Original Research Article

INSIGHTFUL VALORIZATION OF BIOLOGICAL ACTIVITIES OF FLAX SEED (*LINUM USITATISSIMUM*) THROUGH EXPERIMENTAL AND COMPUTER AIDED MECHANISMS

ABSTRACT:

Flax, also known as common flax or linseed, is a flowering plant, *Linum usitatissimum*, in the family Linaceae. The current study used haloperidol-induced catalepsy and reserpine antagonism to investigate *in vivo* anti- Parkinson's activity and *in silico* approaches like docking studies (schrodinger software), Ramchandran plot (procheck), ADME and biological activity score using (molinspiration) online software. The n-hexane extract of *Linum usitatissinum* (HELU) (flax seeds) exhibited various phytochemical constituents like carbohydrates, lignans, alkaloids, phenolic compounds, flavonoids, fatty acids, coumarin derivatives and sterols. Pharmacological tests were studied at 200 mg/kg bd.wt and 400 mg/kg bd.wt, using *in vivo* behavioural effects like muscular rigidity, tremors, akinesia, grip strength, and locomotory activity. The experiment was designed by giving haloperidol to induce catalepsy and Reserpne to induce Parkinson's disease-like symptoms. The increased cataleptic scores were significantly decreased by HELU (200 and 400 mg/kg bd.wt., *p.o.*) in reserpine antagonism model. Docking studies for natural chemicals against PDB ID: 4I6B, 7JOZ, 4XUD, 4OYX were conducted to better understand the ligand-binding affinity of the extract's active ingredients. The results revealed that D-xylose, D-arabinose, L-rhamnose, L-fucose, hesperidin, herbacetin, β-carboline, isoquinoline, ferulic acid, eicosapentanoic acid, docosahexaenoic acid, beta sitosterol, niacin, aesculetin and standard drug levodopa, carbidopa had shown highest glide scores with all the selected proteins which indicate a stronger receptor-ligand binding affinity. Our findings revealed that *Linum usitatissimum* n-hexane extract has strong anti Parkinson's action.

KEYWORDS: *Linum usitatissimum*, levodopa, carbidopa, Docking studies, ADME analysis, Anti-Parkinson's activity.

1. INTRODUCTION

Parkinson's disease is a chronic neurodegenerative condition marked by the death of dopaminergic neurons in the brain's substantia nigra. The presence of intracytoplasmic inclusions from protein aggregates known as Lewy Bodies (LBs) and the reduction of pigmented dopamine-containing neurons in the substantia nigra pars compacta are pathological hallmarks of PD. [1]. Reserpine works by slowing the activity of the nervous system. Haloperidol easily crosses blood brain barrier by virtue of its high lipophilic character [2]. It binds to the internal surface of D_2 receptors located in the substantia nigra pars compacta and decreases central dopaminergic functions and results in reduced coordination of motor activities thus, produces Parkinsonism like symptoms. Catalepsy is defined as a failure to correct externally imposed unusual postures over a prolonged period of time. Levodopa + Carbidopa is used as a standard drug for both the models. The combination of levodopa and carbidopa (30 mg/kg bd.wt., *i.p*) is used to increase the efficiency of Levodopa.

The current medication treatments for Parkinson's disease have a variety of negative effects. As a result, herbal remedies should be regarded as an alternative/complementary treatment option. Flax seed (FS) botanically known as *Linum usitatissium*, belongs to the Linaceae family, is a versatile blue flowered crop. This nutty flavored seed comes in a variety of colors ranging from a reddish brown to a light yellow. It is commonly consumed in three forms: as a whole seed, ground powder, or as oil. FS is composed of 41% fat, 20% protein, 28% dietary fiber, 7.7% moisture, and 4% ash. The active components of FS are dietary fiber (cellulose, mucilage, gums, and lignin), phytochemicals, and omega 3 fatty acids (www.flaxcouncil.ca) *Linum usitatissimum*, commonly called as flax seeds is a native of Canada, Argentina, USA, Poland, Egypt, Czechoslovakia (*Linaceae*) [2]. Flax seeds show cardiovascular, anti-cancer, anti-diabetic activities. However the effect of Flaxseed on learning and memory is unclear so the current study is to use *in-vivo* and *in silico* models to screen the n-hexane extract of *Linum usitatissimum* and to establish the binding affinity for the specific target for its anti-Parkinson's activity.

