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RESEARCH ARTICLE

Phytochemical and *In-Silico* Evaluation of the Pharmaceutically Important Biomolecules Present in Leaf and Seed Extracts of *Mucuna pruriens.*

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ABSTRACT

The present research aimed to identify the bioactive compounds present in two varieties of *Mucuna pruriens* var *utilis* (IIHR Selection 3 and Arka Dhanvantari) and their pharmaceutical importance evaluated through phytochemical and *in-silico* screening. The phytochemical screening of the leaf and seed extracts revealed the presence of alkaloids, tannins, flavonoids, phenols, proteins, terpenoids, glycosides, steroids, saponins etc. A total of 43 compounds were identified in the gas chromatography and mass spectrometry (GC-MS) analysis, and the identified biological active compounds were used for *in-silico* analysis. *In-silico* docking study revealed potent inhibition of the selected compounds: alpha-D-glucopyranoside, ethyl iso-allocholate and sitosterol against uncoupling protein 2 receptor (UCP2) involved in type 2 diabetes, showing that the binding profiles towards key amino acid residues in the active site were similar to that of the commercial alpha-amylase inhibitors: voglibose, acarbose and glibenclamide. Furthermore, the potent inhibition of another selected compound: 3-(4-hydroxyphenyl) propanoic acid against DJ-1 receptor (PARK7) involved in Parkinson's disease was determined by docking and it was observed that the binding profile towards key amino acid residues in the active site was similar to that of commercial levodopa. Therefore, *M. Pruriens* extracts can be explored in managing type-2 diabetes and Parkinson's disease.

Key words: *Mucuna pruriens; GC-MS; Biomolecules; Ligand-based docking; Type-2 diabetes; Parkinson's disease*

INTRODUCTION

Human beings have used plants to treat diverse ailments for thousands of years. According to World Health Organization, most population still rely on traditional medicines for their health requirements and herbal remedies are considered dietary supplements for disease prevention and alternative/complementary medicine (Wooand El-Nezami, 2012). Rural areas of many developing countries depend on traditional medicine because they are relatively safer and cheaper than synthetic or modern medicine (Hassan *et al.,* 2009). India has a rich culture of medicinal herbs and spices, which includes more than 2000 species and a vast geographical area with high potential for Ayurveda, Unani and Siddha traditional medicines. However, only very few, including *Mucuna pruriens* have been comprehensively studied chemically and pharmacologically for their potential medicinal value (Ashish *et al*., 2011).

Mucuna pruriens is an annual twinning crop belonging to the family of *Fabaceae* and India is one of the natural centers of origin in the world (Eilittä and Carsky 2003, Eilittä *et al.,* 2002). It is grown as a food crop, ornamental plant, living mulch, and green manure crop. The seeds of *M. pruriens* contain

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alkaloids, mucunine, mucunadine, mucunadinine, prurieninine, pruriendine, nicotine, beta-sitosterol, glutathione, lecithin, vernolic and gallic acids. They also contain several bioactive substances such as tryptamine, alkylamines, steroids, flavonoids, coumarins, cardenolides, serotonin, oxitriptan, nicotine, and bufotenine (Jellin 2010). The presence of L-3 4-dihydroxy phenylalanine (L-DOPA), which is a constituent of more than 200 indigenous drug formulations, is documented in the seed, stem, leaves and roots of *M. pruriens.*

The physicochemical properties of *M. pruriens* such as molecular mass, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) have been reported by researchers and can be obtained from ProtParam and EXPASy servers (Emanuelsson *et al.,* 2007). The model building or comparative modeling is one of the most robust methods for protein structure prediction and can be supplemented for protein structure prediction of various compounds based on the experimentally determined structure of another homologous protein (Franca 2015).

