Section A-Research paper

Exploring the Neuroprotective Potential of *Spathodea campanulata* leaves: A comprehensive In-silico and In-vivo Study on Anti-Parkinson Activity

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Abstract

Spathodea campanulata commonly known as the African tulip tree belongs to the family Bignoneaceae. The leaves of this tree were found to be rich source of various secondary metabolites and have been used in several ailments in traditional systems of medicine. Hence this study aims to assess the anti-Parkinson efficacy of *Spathodea campanulata* phytoconstituents in leaf extract by in-silico and in-vivo experiments. Molecular docking score and ADMET properties of the phytoconstituents of *Spathodea campanulata* were predicted using schrödinger software. The effect of *Spathodea campanulata* was evaluated in haloperidol induced parkinson's rats, the evaluation of in vivo anti-Parkinson's effect was performed by behavioural studies that include catalepsy and rota rod test and biochemical assays like estimation of catalase, acetylcholinesterase and dopamine. In-silico studies revealed that cerebroside, catapol and specioside had the highest docking scores among all the other selected phytochemicals with protein targets of PD. In-vivo study results showed significant decrease in cataleptic scores, significant increase in fall-off time in rota rod test and biochemical estimations revealed significant increase in the catalase enzyme activity and dopamine levels, whereas significant decrease in acetylcholinesterase enzyme activity in the brain when treated with leaf extract of *Spathodea campanulata*. Therefore, in conclusion study revealed the significant activity of *Spathodea campanulata* leaf extract against Parkinson's disease. Further, the results suggest that *Spathodea campanulata* has a promising therapeutic potential against Parkinson's disease and further research can explore its molecular mechanism and disease- modulating pathways.

Keywords: acetylcholinesterase, *Spathodea campanulata*, anti-Parkinson's, dopamine

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease. More than 6 million people globally are said to be suffering from Parkinson's disease. It is estimated that about 2 % of men and 1.3% of women have lifetime risk for Parkinson's disease (Brown, Lockwood,et al., 2005). Parkinson's disease is uncommon before the age of 50; around 2% of persons over the age of 65 are affected, whereas this figure is more than double (i.e., 5%) in people over the age of 80. It is caused by the depletion of dopaminergic neurons in the substantia nigra pars compacta region (SNpc) of the basal ganglia and other parts of the brain (Teleanu, Niculescu, et al., 2022). Person with PD experience motor symptoms like, bradykinesia, tremor, rigidity, gait instability, postural instability, and non- motor symptoms like cognitive impairment, depression, constipation, dysphagia, mood disorders (Guo, Zhao, et al., 2018). On pathological observation many neural inclusions in the form of Lewy bodies and Lewy neurites are found. Lewy bodies mostly consist of aggregated and misfolded -synuclein species (Tolosa, Garrido, et al., 2021). The pathogenesis of PD is influenced by alpha-synuclein aggregation, mitochondrial dysfunction, oxidative stress, excitotoxicity, and neuroinflammation (Kaur, Mehan, et al., 2019).

The current treatment that is available for Parkinson's disease is foccused on symptomatic relief rather than depletion of the disease progression. Dopamine based treatments are used to reduce motor symptoms. Non dopaminergic therapies are used in the treatment of non motor symptoms. Rehabilation and exercise along with pharmacological treatment have shown to improve symptoms of parkinson's disease. Complicated cases of PD are treated with advanced therapies like levodopa and carbidopa enteral suspension, deep brain simulation. Palliative care is also a part of treatment along with other therapies in late stages of PD (Bloem, Okun, et al., 2021)

Spathodea campanulata P. Beauv. is a large tall tree in the Bignoniaceae family with a history of medicinal use in Africa (Santos, Minatel, et al., 2020). Literature survey on phytochemicals of different parts of *Spathodea campanulata* has shown presence of secondary metabolites like Iridoids, sterols, cerebrosides, flavonoids, and triterpenoids. Various iridoids like Verminoside and Specioside; Triterpenoids like Spathodic acid, 3β-acetoxy oleanolic acid, siaresinolic acid, 3β-acetoxy12-hydroxyoleanan-28, 13-olide, and oleanolic acid, ursolic acid, tomentosolic acid, etc; sterols, such as spathodol, β-sitosterol-3-O-β-Dglucopyranoside, β-sitosterol-3acetate, stigmasterol, cholesterol, and campesterol were found. Other compounds like anthocyanins, cinnamic acid derivatives, carotenoids, monoterpenoids and sesquiterpenoids, diterpenoids (abietatriene), coumarins, aromatic acid, and their esters were found in the flower, stem bark, leaf, and aerial parts of the tree(Magnibo, Nyemb,et al., 2021). It is stated in the traditional system for the treatment of malaria, diabetes, stomach ulcers, wounds, skin infections, and viral disorders. Preclinical investigations have revealed extraordinary effectiveness, lending evidence to the plant's long-standing usage as an antimalarial, wound healing, antidiabetic, antibacterial, and anti-inflammatory drug (Padhy GK., 2021). In this concept the the anti-Parkinson effect of *Spathodea campanulata* was not been evaluated, hence the study aims to explore the effect of *Spathodea campanulata* as antiparkinsons effect.