2. MATERIALS AND METHODS

2.1 Seed collection and drying

Seeds of *Linum usitatissimum* were identified, collected, authenticated by botanist Suresh Babu, New government degree college, Kukatpally. *Linum usitatissimum* seeds were cleaned and dried under shade for about six days and powdered. The powdered material was stored.

2.2 Preparation of n-hexane extract of *Linum usitatissimum* (Soxhlet)

The Soxhlet extractor is a type of continuous extraction of a component from a solid mixture. The powdered material of seeds of *Chenopodium quinoa* were dried and extracted with ethanol by soxhlation technique. As to get efficient extraction, this method allows a continuous extraction process; it is nothing but a series of short macerations. Boiling solvent rise up through the larger side arm. Condensed drop of solvent falls into the porous cup, dissolving out the desired component from a solid mixture. When the smaller side-arm fills to overflowing, it initiates a siphoning action. The solvent, containing the dissolved component, is siphoned into the boiler below residual solvent then drains out of the porous cup, as fresh solvent drops continue to fall into the porous cup. And the cycle repeats. The organic extract obtained was evaporated to dryness by keeping at room temperature. Large amounts of drug can be extracted with a much smaller quantity of solvent. This process of extraction is economical in terms of time, energy and consequently financial investments [3].

Determination of extract yield: The formula for calculating the extract yield was as follows:

$$\text{yield}\ (\%) = \frac{\text{Mass of extract}}{\text{Mass of flax seed powder}} \times 100$$

Figure 1: n-hexane extract of *Linum usitatissimum* by Soxhlet extractor

2.3 Preliminary phytochemical analysis of the extract

The extract was subjected to preliminary phytochemical investigations to identify various phytoconstituents present in the n-hexane extract of seeds of *Linum usitatissimum*.

2.4 Acute toxicity testing

The acute toxicity studies were carried out using OECD 425 guidelines. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India. (Reg.No. 1175/PO/ERe/S/08/CPCSEA).

2.5 Animal housing

The animals (mice and rat) were housed in poly acrylic cages with not more than six animals per cage, with 12 h light/12 h dark cycle. Animals have free access to standard diet and drinking water *ad libitum*. The animals were allowed to acclimatize the laboratory environment for a week before the start of the experiment. The care and maintenance of the animals were carried out as per the approved guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

2.6 *In vivo* methods for evaluation of anti-Parkinson's activity

In vivo evaluation of anti-Parkinson's activity of the n-hexane extract of seeds of *Linum usitatissimum* were carried out in following models.

- Haloperidol induced catalepsy.
- Reserpine antagonism

2.6.1 Haloperidol induced catalepsy

30 healthy Albino rats of either sex weighing 200-250 gm were selected for the study. They are divided in to 5 groups, each group consisting 6 animals (n=6). Group I (normal) received saline (0.9 % NaCl). Group-II (Disease control) received Haloperidol (1 mg/kg bd.wt. *i.p*). Group III received with HELU (200 mg/kg bd.wt. *p.o.*) + Haloperidol (1 mg/kg bd.wt. *i.p*) Group IV received with HELU (400 mg/kg bd.wt. *p.o.*). Group V received with Levodopa + Carbidopa (30 mg/kg bd.wt. *i.p*) + Haloperidol (1 mg/kg bd.wt. *i.p*). Cataleptic scores were measured at every 30 minutes time interval for 2 hours and 30 minutes. Standard bar test will be used to measure the cataleptic scores. Catalepsy was determined by placing an animal on the horizontal metal bar at a height of 10 cm in such a way that the fore-limbs of the animal should be on the horizontal bar while the hind-limb touches the surface for a period of 5 minutes [4].

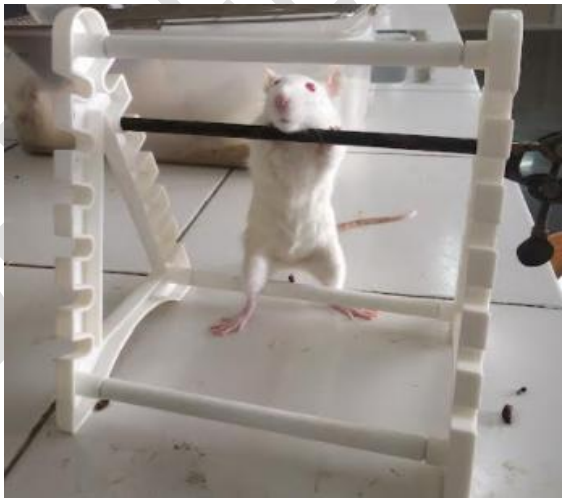


Figure 2: Haloperidol induced catalepsy in Wistar rats

2.6.2 Reserpine Antagonism

30 healthy Albino mice of either sex weighing 20-25 gm were selected for the study. They are divided in to 5 groups, each group consisting 6 animals (n=6). Group I (normal) is administered with saline (0.9 % NaCl) for 5 consecutive days. Group II (disease control) is administered with Reserpine (5 mg/kg bd.wt., *i.p*) for 5 days. Group III is administered with

