Original Research Article

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

Comment [RD1]: An in-vitro Investigation of anti-Parkinson's Activities of Flax Seed (*Linum usitatissimum* L.) using Experimental and Computer Aided Mechanisms

ABSTRACT:

The present research explored *in vivo* anti Parkinson's activity using haloperidol induced catalepsy, reserpine antagonism 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 evaluations were done using 200 mg/kg, bd.wt and 400 mg/kg, bd.wt. The intensity of muscular rigidity, tremors, akinesia, grip strength, and locomotory activity were significantly decreased by HELU (200 and 400 mg/kg bd.wt., *p.o.*) in reserpine antagonism model. Treatment with HELU (200 and 400 mg/kg bd.wt., *p.o.*) has significantly reduced the duration of catalepsy induced by haloperidol as compared to haloperidol induced groups. To understand the ligand-binding affinity of the active constituents of the extract, docking studies were performed for natural compounds against PDB ID: 4I6B, 7JOZ, 4XUD, 4OYX. 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 results unveiled that n-hexane extract of *Linum usitatissimum* possessed significant anti Parkinson's activity.

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

1. INTRODUCTION

Parkinson's disease ( ) is a chronic progressive neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra of the brain. The pathological trademarks of PD are due to the presence of intracytoplasmic inclusions from protein aggregates called Lewy Bodies (LBs) and the depletion of pigmented dopamine containing

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neurons in the region known as substantia nigra pars compacta [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 ( ).

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. In the present study an attempt is made to screen the n-hexane extract of *Linum usitatissimum* for its anti-Parkinson's activity using *in-vivo* and *in silico* models.

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 sidearm. 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].

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Comment [RD30]: **Seed Collection and Identification**

Comment [RD31]: A XXX kg (quantity) of *Linum usitatissimum* seeds were purchased at xxxxx name of place/country seed is available.
The seed was identified, and authenticated by botanist at Suresh Babu, New Government Degree College Kukatpally, xxxx country.

Preparation of Seeds
Linum usitatissimum seeds were cleaned and dried under shade for six days and ground. The powdered seeds was stored in xxxxx at xxxx condition.

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Comment [RD34]: The **n-hexane extraction of *Linum usitatissimum* was carried using** Soxhlet extractor following standard procedures [3].

Comment [RD35]: ***Linum usitatissimum***

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

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

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*.

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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).

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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).

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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.

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- Haloperidol induced catalepsy.
- Reserpine antagonism

Comment [RD58]: using Haloperidol induced catalepsy and Reserpine antagonism models as described below.

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].

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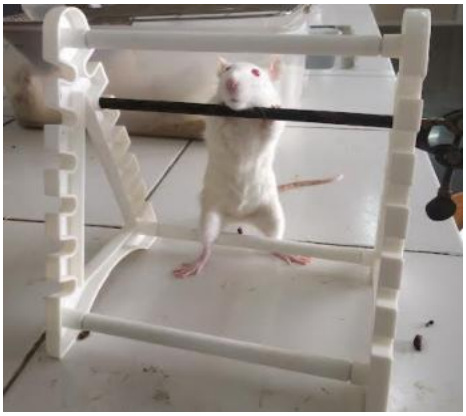

Figure 2: Haloperidol induced catalepsy in Wistar rats

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 5 (mg/kg bd.wt.,) except control and after 30 min, group III, IV and V receives their corresponding treatment during 5 days. On 5th 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].

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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.

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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

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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.

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

= 280/450 x 100

= 62.26%

3.2 Preliminary phytochemical analysis

Comment [RD105]: Constituent

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).

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Comment [RD109]: ,and linoleic acids as the most dominant phytochemical constituent
Of -hexane seed extract of *Linum usitatissimum*

Table 1: Preliminary phytochemical analysis

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

Note: ++ indicates present, -indicates absent

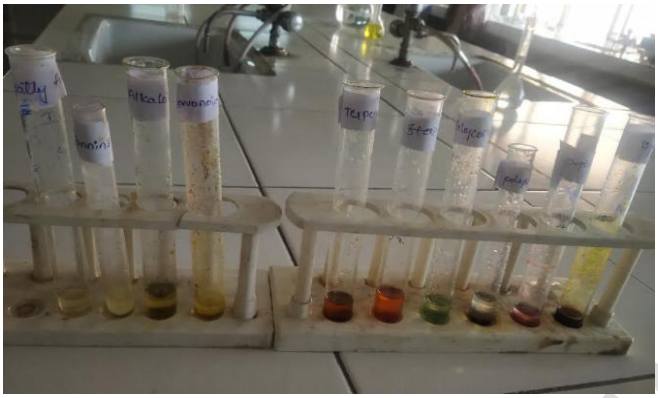

Figure 3: Preliminary Phytochemical analysis

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3.3 Acute toxicity studies

n-hexane extract of heads of *Linum usitatissimum* was tested on Swiss albino mice up to a dose of 2000 mg/kg bd. wt. The animal did not exhibit any signs of toxicity or mortality up to 2000 mg/kg bd. wt. various morphological and behavioural characters were observed during the study. Hence the extract was found to be safe up to 2000 mg/kg bd. wt.