2. Materials and methods

2.1. Collection of plant material and Preparation of extract

The leaves of *Spathodea campanulata* were collected from Ladyhill, Dakshina Kannada District Karnataka, India during the month of October. The plant was then authenticated by Prof Smitha Hegde, Deputy Director, Nitte University, Centre for Science and research, Mangalore. The leaves were cleaned and shade dried for 15 days, further coarsely grinded in an electric grinder and macerated with ethanol for 7 days, followed by filtration. The filtrate was evaporated by placing the extract in water bath, and once the content was evaporated, the dry extract was obtained. The extracted material was then kept in a desiccator for future use.

2.2. Preliminary phytochemical tests

Preliminary qualitative analysis of phytochemicals for presence of flavonoids, tannins, saponins, alkaloids, terpenoids, and steroids were performed in the extracts using standard methods (Shaikh, 2020)

2.3. Selection of animals

Albino wistar rats weighing 175-200 g of either sex was procured from the animal house of NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore. The animals were divided into different groups. The animals were housed in different cages based on their sexes. Cages were kept at standard lab temperature 25 -29º C with an appropriate 12-hour dark and light cycle and were given free access to regular food and water. Each animal was handled carefully without causing any stress. The experimental procedures were done according to CCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal). New Delhi, India, and the research work was permitted and approved by the institutional Animal Ethics Committee and the approval number of the protocol is NGSMIPS/IAEC/AUG-2022/315.

2.4. Anti-Parkinson activity

Albino Wistar rats were randomly divided into 5 groups, each group containing 6 animals (ie. n=6) to assess the anti-Parkinson activity of *Spathodea campanulata* leaf extract. Group I received normal saline, Group II did not receive any treatment, group III received Syndopa (L-dopa + C-dopa) 20 mg/Kg orally, group IV received 200 mg/kg of *S. campanulata* leaf extract and group V received 400 mg/kg of *S. campanulata* leaf extract. The treatment dose 1-hour prior was administered, followed by administering haloperidol (1mg/kg, i.p) for 21 days to group II III, IV and V. The disease group receives one dose of haloperidol (1mg/kg i.p). The Anti-Parkinson activity of the treatment groups was evaluated by behavioral studies and biochemical assay.

2.5. Behavioural studies

2.5.1. Haloperidol induced catalepsy

Catalepsy was measured by the amount of time the rat remained in an intact position with both front limbs raised and resting on a bar. Catalepsy is said to stop when both front paws were withdrawn from the bar or the animal moved its head in an exploring way. All observations were conducted in a peaceful environment.The test compound of low (200 mg/ kg) and high doses (400 mg/ kg) or the standard (Syndopa 20 mg /kg) was injected (p.o.)(Waku, Magalhães, et al., 2021) into groups of 6 rats each. Another 2 groups of rats will be taken as control and disease groups. Each of the rats were first placed on the table and scored based on the below criteria The rats were subjected to this scoring after 15 min, 30 min, 60 min, 90 min and 120 min of administration of haloperidol. Normal movement of rats when placed on the table, score = 0; The movement of rats only when touched or pushed, score = 0.5; Rat placed on the table with front paws set alternately on a 3cm high block fails to correct the posture in 10 seconds; score = 0.5 for each paw with a total of 1 for this stage. If the rat fails to remove its front paws when placed alternately on a 9cm block, score = 1 for each paw with a total score of 2 for this stage (Waku, Magalhães, et al., 2021).

2.5.2. Rota rod test

Rotarod test is an initial screening method for neuromuscular impairment which is used to assess motor coordination. Here the rats are balanced on a rotating rod and their latency to fall is recorded as the endpoint. The rod will be rotating at 20 rpm. The test compound of low (200 mg/ kg) and high doses (400 mg/ kg) or the standard (Syndopa 20 mg /kg) was injected (p.o.)(Waku, Magalhães, et al.,2021) into groups of 6 rats each. Another 2 groups of rats will be taken as control and disease groups. Then, the animals were exposed to a horizontal rod whose diameter is 2.5 cm and height is 25cm from the floor. The animals were then kept for observation for 5 minutes. Then the fall-off time for each rat, before the drug treatment and after the administration of the drug was recorded (Dayan ,1998).