HELU (200 mg/kg bd.wt. *p.o.*) + Reserpine (5 mg/kg bd.wt. *i.p*) for 5 days. Group IV is administered with HELU (400 mg/kg bd.wt. *p.o.*) + Reserpine (5 mg/kg bd.wt. *i.p*) for 5 days. Group V is administered with standard drug combination Levodopa + carbidopa (30 mg/kg bd.wt., *i.p*) + Reserpine (5 mg/kg bd.wt., *i.p*) for 5 days. During the experiment all the groups administered with reserpine (5mg/kg bd.wt.,) except control and after 30 min, group III, IV and V receives their corresponding treatment during 5 days. On 5^{th} day various parameters like muscular rigidity, rearing's, tremors, akinesia and grip strength were measured in all the animal groups by placing the animals singly on the floor of a Perspex container (30*26*20 cm height) for a period of 10 min time duration [4].

2.7 Molecular Docking Studies

2.7.1 Structure based drug design

Initially the protein downloaded from PDB was prepared by removing chain B. Water molecules present in both the chains are removed. Energy minimization was done. Later molecules drawn using chemdraw were converted to mol format and ligprep was created. Grid generation was done by removing crystal ligand and the structures were docked against protein 4I6B, 7JOZ, 4XUD and 4YOX.

2.7.2 Selection of proteins - Schrodinger XP-docking

The proteins selected in the present study are Alpha synuclein inhibitor (PDB ID: 4I6B), D_2 receptor promoter (PDB ID: 7JOZ), COMT inhibitor (PDB: 4XUD) and Adenyl cyclase inhibitor (PDB ID: 4YOX).

2.7.3 Ramachandran plot

Ramachandran plot has been generated from PROCHECK validation server which was used to access the quality of the model by looking into the allowed and disallowed regions of the plot [5].

2.7.4 *In silico* ADME study using molinspiration

The ADME properties of selected active constituents of *Linum usitatissimum* were evaluated using the tool Molinspiration Cheminformatics server (http://www.molinspiration.com). There are several pharmacokinetic parameters and physicochemical descriptors which were evaluated for herbal extracts through application of the tool Molinspiration. These properties are mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and of course presence of various pharmacophoric features that influence the behaviour of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others. The

Lipinski rule of five deals four simple physicochemical parameter ranges (MWT ≤ 500, log P ≤ 5, H-bond donor's ≤ 5, Hbond acceptors ≤ 10) [6].

2.7.5 Bioactivity score using molinspiration

The bioactivity score of selected active constituents of *Linum usitatissimum* were also evaluated using the tool Molinspiration Cheminformatics server (http://www.molinspiration.com). In this computational chemistry technique large chemical databases are analysed in order to identify possible new drug candidates. Only SMILES or SD file structures of active molecules are sufficient for the training, no information about the active site or binding mode is necessary. This is particularly useful in projects where structure-based approach cannot be applied because information about 3D receptor structure is not available [7].

2.8 Statistical analysis

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, negative control, and standard by using Dunnett's t-test. Significant values are expressed as control group (**=p<0.01, *=p<0.05), negative control (A=p<0.01, B=p<0.05) and standard (a=p< 0.01, b=p< 0.05), ns- nonsignificant.

3. RESULTS

3.1 Preparation of n-hexane seed extract of *Linum usitatissimum*

The n-hexane extract of seed of *Linum usitatissimum* was prepared by soxhlation technique. The percentage yield of n-hexane seed of extract of *Linum usitatissimum* was calculated by using the following formula.

% Yield of extract= Amount of extract obtained/Amount of powder used X 100.

= 280/450 x 100

= 62.26%

3.2 Preliminary phytochemical analysis

The preliminary phytochemical investigation of n-hexane extract of seed of *Linum usitatissimum* revealed the presence of bioactive compounds of Alkaloids, flavonoids, Lignans, Glycosides, α-linoleic acid, linoleic acids were the most prominent (Table 1).