For L-DOPA isolated from *Mucuna pruriens*, the position-specific iterated BLAST (PSI-BLAST) against Protein Data Bank (PDB) was studied by (Wang *et al.,* 2015) to identify their homologous structures based on the maximum identity with high score and lower e-value, which shows the quantitative structure-activity relationship between the bioactive targets as one of the promising tools in drug discovery, while the 3D structure was predicted using automated Swiss-Model server by Arnold *et al.,* 2006 and Biasini *et al.,* 2014. Globally, medicinal plants are used as crude extracts, and most of the potent and active substances are employed as isolated compounds hence the present study was designed to identify the bioactive compounds present in *M. Pruriens* and to evaluate the selected secondary metabolites with pharmaceutical importance, through phytochemical and *in-silico* screening. The presence of different phytochemical compounds was confirmed, of which eight amongst them were narrowed down to their pharmacological activity, and it was observed that they possess either antidiabetic or anti-Parkinson's activity.

MATERIALS AND METHODS

Source of plant samples and chemicals

Seeds of two varieties of *Mucuna pruriens* var *utilis:* [IIHR Selection 3 (S3) and Arka Dhanvantari AD)] were collected from the Indian Institute of Horticultural Research (IIHR) Bengaluru, India (Figure 1). Identification of the seeds was carried out by IIHR. The plants were raised and maintained in the greenhouse for further analysis (Figure 2). Analytical grade chemicals used for preparing the stock solutions were purchased from Sigma-Aldrich chemicals and Himedia laboratories, India.

Fig 1. Source of crude seed extracts for phytochemical analysis.

A- Seeds of *M. pruriens* var *utilis* (*IIHR selection 3*).

B- Seeds of *M. pruriens* var *utilis* (*Arka Dhanvantari*).

Phytochemical screening

The seeds and leaves of S3 variety and AD variety were ground into a fine powder and crude extracts were obtained with seven different solvents: ethanol, methanol, chloroform, diethyl ether, hexane, ethyl acetate and water. The crude extracts were evaluated for the presence of active phyto-constituents such as alkaloids, tannins, flavonoids, phenols, proteins, terpenoids, reducing sugars, glycosides, carbohydrates and saponins using standard protocol (Siddiqui and Ali 1997). Detection of alkaloids was carried out using wagner's and dragendroff's tests. Detection of tannins, flavonoids, and phenols were carried out using ferric chloride, lead acetate, and ferric chloride test respectively. Test for reducing sugar was also conducted.To detect the presence of proteins, ninhydrin and millon's tests were carried out. Salkowaski and copper acetate tests were carried out to detect terpenoids and steroids. For detection of saponins, froth tests were conducted. To test for the presence of carbohydrates and glycosides, Molisch's and Legal's tests were conducted.

Fig 2. Source of leaf extracts.

A- 15 days old (IIHR selection 3)plants in the greenhouse.

B- 95 days old (Arka Dhanvantari) plants with matured pods in the greenhouse.

Gas Chromatography – Mass Spectrometry Analysis (GC-MS)

Preparation of the extract for GC-MS analysis

The extract was prepared according to the protocol described by Rahul *et al.* (2012) with appropriate modification. Five (5) g of the powdered seed was soaked in 25 mL methanol for 48 hours. The extract was centrifuged at 5000 xg for 5 min, then filtered through Whatman filter paper No. 41 along with 0.5 g sodium sulfate to remove any sediments and traces of water in the filtrate, and finally passed through a polyvinylidinedifluoride membrane filter of 0.22 micron (PDVF, Thermo Fisher India). The extract contained both polar and non-polar phytocomponents of the plant material used.

Analysis of extract through GC–MS

GC-MS analysis of methanol seed extract of *M. pruriens* was performed using Trace GC Ultra gas chromatograph connected to a Quantum XLS mass spectrometer (MS-DSQII, Detector-ECD): (GC-Thermo Trace GC Ultra Thermo Scientific, USA). GC was equipped with TG-5MS capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) consisting of a stationary phase 5% phenyl and 95% methyl polysiloxane. The injection was carried out in CT split-less mode at an injector temperature of 260 °C. Helium gas was used as a carrier gas with a 1.0 mL/min flow rate. The oven temperature programming was as follows: the initial oven temperature was held at 70 °C for 2.0 min, and then increased to 210 °C at a rate of 20° C/min, and then increased to 290 °C at a rate of 10° C/min held for 13.0 min. The ion source and transfer line temperature were 220 °C and 290 °C, respectively.