2.6. Biochemical assay

2.6.1. Preparation of rat brain homogenate

The excised animal brain tissue was weighed, placed in an ice bath, cleaned with ice cold saline, and homogenised with 20:1 (w/v) of tissue and phosphate buffer (pH 8.0 0.1M) ratio was employed, which was then put in a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 10 minutes at 3000 rpm, and the resultant supernatant liquid was used to estimate biochemical parameters of the brain.

2.6.2. Estimation of catalase (Neagu, Paun, et al., 2015)

Catalase is used to evaluate the oxidative stress level in rat brains. The test mixture contains 50µl of 1 M Tris-HCl buffer with a pH of 8.0, 5mM EDTA, 900µl of 10 Mm H_2O_2, 30µl of distilled water, and 20µl of brain supernatant are included. The rate of H_2O_2 breakdown is measured spectrophotometrically at 240 nm.

2.6.3. Estimation of acetylcholinesterase (Dayan,1998)

AchE serves as a marker for cholinergic neuron loss in the forebrain. Elman's technique was used to measure AchE activity. The test mixture comprises of 0.05 mL supernatant, 3 mL sodium phosphate buffer (pH 8), 0.1 mL acetylcholine iodide, and 0.1 mL DTNB. The absorbance at 412nm will be monitored every 2 minutes at 30-second intervals. The results will be expressed in micromoles of acetylcholine iodide hydrolyzed per minute per mg of protein.

2.6.4. Estimation of dopamine (Ansari, Singh, et al., 2022)

1 ml of rat brain supernatant was taken in 25 ml flask. 1ml of 4N HCl and 0.02N brominating mixture was added to the flask. The flask is thoroughly shaken and let to bromine completely for 5 minutes. Then, 1 ml of potassium iodide in 0.1N solution was added to each flask and completely brominated to yield 25 ml. The resulting yellow solution is measured spectroscopically at 280nm using distilled water as the reference. The calibration curve was used to determine dopamine content in the rat brain supernatant.

2.7. Statistical analysis

All the data are expressed in the form mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) which will be followed by the Dunnett's test using Graph Pad: Prism computer software version 9. A p-value of less than 0.05 was taken as statistically significant.

2.8. In-silico studies

2.8.1. Selection of phytoconstituents

In this study phytochemicals present in *Spathodea campanulata* was obtained through literature(Padhy GK, 2021). The canonical smiles of these phytochemicals were obtained from chemical database Pub chem (www.pubchem.com).The phytochemicals from *Spathodea campanulata* leaves were subjected to ADMET profile screening and the phytochemicals like verminoside, apigenin, catapol, diosmethin, luteolin, spathodic acid, specioside and cerebroside was selected for docking studies, since they had better ADMET scores compared to other phytochemicals.

2.8.2. Selection of protein targets for Parkinson's disease

Disgenet (www.disgenet.org) was used to obtain various genes that are associated to PD. Swiss target prediction (www.swisstargetprediction.ch) which is an online platform that predicts the most probable targets for small molecule. The final common targets obtained were selected and the PDB id of those targets were obtained by protein data bank (www.rcsb.org).

2.8.3. Molecular docking and ADMET properties prediction

The ligand was prepared using ligprep schrodinger. The protein was prepared using protein preparation wizard schrodinger. The grid for the target protein was prepared using glide grid. Then the ligands were docked to the selected target protein using glide dock schrodinger. The ADMET properties of the compounds were predicted using QikProp schrödinger.

3. Results and discussion

The extract of the *Spathodea campanulata* leaves was prepared by maceration method. A total of 454 grams of the dry leaves was coarsely powdered and subjected to maceration in 95% ethanol. The percentage yield of *Spathodea campanulata* leaf extract was found to be 9.92 %.Qualitative phytochemical investigation of *Spathodea campanulata* leaf extract is presented in Table 1, revealed various secondary phytochemicals like alkaloids, carbohydrates, flavonoids, saponins, tannins, glycosides, triterpenoids, steroids and phenols.

Table 1: Results of preliminary phytochemical analyses of *Spathodea campanulata*

Sl.no	Chemical Tests	Maceration extract of *Spathodea campanulata*
1.	**Alkaloids** a) Dragendorff's test b) Wagner's test c) Mayer's test d) Hager's test	 + + + +
2.	**Carbohydrates** a) Molisch's test b) Benedict's test	 + +
3.	**Saponins** a) Froth formation test	 +
4.	**Steroids** a) Liebermann's test b) Salkwoski's test	 + +
5.	**Tannins/ Phenolic compounds** a) Ferric chloride test b) Lead acetate test c) Gelatin test	 + + +
6.	**Flavanoids** a) Shinoda's test b) Alkaline reagent tests	 + +
7.	**Triterpenoids** a) Liebermann's Burchard test	 +
8.	**Glycosides** a) Keller-Killiani test	 +
9.	**Proteins** a) Biuret test b) Millon's test	 + +

Note: + present, - absent

3.1. Anti-Parkinson activity

In this study the two doses of *S.campanulata* ie; 200 mg/kg and 400 mg/kg was selected based on references of acute oral toxicity study conducted by studies that were conducted on the same extract for evaluation different pharmacological activity (Padhy GK., 2021).