Table 1: Preliminary phytochemical analysis

Phytochemical constituents	Results
Carbohydrates	+
Alkaloids	+
Flavonoids	++
Lignans	++
Glycosides	++
Tannin's	+
terpenoid’s	+
Fatty acids	++

Note: ++ indicates present, -indicates absent

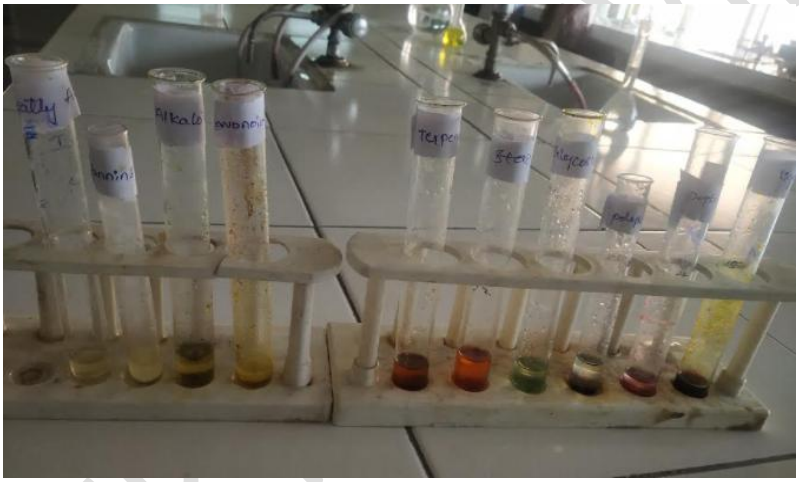


Figure 3: Preliminary Phytochemical analysis

3.3 Acute toxicity studies

On Swiss albino mice, N-hexane extract of *Linum usitatissimum* heads was evaluated up to a dose of 2000 mg/kg bd. wt. Up to 2000 mg/kg bd. wt., the animal showed no symptoms of toxicity or fatality. During the research, many physical and behavioural characteristics were observed. As a result, up to 2000 mg/kg bd. wt. of the extract was proven to be safe.

3.4 *In vivo* anti Parkinson’s activity

The n-hexane extract of seeds of *Linum usitatissimum* was screened for its anti-Parkinson’s activity using the following models.

3.4.1 Haloperidol induced catalepsy

Haloperidol-treated groups scored higher on the cataleptic scale. The cataleptic score was observed to be lower in those treated with the HELU plus a conventional medication (levodopa+carbidopa 30 mg/kg, i.p.).

Table 2: Effect of HELU on Haloperidol Induced Catalepsy in Wistar Rats

Treatment	Duration of Catalepsy (Seconds)				
	30 min	60 min	90 min	120 min	150 min
Control (saline)	8.25±0.6	10.7±0.42	12.4±0.1	12.1±0.1	10.8±0.14
(Disease Control) Haloperidol (1 mg/kg bd.wt. *i.p)*	110±0.86*	98.2±6.42*	88±0.9*	78±0.75*	60±0.89*
HELU (200 mg/kg bd.wt. *p.o.*)+Haloperidol	98±0.2*^{aA}	77±0.5*^{aA}	43±0.8*^{aA}	38±0.6*^{aA}	25±0.32*^{aA}
HELU (400 mg/kg bd.wt. *p.o.*)+Haloperidol	72±0.89* ^{aA}	50.2±0.1*^{aA}	28±0.2*^{aA}	26±0.4*^{aA}	20±0.30*^{Aa}
Levodopa + Carbidopa (30 mg/kg bd.wt. *i.p*)+Haloperidol	34±0.7* ^{a}	28±0.2* ^{a}	15±0.6*^{a}	16.0±0.72*^{a}	14±0.40*^{a}

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, disease control and standard. (By using Dunnett's test) significant values were expressed as control group (*p<0.0001), disease control (a=p<0.01) and, standard (A=p<0.01).

3.4.2 Reserpine antagonism

Reserpine significantly decreases the intensity of muscular rigidity, tremors, and increases the intensity of grip strength and akinesia in this model.