Identification of the compounds

The various compounds present in the methanol seed extract of *M. Pruriens* were detected by GC-MS. Identification and interpretation of each compound's mass spectrum was carried out using the database of National Institute Standard and Technology (NIST) available in the instrument. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library.

Table 1a. Phytochemical analysis of *M. pruriens* (S3) seed extract

Test	Reagents	Solvents extracts						
		Ethanol	Methanol	Chloroform	Hexane	Water	Diethyl ether	Ethyl acetate
Alkaloids	Dragendroff	-	-	+	+	+	+	-
	Wagner	-	+	+	+	+	+	-
Reducing sugars	Benedict	+	+	-	+	+	+	+
Proteins	Ninhydrin	+	+	+	+	+	+	+
	Millon's reagent	+	+	+	+	+	+	+
Terpenoids	Salkowaski test	+	+	-	+	-	+	+
	Copper acetate test	+	-	+	-	+	+	-
Steroids	Salkowaski test	-	-	+	+	-	+	+
Tannins	Ferric chloride	+	+	-	-	+	+	+
Phenols	Ferric chloride	+	+	+	+	+	+	+
Flavonoids	Lead acetate	+	-	-	-	-	+	-
Saponins	Frothtest	+	-	-	-	+	-	-
	Foam test	+	-	-	-	+	-	-
Carbohydrates	Molisch's test	+	+	-	+	+	+	+
Glycosides	Legal's test	+	+	+	-	+	-	-

+: Presence of phytochemicals **-:** Absence of phytochemicals

In silico analysis of the biomolecules present in M. pruriens extract

The names, molecular formula, molecular weight, and structure of the compounds in the methanol seed extract of *M. pruriens* identified using GC-MS analysis and the NIST library were ascertained *via* PubChem database and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/).

Screening of ligands for pharmacological activity

The three-dimensional (3D) structures of the biomolecules having enzymatic inhibitory activity were obtained from PubChem compound-specific database. Screening of the pharmacological activity for the selected structures was carried out using the QikProp module from the Schrodinger 9.3 and Prediction of Activity Spectra for Substances (PASS).

Ligand-based docking using GLIDE (Grid-based Ligand Docking with Energetics)

To carry out glide analysis, 3D conformation structures of the ligands for both antidiabetic and anti-Parkinson's disease were first obtained from Pubchem in .sdf format. The receptors were prepared, grid generation was done, followed by preparation of the ligands, and finally, docking analysis was carried out.

Analysis of docking result

GLIDE XP visualizer of Schrodinger software was used in viewing the docking results and incorporated them into the project table as XP descriptor file in.xpdes format. The analyzed results were saved in .csv format.

RESULTS AND DISCUSSION

Phytochemical analysis of M. pruriens

The leaf and seed extracts of the two varieties of *M. pruriens* [IIHR selection 3 (S3), and *Arka Dhanvantari* (AD)] were subjected to preliminary phytochemical screening using different qualitative tests. The results indicated the presence of alkaloids, tannins, flavonoids, phenols, proteins, terpenoids, reducing sugars, glycosides, carbohydrates, steroids, and saponins, as shown in tables 1a & b and 2a & b.

GC-MS analysis of seed extract of M. pruriens

The methanol seed extract of *M. pruriens* was subjected to GC-MS analysis using Trace GC Ultra gas chromatograph. The compounds identified are recorded in table 3.

Table 1b. Phytochemical analysis of *M. pruriens* (AD) seed extract

Test	Reagents	Solvents extracts						
		Ethanol	Methanol	Chloroform	Hexane	Water	Diethyl ether	Ethyl acetate
Alkaloids	Dragendroff	+	+	+	+	+	+	-
	Wagner	-	+	+	+	+	+	-
Reducing sugars	Benedict	+	+	-	+	+	+	+
Proteins	Ninhydrin	+	+	+	+	+	+	+
	Millon's reagent	+	+	+	+	+	+	+
Terpenoids	Salkowaski test	-	-	-	+	-	+	+
	Copper acetate test	+	-	+	-	+	+	-
Steroids	Salkowaski test	-	-	+	+	-	+	+
Tannins	Ferricchloride	+	+	-	-	+	-	-
Phenols	Ferric chloride	+	+	-	-	+	-	-
Flavonoids	Lead acetate	-	-	+	-	-	-	-
Saponins	Frothtest	+	-	+	-	+	-	-
	Foam test	+	-	-	-	+	-	-
Carbohydrates	Molisch's test	+	+	-	+	+	+	+
Glycosides	Legal's test	-	-	-	+	-	-	-

