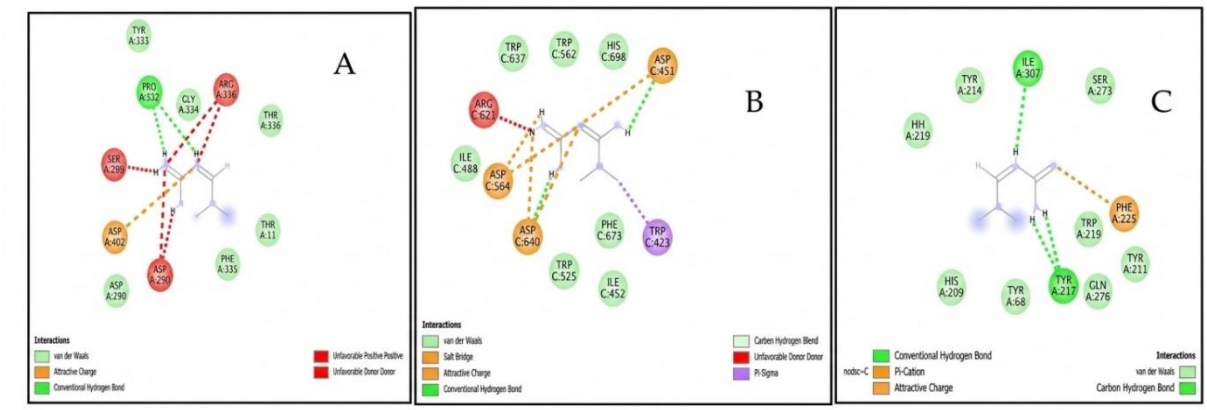


Fig. 5 Metformin docked to α-amylase (A), α-glucosidase (B) and Dipeptidyl Peptidase-IV (C)

DISCUSSION

The dominance of oleic acid (12.78%), trans-13-octadecenoic acid (8.69%), and n-hexadecanoic acid (6.43%) aligns with previous studies reporting these compounds as major constituents in plant-derived oils with anti-inflammatory, antioxidant, and antimicrobial activities [17,18]. The occurrence of oleic acid in particular corroborates findings by [19] identified it as a key lipid conferring membrane-stabilizing and hypocholesterolemic properties in medicinal plants. The identification of 1,3-dioxolane (9.31%) is noteworthy, as this compound class has been linked to antifungal and antibacterial activity, consistent with earlier GC–MS reports by [20] in *Eclipta alba* extracts. Similarly, the detection of citral (1.43%) and p-menth-8(10)-en-9-ol (0.67%) suggests the extract may possess aromatic terpenoid components, which are known to contribute to flavor, fragrance, and therapeutic bioactivity, in agreement with findings by [21]. The predominance of long-chain fatty acids such as hexadecenoic acid, methyl stearate, and cyclopentaneundecanoic acid indicates that the extract could serve as a potential source of natural lipophilic antioxidants. This observation supports reports by [22], who demonstrated that hexadecanoic acid derivatives exhibit bactericidal and anti-inflammatory effects by modulating lipid peroxidation and nitric oxide synthesis. The presence of minor constituents such as benzoic acid (1.09%), 2,5-furandione (0.54%), and 1H-indene (0.43%) further reinforces the chemical diversity of the extract. Benzoic acid derivatives are recognized natural preservatives with antifungal potential [23]

Fatty acids (oleic acid, octadecenoic acid, hexadecenoic acid) repeatedly show strong binding, suggesting they may play a role in enzyme inhibition. Terpenoid-like compounds (citral, menthol derivatives) also demonstrated good activity. Metformin, while effective clinically, showed weaker binding scores compared to many natural compounds, highlighting the potential of these phytochemicals as complementary or alternative inhibitors. Previous studies by [24] reported that oleic acid exhibits inhibitory activity against α-glucosidase, suggesting its role in reducing postprandial hyperglycemia. Additionally, [25] also highlighted oleic acid derivatives as natural enzyme inhibitors with antidiabetic potential. While direct studies on 3,7,11-tridecatrienoic acid are limited, [26] emphasized that polyunsaturated fatty acids (PUFAs) can regulate glucose metabolism and enzyme activity. Overall, the chemical composition pattern obtained is consistent with those observed in bioactive extracts from tropical medicinal plants, confirming that the solvent extraction method employed (ethanol followed by petroleum ether) effectively isolates both polar and non-polar secondary metabolites. The significant representation of oleic and linoleic acid analogues highlights the extract's potential for nutraceutical and pharmaceutical applications, particularly in oxidative stress–related conditions.