Table 3: Effect of HELU on reserpine antagonism in mice

Treatment	Muscular Rigidity (secs)	Tremors (score)	Akinesia (Number of steps taken	Locomotory (activity in 10 min)	Grip strength (Latency to

			with forelimbs)		fall in seconds)
Control	30±0.8	0	50±0.62	306±0.3	162±0.21
Disease control Reserpine (5 mg/kg bd.wt)	7±0.06*	8±0.6*	8±0.31*	112±0.45*	22.2±0.48*
HELU (200 mg/kg bd.wt.)+Reserpine	12±0.63*^{aA}	4.18±0.86*^{aA}	27±0.26*^{aA}	162±0.56*^{aA}	62±0.52*^{aA}
HELU (400 mg/kg bd.wt.)+Reserpine	23±0.84*$^{a\ ns}$	2±0.8*^{aA}	38±0.50*^{aA}	202±0.2*^{aA}	90±0.02*^{aA}
Levodopa+Carbidopa (30 mg/kg bd.wt.)+Reserpine	28.01±0.6*^{a}	1±0.8*^{a}	45.01±0.78*^{a}	282±0.4*^{a}	103±0.79*^{a}

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, disease control and standard. (By using Dunnett's test) significant values were expressed as control (*p<0.0001), disease control (a=p<0.01) and standard (A=p<0.01), ns=non-significant.

3.4.3 Molecular docking

Table 4: Schrodinger XP Docking Score

S.NO	Compounds	4I6B	7JOZ	4XUD	4OYX
1.	D-xylose	-9.15	-6.34	-5.77	-8.36
2.	D-arabinose	-8.10	-6.37	-5.68	-8.68
3.	L-rhamnose	-8.16	-6.77	-5.63	-6.70
4.	L-fucose	-8.71	-6.52	-5.11	-8.23
5.	Herbacetin	-11.74	-7.03	-1.76	-8.83

6.	Hesperidin	-9.50	-6.01	-3.03	-7.00
7.	Eicosapentaenoic acid	-7.00	-5.93	-1.08	-4.32
8.	Docosahexaenoic acid	-5.21	-5.33	-	-3.59
9.	Beta-carboline	-8.52	-5.37	-4.35	-7.49
10.	Iso-quinoline	-8.13	-5.14	-3.84	-6.40
11.	Ferulic acid	-8.65	-5.53	-5.34	-5.76
12.	Beta sitosterol	-5.96	-4.68	-3.76	-3.66
13.	Aesculetin	-8.92	-6.43	-5.40	-7.43
14.	Niacin	-6.80	-5.86	-4.75	-5.88
15.	Levodopa	-7.50	-5.88	-	-8.70
16.	Carbidopa	-6.55	-5.74	-	-8.58

G score = glide score, The more negative the glide score, the more favourable the binding.

3.4.4 Ramachandran plot Analysis

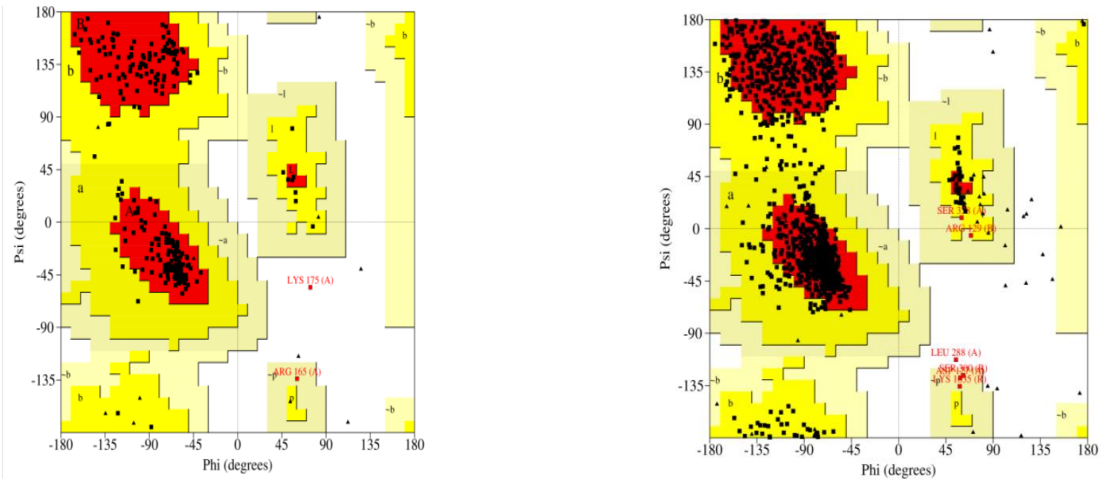
Protein 4I6B, 7JOZ, 4XUD and 4OYX were analysed for Ramachandran plot to know amino acid presence in different regions of respective protein tabulated in table 5 and pictorial representation by figure below.