+: Presence of phytochemicals **-:** Absence of phytochemicals

Screening of identified compounds from M. pruriens methanol seed extract for pharmacological activity

From the above-listed compounds in table 3, eight were identified as having either antidiabetic or anti-Parkinson's activity. Their respective 3D conformation structures and biological activity were obtained from PDB and PASS database, respectively (Table 4).

Uncoupling protein 2 (UCP2) structure modelling

UCP2 protein was modelled using Discovery Studio version 2. 0 software in order to get a correct structure for it since it was not available in PDB. After modelling, the active site for ucp2 was found using CASTp (Computed Atlas of Surface Topography of Proteins), then docking was carried out and the output of receptor grid generated was X: 11.16, Y: 11.69, Z: 31.94 co-ordinates, finally Ramachandran plot was obtained (figure 3).

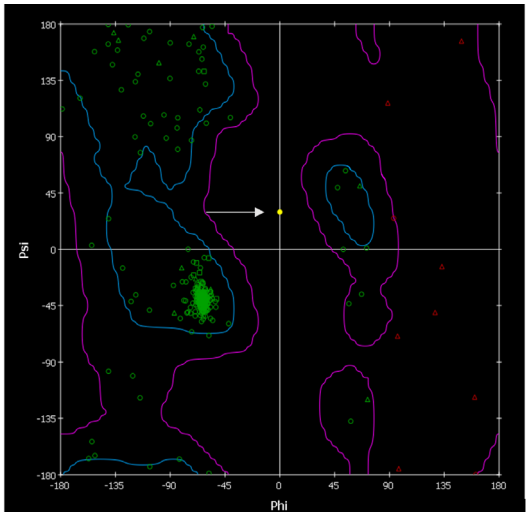


Fig 3.Ramachandran plot of UCP2 structure modelling

Table 2a. Phytochemical analysis of *M. pruriens* (S3) leaf extract

Test	Reagents used	Solvents extracts					
		Ethanol	Methanol	Chloroform	Water	Hexane	Diethyl ether
Alkaloids	Dragendroff	-	-	+	-	+	+
	Wagner	-	+	+	-	+	+
Terpenoids	Salkowaski test	+	+	+	+	+	+
Tannins	Ferric chloride	+	+	-	+	+	-
Phenols	Ferric chloride	+	+	-	+	-	-
Flavonoids	Lead acetate	+	+	-	+	+	+
Saponins	Froth test	+	-	-	+	-	-
	Foam test	+	-	-	+	-	-
Glycosides	Legal's test	+	+	+	+	+	-

+: Presence of Phytochemicals **-:** Absence of phytochemicals.

Medicinal plants are used in different countries as they are the source of many potent drugs. The use of plants as a source of medicine has been inherited as an important component of the health care system. India is the largest producer of medicinal herbs and is therefore called the botanical garden of the world (Sathyaprabha 2010). Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic and infectious diseases.

Successful determination of bioactive compounds from plant materials largely depends on the type of solvent used in the extraction procedure. The solvents can also contribute to the variation since it has been proven that the extraction of biologically active compounds from plants depends upon the polarity of the solvents used (Ghosh *et al.,* 2012).