Comment [RD111]: The **acute toxicity of** n-hexane extract of *Linum usitatissimum* on Swiss albino mice up to a dose of 2000 mg/kg bd. wt. Is presented in Table xxxxx.

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3.4 *In vivo* anti Parkinson's activity

Comment [RD113]: anti-Parkinson's

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

Comment [RD114]: Just present the result

3.4.1 Haloperidol induced catalepsy

The cataleptic score of haloperidol treated groups was found to be higher. But in groups treated with the HELU and standard drug (levodopa+carbidopa 30 mg/kg, *i.p*) the cataleptic score was found to be reduced.

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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.

Comment [RD117]: (Table 3).

Table 3: Effect of HELU on reserpine antagonism in mice

Treatment	Muscular Rigidity (secs)	Tremors (score)	Akinesia (Number of steps taken with forelimbs)	Locomotory (activity in 10 min)	Grip strength (Latency to 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	23±0.84*$^{a\ ns}$	2±0.8*^{aA}	38±0.50*^{aA}	202±0.2*^{aA}	90±0.02*^{aA}

bd.wt.)+Reserpine					
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.

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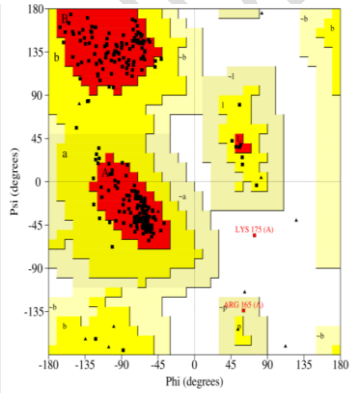
Comment [RD120]: The proteins 4I6B, 7JOZ, 4XUD, and 4OYX were analyzed on the Ramachandran plot to know amino acid present as tabulated in Table 5 and pictorial representation by the Figure below.

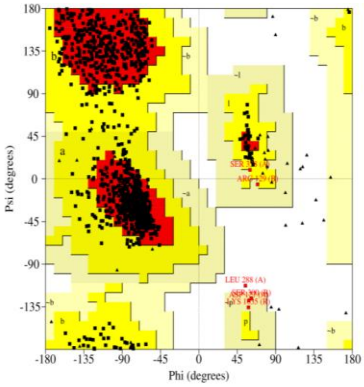
Table 5: Ramachandran plot status with protein with 4I6B, 7JOZ, 4XUD and 4OYX.

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

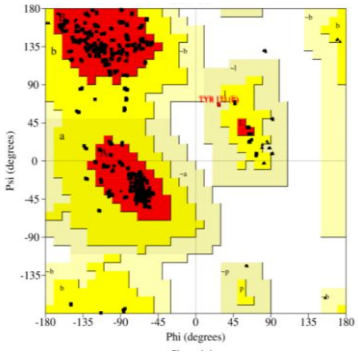
a) 4I6B b) 7JOZ

c) 4XUD d) 4YOX

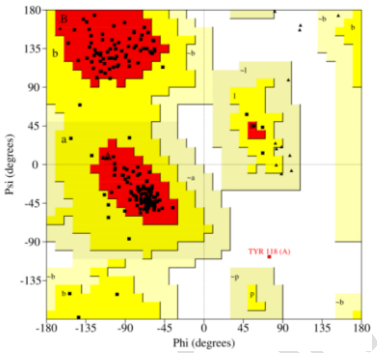




a) 4I6B b)7JOZ



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 modulator**	**Kinase inhibitor**	**Nuclear receptor ligand**	**Protease inhibitor**	**Enzyme inhibitor**
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, induces catalepsy due to blockade of dopamine receptors in the striatum. Fatty acids in haloperidol induced catalepsy elicits multiple effects including hypothermia, catalepsy and reduced motor activity, as well as actions on the hypothalamic–pituitary axis including modulation of growth hormone, luteinizing hormone, and prolactin secretion and also corrects the 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. A cyanogenic glycoside Linamarin in the combination of treatment evaluated and improved the open field and swim tests to identify locomotor and hippocampal alterations in adult male Wistar rats. The flavonoids such as Herbacetin, a novel flavonoid found in *Linum*

Comment [RD121]: Parkinson's disease is a condition that imposes several motor deficits, often followed by cognitive dysfunction. The progression of the disease is also known to induce tremors, muscular rigidity, akinesia, and postural instability [8]. The haloperidol showed cataleptic behavior. Haloperidol is a non-selective D2 dopamine antagonist, that induces catalepsy due to the blockade of dopamine receptors in the striatum.

Comment [RD122]: haloperidol-induced

Comment [RD123]: elicit

Comment [RD124]: , and

Comment [RD125]: hypothalamic-pituitary

Comment [RD126]: build-up

Usitatissimum, its high-fat and cholesterol-enriched diet induced obesity has been associated with the development of glucose intolerance, hyperglycaemia, insulin resistance, hepatic steatosis, and dyslipidaemia [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 showed 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]. The cyclic peptides specifically reduce the toxicity of human –synuclein and prevent dopaminergic neuronal loss [13]. 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].