Table 5: Ramachandran plot status with protein with 4I6B, 7JOZ, 4XUD and 4OYX.

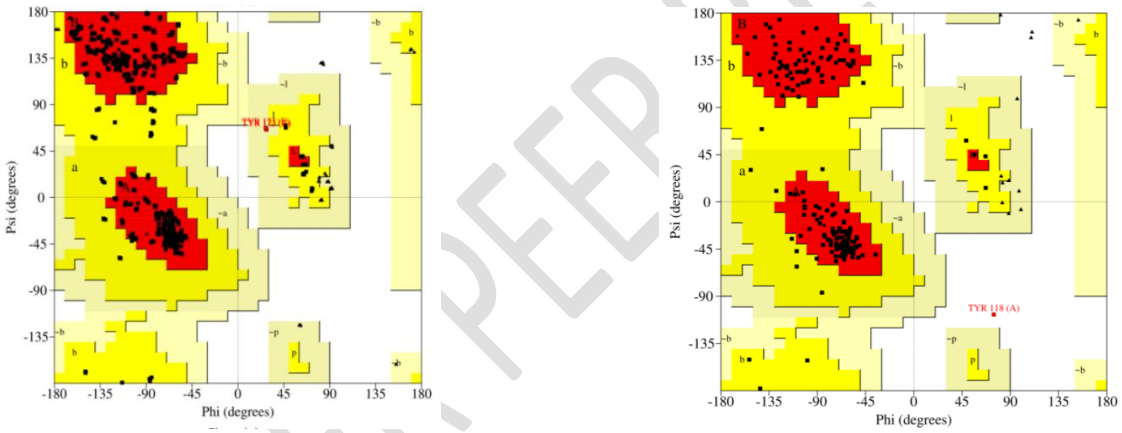
Residues	**4I6B**	**7JOZ**	**4XUD**	**4OYX**
Most favourable region (%)	88.9	86.0	91.2	90.9
Additional allowed regions (%)	10.3	13.5	8.3	8.6
Generously allowed regions (%)	0.4	0.4	0.0	0.5
Disallowed regions (%)	0.4	0.1	0.5	0.0

a) 4I6B b) 7JOZ

c) 4XUD d) 4YOX



a) 4I6B b)7JOZ



c) 4XUD d) 4YOX

Figure 4: Ramachandran plot of protein 4I6B, 7JOZ, 4XUD and 4OYX.

Table 6: ADME properties of compounds from *Linum usitatissimum* by molinspiration

	Compound	MW	nON	nOHNH	nV	nrotb	TPSA	miLogP
1.	D-xylose	150.13	5	4	0	0	90.15	-2.22
2.	D-Arabinose	150.13	5	4	0	4	97.98	-2.22

3.	L-Rhamnose	164.16	5	4	0	0	90.15	-1.64
4.	L-fucose	164.16	5	4	0	0	90.15	-1.64
5.	Herbacetin	302.24	7	5	0	1	131.35	1.91
6.	Hesperidin	610.57	15	8	3	7	234.30	-0.55
7.	Beta-carboline	198.22	3	2	0	1	48.91	2.17
8.	Isoquinoline	129.16	1	0	0	0	12.89	2.05
9.	Ferulic acid	194.19	4	2	0	3	66.76	1.25
10.	Eicosapentaenoic acid	302.46	2	1	1	13	37.30	5.40
11.	Docosahexaenoic acid	328.50	2	1	1	14	37.30	5.68
12.	Beta sitosterol	414.72	1	1	1	6	20.23	8.62
13.	Aesculetin	178.14	4	2	0	0	70.67	1.02
14.	Niacin	123.11	3	1	0	1	50.19	0.27
15.	Levodopa	197.19	5	5	0	3	103.78	-2.20
16.	Carbidopa	226.23	6	6	1	4	115.81	-2.81

Table 7: Bioactive score of compounds from *Linum usitatissimum* by molinspiration