In the present study, the leaf and seed extracts of *M. pruriens* (S3 and AD variety) were subjected to qualitative phytochemical screening. Seven different solvents: ethanol, methanol, chloroform, diethyl ether, hexane, ethyl acetate and water were used. The presence of alkaloids, tannins, flavonoids, phenols, proteins, terpenoids, reducing sugars, glycosides, carbohydrates, steroids and saponins were documented and these results were similar to the results of Kumar *et al.* (2009) in *M. pruriens.* Sundaram *et al.* (2013) recorded the presence of alkaloids, steroids, terpenoids, tannins, flavonoids, phenols, glycosides and saponins in the ethanolic extract of *M. bracteata*. Manalisha and Chandra (2012) also reported similar findings in the phytochemical evaluation of *M. pruriens* var *linn*. The chloroform and hexane seed extracts of germplasm of *M. pruriens* used in the qualitative phytochemical analysis showed the presence of alkaloids, steroids, flavonoids, resins, phenols, and saponins (Sundaram *et al.,* 2013).

Table 2b. Phytochemical analysis of *M. pruriens* (AD) leaf extract

Test	Reagents used	Solvents extracts					
		Ethanol	Methanol	Chloroform	Water	Hexane	Diethyl ether
Alkaloids	Dragendroff	-	-	+	-	+	+
	Wagner	-	-	+	-	-	+
Terpenoids	Salkowaski test	+	+	+	+	+	+
Tannins	Ferric chloride	+	+	-	+	-	-
Phenols	Ferric chloride	+	+	-	+	-	-
Flavonoids	Lead acetate	+	+	-	+	+	+
Saponins	Froth test	+	-	-	+	-	-
	Foam test	+	-	-	+	-	-
Glycosides	Legal's test	+	+	+	+	-	-

+: Presence of phytochemicals **-:** Absence of phytochemicals.

In the phytochemical screening carried out on the leaf extracts, the presence of alkaloids, terpenoids, tannins, phenols, flavonoids, saponins and glycosides were recorded. However, the diethyl ether leaf extract of both S3 and AD variety revealed the presence of alkaloids, flavonoids and terpenoids, respectively, while saponins, tannins, carbohydrates, reducing sugar, steroids, phenols, proteins and glycosides were absent. Diethyl ether being a non-polar solvent, have revealed the presence of alkaloids, terpenoids,

and flavonoids (Tiwari *et al.,* 2009)particularly cancer. However, computational drug design of boron-containing therapeutics and diagnostics is hampered by the fact that many software packages used for this purpose lack parameters for all or part of the various types of boron atoms. In the present paper, we describe simple and efficient strategies to overcome this problem, which are based on the replacement of boron atom types with carbon atom types. The developed methods were validated by docking closo- and nido-carboranyl antifolates into the active site of a human dihydrofolate reductase (hDHFR, while leaf extract using polar solvents like ethanol reported the presence of flavonoids, tannins, saponins, cardiac glycosides, reducing sugars, steroids, and/or triterpenoids and glycosides (Agbafor and Nwachukwu 2011; Eze *et al.,* 2012). A similar outcome in the phytochemical screening of *Mucuna pruriens* leaves using methanol and chloroform was also reported by Okere *et al.*(2014).