The binding energy analysis identified top five compounds with notable affinities toward carbohydrate-metabolizing enzymes (Table 3). Among these, *Trans-13-Octadecenoic acid* and *11,13-Dimethyl-12-tetradecen-1-ol* exhibited the strongest interactions with α-glucosidase (−6.4 kcal/mol), suggesting potential utility as inhibitors of postprandial glucose release. Similarly, *Hexadecenoic acid* demonstrated comparable binding strength (−6.3 kcal/mol) to the same enzyme, reinforcing the relevance of fatty acid derivatives in modulating glucosidase activity. Interestingly, *3,7,11-Tridecatrienoic acid* displayed multi-target binding to α-amylase and Dipeptidyl Peptidase-IV with energies ranging from −6.4 to −6.0 kcal/mol, indicating broader inhibitory potential

across complementary pathways of carbohydrate hydrolysis. In contrast, *6-Octadecenoic acid* showed moderate affinity (−5.8 kcal/mol) toward α-amylase, which, while weaker than the top candidates, remains biologically significant. Consistent with previous findings**,** the leaf extract demonstrated *in vitro* α-amylase inhibitory activity comparable to the standard drug**,** acarbose [27]**.** Overall, these findings highlight unsaturated fatty acids and related derivatives as promising scaffolds for enzyme inhibition. The predominance of α-glucosidase inhibition suggests potential therapeutic relevance in glycemic control, though further experimental validation is required to assess bioavailability, specificity, and safety.

The docking Figures (Figure 2-5) illustrated hydrogen bonding, hydrophobic interactions, and van der Waals forces that stabilize these complexes. Studies by [28] demonstrated that palmitoleic acid improves insulin sensitivity and modulates glucose metabolism, indirectly supporting its inhibitory role on carbohydrate-metabolizing enzymes. Altogether, the results suggested that natural compounds such as tridecatrienoic acid, hexadecenoic acid, and oleic acid derivatives may serve as promising leads for antidiabetic drug development. Their binding affinities are stronger than Metformin in silico, indicating potential biological relevance. According to [29], Metformin's mechanism indicated that it primarily acts via AMPK activation rather than direct enzyme inhibition. This explains why the docking results showed weaker binding energies compared to natural fatty acids.

CONCLUSION

The findings of this study demonstrate that *Mucuna pruriens* leaf extract contains diverse bioactive compounds with promising antidiabetic potential. GC–MS analysis identified thirty-three phytoconstituents, predominantly unsaturated fatty acids and their derivatives, while molecular docking revealed that several compounds exhibited stronger binding affinities than metformin against α-amylase, α-glucosidase, and Dipeptidyl Peptidase-IV. In particular, trans-13-octadecenoic acid, 11,13-dimethyl-12-tetradecen-1-ol, and 3,7,11-tridecatrienoic acid emerged as the most promising candidates for enzyme inhibition. These findings provide mechanistic support for the traditional use of *Mucuna pruriens* in diabetes management and suggest that its bioactive constituents may serve as potential lead compounds for the development of novel plant-derived antidiabetic agents. However, further in vitro, in vivo, and clinical studies are required to validate their efficacy, safety, and pharmacological potential.

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AUTHORS' CONTRIBUTION

CPU – Data acquisition, manuscript preparation, literature search, manuscript editing. SCM – Extract analysis, Data analysis, manuscript review. CEU – Concept and design, manuscript review, manuscript final approval. CCD – Laboratory analysis, resource planning. All authors read and approved the final manuscript

CONFLICT OF INTEREST

The authors of this work declare that they have no conflict of interest

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