	Compounds	**GPCR Ligand**	**Ion channel modula**	**Kinase inhibitor**	**Nuclear receptor ligand**	**Protease inhibit**	**Enzyme inhibitor**

			tor			or	
1.	D-Xylose	-0.77	-0.18	-1.34	-1.61	-0.83	0.25
2.	D-Arabinose	-1.06	-0.35	-1.35	-1.17	-0.71	-0.01
3.	D-Galactose	-0.44	0.02	-0.80	-0.85	-0.51	0.40
4.	L-Rhamnose	-0.75	-0.15	-1.11	-1.11	-0.61	0.20
5.	L-fucose	-0.75	-0.15	-1.11	-1.11	-0.61	0.20
6.	Hesperidin	-0.01	-0.59	-0.36	-0.20	-0.00	0.06
7.	Herbacetin	-0.08	-0.17	0.30	0.34	-0.24	0.32
8.	Beta-carboline	0.22	0.79	0.48	-0.33	-0.22	0.51
9.	Isoquinoline	-0.71	0.20	-0.37	-1.44	-0.87	-0.23
10.	Ferulic acid	-0.47	-0.30	-0.72	-0.14	-0.81	-0.12
11.	Beta sitosterol	0.14	0.04	-0.51	0.73	0.07	0.51
12.	Eicosapentaenoic acid	0.36	0.21	-0.11	0.39	0.20	0.38
13.	Docosahexaenoic acid	0.34	0.18	-0.09	0.37	0.21	0.34
14.	Aesculetin	-1.05	-0.61	-1.06	-0.81	-1.17	-0.22
15.	Levodopa	-0.04	0.39	-0.60	-0.17	-0.01	0.29
16.	Carbidopa	-0.19	-0.39	-0.63	-0.56	-0.12	0.04

4. DISCUSSION

Parkinson's disease imposes several motor deficits, often followed by cognitive dysfunction along with the progression of the disease and along with tremors, muscular rigidity, akinesia and postural instability. The haloperidol showed cataleptic behaviour. Haloperidol is a non-selective D_2 dopamine antagonist that causes catalepsy in the striatum by blocking dopamine receptors. Multiple effects of fatty acids in haloperidol-induced catalepsy include hypothermia, catalepsy, and reduced motor activity, as well as actions on the hypothalamic–pituitary axis, such as modulation of growth hormone, luteinizing hormone, and prolactin secretion, and correction of imposed postures in catalepsy [8]. Oleic acid was a mediator of alpha-synuclein or the toxic build up in the brain that causes Parkinson's disease. Linamarin, a cyanogenic glycoside, was used in a combination of treatments to evaluate and improve open field and swim tests in adult male Wistar rats to identify locomotor and hippocampal abnormalities. Flavonoids like Herbacetin, a new flavonoid discovered in *Linum usitatissimum*, have been linked to the development of glucose intolerance, hyperglycemia, insulin resistance, hepatic steatosis, and dyslipidaemia in people who eat a high-fat, cholesterol-rich diet [9].

Reserpine antagonism shows stereotypic behaviour in the mice which was characterized by decrease in the frequency of horizontal movements like rearing, grooming, muscular rigidity, Akinesia [10]. The phytochemical constituents identified in n-hexane extract of *Linum usitatissimum* are Fatty acids, Alkaloids, Flavonoids, Cyanogenic glycosides, Lignans, Sterols and Terpenoids and Anti-oxidants. omega-3 fatty acids prevent neuronal cell death in reserpine antagonism model of Parkinson's disease. The Beta carboline alkaloids show the mono amine oxidative inhibition and reduces muscular rigidity in reserpine models [11]. Alpha Linoleic Acid reduce the risk of cancer and cardiovascular diseases and decrease the production of arachidonic acids and other pro-inflammatory eicosanoids. It involves in the protection of dopaminergic neurons, reduce mitochondrial dysfunction and strengthen antioxidant defences making α-Linoleic Acid a viable neuroprotective agent in Parkinson's disease [12]. Cyclic peptides specifically reduce the toxicity of human -synuclein. These cyclic peptide prevent dopaminergic neuronal loss. Flavonoids like Quercetin has consistently been shown to protect against oxidative stress and dopamine depletion, improve motor balance and coordination, and maintain the resting membrane potential of neurons in reserpine antagonism in mice [13].