Table 3. Compounds identified in methanol seed extract of *M. pruriens* by GC-MS

RT (min)	Name of compound	Peak area (%)	Molecular weight g/mol	Molecular formula
2.28	2,4,6-Cycloheptatrien-1-one	2.65	106.12194	C_7H_6O
2.29	3,5-bis-trimethylsilyl-	2.65	204.417500	$C_7H_{20}N_2OSi_2$
3.38	d-Mannose	0.92	180.155880	$C_6H_{12}O_6$
3.98	α-D-Glucopyranoside	0.92	180.15588	$C_6H_{12}O_6$
4.65	delta5-pregnenediol	1.13	318.49346	$C_{21}H_{34}O_2$
4.68	1H-Azonine	1.13	119.16376	C_8H_9N
5.50	DL-2,6-Diaminoheptanedioic acid	0.09	199.132462	$C_7H_{14}N_2O_4$
5.52	dl-2,6-Diaminoheptanedioic acid	0.09	190.197060	$C_7H_{14}N_2O_4$
5.78	3,4-dihydroxy-5-(1,2,3,4-tetrahydroxybutyl) oxolan-2-one	0.15	238.19196	$C_8H_{14}O_8$
5.79	6-hydroxy-4-methyl-4-hexenal	0.15	128.16898	$C_7H_{12}O_2$
6.34	6-diazahomoadamantan-9-ol	0.07	299.370840	$C_{16}H_{21}N_5O$
7.42	2,7-Anhydro-l-galacto-heptulofuranose	6.44	434.48104	$C_{26}H_{26}O_6$
7.45	1,2-diamino-1,2-dideoxy-D-glucitol	6.44	180.20224	$C_6H_{16}N_2O_4$
8.34	Desulphosinigrin	0.77	279.310080	$C_{10}H_{17}NO_6S$
8.36	Digitoxin; Digitoxoside	0.77	764.93906	$C_{41}H_{64}O_{13}$
8.38	Ethyl ester	0.77	242.698700	$C_{12}H_{15}ClO_3$
8.39	Octaethylene glycol monododecyl ether	0.77	538.75472	$C_{28}H_{58}O_9$
8.96	Geldaramycin	0.35	560.635900	$C_{29}H_{40}N_2O_9$
8.97	2-Bromotetradecanoic acid	0.35	307.266980	$C_{14}H_{27}BrO_2$
8.98	Propane-3-diol, (N-1,3-dihydroxypropylaminocyclitol)	0.35	400.517700	$C_{19}H_{20}N_4O_2S_2$
8.99	9,10-Secocholesta-5	0.35	468.711020	$C_{31}H_{48}O_3$
9.01	3-octyl- (Ricinoleic acid)	0.35	298.46076	$C_{18}H_{34}O_3$
9.88	Hexadecanoic acid	3.95	256.424080	$C_{16}H_{32}O_2$
10.19	Palmitic anhydride	11.31	494.83288	$C_{32}H_{62}O_3$
10.29	l-(+)-Ascorbic acid 2	11.31	176.124120	$C_6H_8O_6$
11.41	9-cis,11-trans-Octadecadienoate	8.66	280.44548	$C_{18}H_{32}O_2$
11.43	11-trans-Octadecadienoate	8.66	294.472060	$C_{19}H_{34}O_2$
11.45	Methyl ester	8.66	100.115820	$C_5H_8O_2$
11.86	6, 9,12-Octadecatrienoic acid (Z, Z,Z)	27.69	278.4296	$C_{18}H_{30}O_2$
11.87	6, 9,12-Octadecatrienoic acid	27.69	434.56716	$C_{28}H_{34}O_4$
11.89	Isopropyl linoleate	27.69	322.52522	$C_{21}H_{38}O_2$

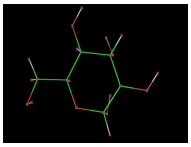
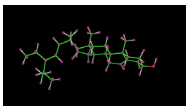
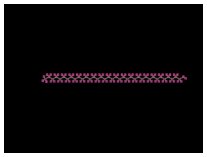
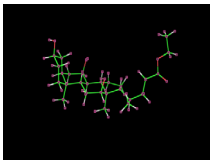
12.84	1-Heptatriacotanol	0.02	536.99874	$C_{37}H_{76}O$
13.50	Oleoyl chloride	4.09	300.907020	$C_{18}H_{33}ClO$
14.43	9,12,15-Octadecatrienoic acid	0.03	278.429600	$C_{18}H_{30}O_2$
16.62	Linoleic acid ethyl ester	14.66	256.424080	$C_{16}H_{32}O_2$
18.45	Ethyl iso-allocholate	0.44	536.99874	$C_{37}H_{76}O$
18.47	Glycine: 2-Aminoacetic acid	0.44	75.0666 00	$C_2H_5NO_2$
18.76	Trilinolein	0.54	879.384420	$C_{57}H_{98}O_6$
19.10	Acetic acid	1.41	60.05196	$C_2H_4O_2$ or CH_3COOH or $C_2H_4O_2$
19.73	3-(4-Hydroxyphenyl) propionic acid	1.01	166.173900	$C_9H_{10}O_3$
21.49	α-sitosterol	2.85	414.706700	$C_{29}H_{50}O$
29.40	3-Methyl-2-(2-oxopropyl)	0.83	232.23198	$C_{13}H_{12}O_4$
32.00	1,2-Benzenedicarboxylic acid, diisooctyl ester	4.04	390.55612	$C_{24}H_{38}O_4$ or $(C_8H_{17}COO)_2C_6H_4$