Comment [RD127]: diet-induced

Comment [RD128]: hyperglycemia

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Comment [RD130]: the n-hexane

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Comment [RD133]: the reserpine

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4.1 Molecular docking

Molecular docking continues to holds great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. The docking analysis of isolated compounds from n-*hexane extract of Linum usitatissinum and* standard drug combination like levodopa and carbidopa were carried out using Schrödinger software. 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, beta sitosterol, and aesculetin against all selected PDB ID: 4I6B, 7JOZ 4XUD 4OYX. The glide scores of the D-xylose, D-arabinose, L-rhamnose, L-fucose, and aesculetin were found to be

Comment [RD144]: hold

Comment [RD145]: screens small (delete space)

Comment [RD146]: an n-*hexane*

Comment [RD147]: combinations

Comment [RD148]: was

Comment [RD149]: beta-carboline

Comment [RD150]: beta-sitosterol,

Comment [RD151]: , and

Comment [RD152]: beta-carboline,

more than the glide score of standard drug combination levodopa and carbidopa stating that the compounds might have same affinity to bind to the proteins. These results clearly indicate that the chemical constituents mentioned above might have shown similar mechanism to that of the standard drug levodopa and carbidopa as anti-Parkinson's . The proteins identified namely PDB ID: 4I6B, 7JOZ 4XUD 4OYX are modeled and the qualities of the 3D model were evaluated using the PROCHECK program and assessed using the Ramachandran plot. It is evident from the Ramachandran plot that predicted models have most favorable regions, additionally allowed regions, generally allowed regions and disallowed regions. Such a percentage distribution of the protein residues determined by Ramachandran plot shows that the predicted models are of good quality. According to Ramachandran plot a good quality model would be expected to have over 90% in the most favoured region. Proteins like PDB ID: 4I6B, 7JOZ 4XUD and 4OYX showed almost 90% favoured a region which clearly indicates that the selected models in the present study are of good quality [14].

Comment [RD153]: the same

Comment [RD154]: a similar

Comment [RD155]: 4OYX,

Comment [RD156]: the most

Comment [RD157]: , and

Comment [RD158]: the Ramachandran

Comment [RD159]: the Ramachandran plot,

Comment [RD160]: , and

4.2 Mol inspiration ADME analysis

Molinspiration molecular properties were calculated on the bases of Lipinski's rule and its components. Lipinski's rule of five is to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it an orally active drug in humans. In the present study, all the compounds that are docked have lower molecular weight so that they are easily absorbed, diffused 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, beta sitosterol, niacin, aesculetin, levodopa and carbidopa with one violation and Hesperidin with 3 violations out of five. Any compound with zero violation clearly indicates the probability of its higher oral bioavailability. Topological polar surface area (TPSA) allows the prediction of transport properties of drug candidates in the intestines and blood-brain barrier. The TPSA score in all the selected active constituents of the extract and standard drug carbidopa and levodopa were found to be less than 140 which clearly indicated better permeability into the tissues. Molinspiration ADME enables the computation of key physicochemical, pharmacokinetic, drug-like and related parameters for one or multiple molecules. Number of H-bond acceptors should be in a range of 0-10 and number of H-bond donors should be 0-5. All the selected active constituents in the present study were found to be within the range.

Comment [RD161]: basis

Comment [RD162]: , and

Comment [RD163]: beta-carboline,

Comment [RD164]: beta-sitosterol

Comment [RD165]: , and

Comment [RD166]: , and

Comment [RD167]: The number

Comment [RD168]: the number

A negative value for ilogP means the compound has a higher affinity for the aqueous phase (it is more hydrophilic); when ilogP equals 0 the compound is equally partitioned between the lipid and aqueous phases; a positive value for ilogP denotes a higher concentration in the lipid phase (i.e., the compound is more lipophilic). In the present study almost all the active constituents herbacetin, beta carboline, isoquinoline, ferulic acid, docosahexaenoic acid, beta 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].

Comment [RD169]: beta-carboline,

Comment [RD170]: beta-sitosterol,

Comment [RD171]: beta-carboline

Comment [RD172]: beta-sitosterol,

4.3 Bioactivity score of using molinspiration

A few compounds of *n-hexane extract of Linum usitatissinum* were subjected to bioactivity score using mol inspiration. The scores for the selected compounds can be interpreted as Active (bioactivity score > 0), moderately active (bioactivity score: -5.0-0.0) and inactive (bioactivity score < -5.0). All the compounds of *n-hexane extract of Linum usitatissinum* were found to be active towards enzyme inhibitor. The results indicated that the compounds exhibited active to moderate score towards GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor and protease inhibitor.

Comment [RD173]: inhibitors.

Comment [RD174]: scores

Comment [RD175]: a kinase inhibitor

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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.

Comment [RD177]: exhibited

Comment [RD178]: extract-specific

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Comment [RD179]: Not cited? Please verify this.