4.1 Molecular docking

Molecular docking, which screens small compounds by orienting and scoring them in the binding site of a protein, continues to offer considerable promise in the field of computer-based drug creation. Using Schrödinger software, identified molecules from n-hexane extract of Linum usitatissinum and common medication combinations like levodopa and carbidopa were docked. The various constituents identified in the plant extract are D-xylose, D-arabinose, L-rhamnose, L-fucose, hesperidin, herbacetin, beta carboline, isoquinoline, ferulic acid, eicosapentanoic acid, docosahexaenoic acid, beta sitosterol, niacin, asculetin and standard drugs levodopa, carbidopa were subjected to docking against PDB ID: 4I6B, 7JOZ 4XUD 4OYX. The highest glide scores were observed with D-xylose, D-arabinose, L-rhamnose, L-fucose, beta carboline, D-galactose, L-rhamnose hesperidin, β-sitosterol, and aesculetin against all selected PDB ID: 4I6B, 7JOZ 4XUD 4OYX. Molecular docking, which screens small compounds by orienting and scoring them in the binding site of a protein, continues to offer considerable promise in the field of computer-assisted drug creation. Using Schrödinger software, identified molecules from Linum usitatissinum n-hexane extract and common medication combinations such as levodopa and carbidopa were docked. The proteins found, PDB ID: 4I6B, 7JOZ 4XUD 4OYX, were modelled, and the 3D model's quality was checked using the PROCHECK tool and the Ramachandran plot. Predicted models have most favourable regions, extra allowed regions, usually allowed regions, and banned regions, as seen by the Ramachandran plot. The Ramachandran plot reveals a fair percentage distribution of protein residues, indicating that the predicted models are of acceptable quality. A good quality model, according to the Ramachandran plot, should have above 90% in the most favoured zone. Proteins with PDB ID: 4I6B, 7JOZ, 4XUD, and 4OYX showed over 90% preference for a region, indicating that the models used in this study are of high quality [14].

4.2 Mol inspiration ADME analysis

The molecular characteristics of molinspiration were computed using Lipinski's rule and its components. The goal of Lipinski's rule of five is to discover if a chemical compound with a specific pharmacological or biological activity has chemical and physical qualities that would allow it to be used as an orally active medication in humans. All of the docked chemicals in this study have a smaller molecular weight, making them more easily absorbed, dispersed, and transported. The selected active constituents like D-xylose, D-arabinose, L-rhamnose, L-fucose, hesperidin, herbacetin, beta carboline, isoquinoline, ferulic acid, eicosapentanoic acid,

docosahexaenoic acid, β-sitosterol, niacin, aesculetin, levodopa and carbidopa with one violation and Hesperidin with 3 violations out of five. Any substance with zero violation has a higher chance of becoming bioavailable when taken orally. The topological polar surface area (TPSA) provides for the prediction of drug candidate transport capabilities in the intestines and across the blood-brain barrier. The TPSA score for all of the extract's active ingredients, as well as the conventional drugs carbidopa and levodopa, was less than 140, indicating superior permeability into the tissues. Molinspiration ADME allows one or more molecules to have their critical physicochemical, pharmacokinetic, drug-like, and associated properties computed. The number of H-bond acceptors should be between 0 and 10, and the number of H-bond donors should be between 0 and 5. In this analysis, all of the active ingredients were found to be within the acceptable range.

When ilogP equals 0, the compound is equally partitioned between the lipid and aqueous phases; when ilogP equals 1, the compound has a higher affinity for the aqueous phase (it is more hydrophilic); when ilogP equals 1, the compound has a higher concentration in the lipid phase (it is more lipophilic); when ilogP equals 1, the compound has a higher concentration in the aqueous phase (it is more lipophilic (i.e., the compound is more lipophilic). In the present study almost all the active constituents herbacetin, beta carboline, isoquinoline, ferulic acid, docosahexaenoic acid, β-sitosterol, have shown a positive ilogP value clearly indicating a higher concentration in the lipid phase except D-xylose, D-arabinose, L-rhamnose, L-fucose, hesperidin, herbacetin, beta carboline, isoquinoline, ferulic acid, eicosapentanoic acid, docosahexaenoic acid, beta sitosterol, niacin, aesculetin and standard drugs levodopa, carbidopa which have shown a negative ilogP value indicating a higher concentration in the aqueous phase [15].

4.3 Bioactivity score of using molinspiration

Molecular docking was used to score a few chemicals from a *Linum usitatissinum* n-hexane extract. The selected chemicals' scores can be classified as active (bioactivity score > 0), moderately active (bioactivity score: -5.0-0.0), or inert (bioactivity score -5.0). All of the components in the *Linum usitatissinum* n-hexane extract were shown to be enzyme inhibitors. The compounds had an active to moderate score for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor, and protease inhibitor, according to the data.

5. CONCLUSION

In rodent models, the n-hexane extract of *Linum usitatissimum* seeds has anti- Parkinson's activity. More research is needed to separate the extract specific phytochemical ingredients and to determine the exact mechanism of its anti- Parkinson's efficacy.

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CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

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