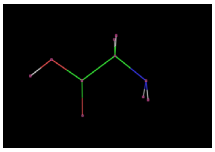
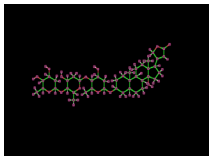
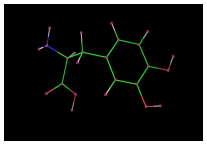
Docking analysis

Docking analysis was carried out on four selected ligands for Parkinson's disease and diabetes *via* Schrodinger version 9.3 and compared with three commercial compounds. The glide docking output is recorded in table 5.

The medicinal value of plants lies in the presence of bioactive compounds found in the leaves, seeds, bark, roots,etc, which produce a definite physiological action on the human body. The bioactive compounds obtained from plants include alkaloids, flavonoids, tannins, phenolic compounds,etc and they are the main drivers of the pharmacological actions of medicinal plants (Hussein and El-Anssary 2018). The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new biologically active agents from higher plants (Duraipandiyan *et al.* 2006). Therefore, plants are valuable sources of a vast array of chemical compounds they synthesize and accumulate in various parts and the knowledge of such chemical constituents is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in discovering new sources of economic materials such as oils, gums, precursors for the synthesis of complex chemical substances etc., (Sheeja and Lawrence 2018).

Table 4. PDB 3D structures of selected compounds

	Name of compound	Biological activity	3D conformation Structure
1	α-D-Glucopyranoside	Antidiabetic activity	
2	α-sitosterol	Antidiabetic activity	
3	1-Heptatriacotanol	Inhibitory and antihypercholesterolemic activities	
4	Ethyl iso-allocholate	Hypoglycaemic and amylase inhibitory activities	

5	(2S)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid	Anti-parkinson's activity	
6	Glycine: 2-Aminoacetic acid	An inhibitory neurotransmitter in the central nervous system	
7	Digitoxin; Digitoxoside	A cardiac glycoside	
8	(3-(4-hydroxyphenyl) propanoic acid)	Anti-Parkinson's activity	

A total of 43 compounds were selected from the GC-MS result of the methanol seed extract composition of *M. pruriens*. The presence of the phytochemical compounds was confirmed and when eight (8) amongst them were narrowed down to their pharmacological activity, it was observed that they possess either antidiabetic or anti-Parkinson's activity namely: α-D-glucopyranoside, ethyl iso-allocholate, α-sitosterol, 3-(4-Hydroxyphenyl) propionic acid, digitoxin, 2-aminoacetic acid and 1-Heptatriacotanol. The compounds identified is similar to the compounds recorded by Bhaskar *et al*. (2011) where the GC-MS analysis showed the presence of bioactive compounds such as n-hexadecanoic acid, squalene, oleic acid, ascorbic acid, octadecanoic acid, n-Decanoic acid, 9,12-Octadecadienoic acid (Z, Z)-, eicosanoic acid, ethyl ester, 1,2-Benzene dicarboxylic acid, diisooctylester, etc in the methanol seed extract of *M. pruriens*.

Gas chromatography-mass spectrometry has been firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species. It is among the most widely used because of its ability to separate complex mixtures of metabolites with high efficiency and at low cost (Zhang *et al.,* 2012). The large compounds are fragmented into small compounds giving rise to an appearance of peaks at different m/z ratios. The nature of the fragments often provides a clue to the molecular structure. These mass spectra are revealed as the fingerprints of the resulting compounds, which can be identified from the data library and without a molecular ion peak as a reference, the difficulty of interpreting a mass spectrum increases markedly. Fortunately, most organic compounds give mass spectra that include a molecular ion (Vijlder *et al.,* 2018).

Table 5. Glide docking output of selected compounds for antidiabetic and anti-Parkinson's activities compared with commercial compounds

PUBCHEM ID	Entry name	Glide score (Kcal/mol)	H-bond
6047	Levodopa (L-DOPA)	-3.101	5
6560213	α-D-glucopyranoside	-4.879478	4
6452096	Ethyl iso-allocholate	-3.96628	2
222284	Sitosterol	-3.058685	2
41774	Acarbose	-5.597681	6
444020	Voglibose	-4.146658	4
3488	Glibenclamide	-3.96628	2

Plants are potent biochemical factories and plant cell culture systems represent a potential renewable source of valuable medicinal compounds. Secondary metabolites from plants may be used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as

models for pharmacologically active compounds in drug synthesis in which the active moiety will be identified and used as the lead molecule for drug design (Mukta *et al., 2017)*. We carried out the *in-silico* analysis using the biological active secondary metabolites identified from the GC-MS screening. The potent inhibition of the identified compounds *viz.,* alpha-D-glucopyranoside, ethyl iso-allocholate and sitosterol against uncoupling protein2 (UCP2) involved in type-2 diabetes was determined by a docking study in which their binding profiles towards key amino acid residues in the active site were observed to be similar to that of the commercial alpha-amylase inhibitors such asvoglibose, acarbose, and glibenclamide.

Also, the potent inhibition of the identified compound 3-(4-hydroxyphenyl) propanoic acid against DJ-1 receptor involved in Parkinson's disease was determined by docking study and it was observed that the binding profile towards key amino acid residues in the active site was similar to that of the commercial levodopa. Fred-Jaiyesimi *et al.* (2009) recorded 69.8 % of amylase inhibitory activity from sitosterol in their study on hypoglycaemic and alpha-glucosidase inhibitory activities on leaves of *Spondiasmombin Linn.* Also, in the study carried out by Oku *et al.* (2000)*6'-dihydroxy-4'-methylpropiophenone-2'-O-(6-O-methoxycarbonyl* on antidiabetic effect of T-1095, an inhibitor of Na (+)-glucose co-transporter in neonatally streptozotocin-treated rats, they recorded the therapeutic potential of alpha-D-glucopyranoside as a means of ameliorating abnormal glucose metabolism *via* diminished glucose toxicity. Therefore, the compounds we identified in the *M. pruriens* extracts used for this study can be considered as good approach in discovering new series of effective α-amylase inhibitors which will be useful in the management of type-2 diabetes.

GLIDE is a well-known ligand docking programme developed by Schrodinger. It was designed to be computationally fast to screen large libraries using the positional, orientational, and conformational space available to the ligand (Friesner *et al.* 2004). In this study, glide grouping of antidiabetic activity of the selected compounds was determined according to the ligand interaction with target proteins when *M. pruriens* ligands were compared with three commercial ligands: Group 1; Acarbose. Group 2; Alpha-D-Glucopyranoside & Voglibose and Group 3; Glibenclamide, ethyl iso-allocholate & alpha-sitosterol. This shows that *M. pruriens* seed extract has strong antidiabetic properties. L-DOPA is the naturally occurring form of dihydroxyphenylalanine and the immediate precursor of dopamine. It can be taken orally as it has the ability to cross the blood-brain barrier. Dopaminergic neurons can also be rapidly taken up and converted to dopamine. Hence, it is used to treat Parkinson's disorders and usually administered with agents that inhibit its conversion to dopamine outside of the central nervous system.

Conclusion

The medicinal / pharmacological property of *Mucuna pruriens* is attributed to the presence of various bioactive compounds. The findings recorded in the present research will be useful to scholars and scientists working in pharmacology to design anti-Parkinson's and anti-diabetic drugs. Therefore, this study supports the management of diseases like type-2 diabetes and Parkinson's disorders with plant-based compounds as they are effective, easily accessible, and have no known adverse effects.

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Conflict of interest

The authors hereby declare no conflict of interest.

Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests:

There were no conflict of interest in the publication of this content.

Author contributions

Research grant- West Africa Agricultural Productivity Programme and National Root Crops Research Institute Umudike Nigeria. Idea conceptualization- Gnanam R, Experiments- Uma D,Santhanakrishnan V. P, Guidance- Renuka R, Writing original draft- Reuben-Kalu J.I,Writing , reviewing &editing –Renuka R,Alum E A

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