3.2. Behavioural studies

3.2.1. Haloperidol induced catalepsy

Haloperidol-induced catalepsy is one of the animal models used to evaluate extrapyramidal side effects like akinesia, rigidity, and tremors. Haloperidol generates animal model of Parkinsonism by interfering with intracellular catecholamine storage, which results in dopamine depletion in nerve terminals.

The evaluation of catatonic activity of the group V and group IV exhibited significant dose dependent decrease in catatonic activity when compared with the group II. The group IV was statistically significant ($p<0.05$) when compared with group III and group I and group V was statistically significant ($p<0.05$) when compared with group I. The group V of plant extract showed better activity compared to group IV. The group II showed significant increase in cataleptic score indicating that the inducing agent (Haloperidol) had the ability to produce catalepsy in these animals. In the results, the group V and group III had similar cataleptic scores when compared to group I and group II indicating the capability of the plant extract to improve catalepsy is similar to the standard drugs actions.

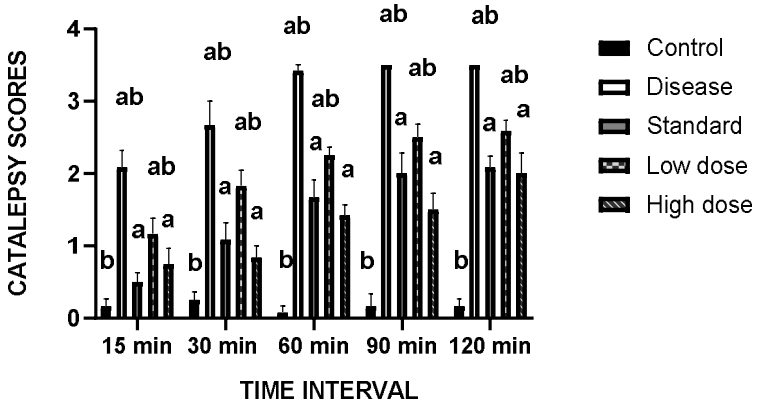

Figure 1: Effect of *Spathodea campanulata* leaf ethanolic extract on Voluntary activity (in score) in Haloperidol induced Catalepsy. The values are expressed as mean ± SEM (n=6) $p< 0.05$ is considered as statistically significant. a = $p < 0.05$ when compared to control group and b = $p < 0.05$ when compared to standard group. Here Control denotes Group I: Normal Control group, Disease denotes Group II: Disease control group, Standard denotes Group III: Standard control group, Low dose denotes Group IV: Low dose group and High dose denotes.

The rats from the control group showed no cataleptic effect suggests that there's no cataleptic actions on normal condition. The rats from disease group showed significant increase in the cataleptic scores thus indicating the inhibitory actions of haloperidol on the dopaminergic system. The rats of standard group when pre-treated with Syndopa showed significant decrease in cataleptic scores when compared to disease group. The rats of low dose and high dose which were pre-treated with 200mg/kg and 400mg/kg of ethanolic extract of leaves of *Spathodea campanulata* showed significant decrease in the cataleptic scores when compared to disease group. *Spathodea campanulata* leaf extract showed potential in the experimental study for improving catatonic activity, suggesting that it may have regenerative or healing properties. This action could be because of neuroprotective action of this extract against the actions of haloperidol.

3.2.2. Rotarod

Rotarod is used to measure their "minimal extrapyramidal deficit," which includes muscular control and balance. Evaluation of motor co-ordination of the ethanolic extract of *Spathodea campanulata* suggested that groups IV and group V showed dose dependent significant increase in fall-off time when compared to group II. The group IV was statistically significant ($p < 0.05$) when compared to group I and group III and group V was statistically significant ($p < 0.05$) when compared to group I. The group V showed better activity compared to group IV. The group II showed significant decrease in the fall-off time indicating that the inducing agent (Haloperidol) had the ability to disrupt the motor co-ordination of the rats. In the results group IV and group III, the group V and group III had similar fall-off time and significant results when compared to group I and group II indicating the capability of the plant extract to improve the motor co-ordination is similar to the standard drugs actions.

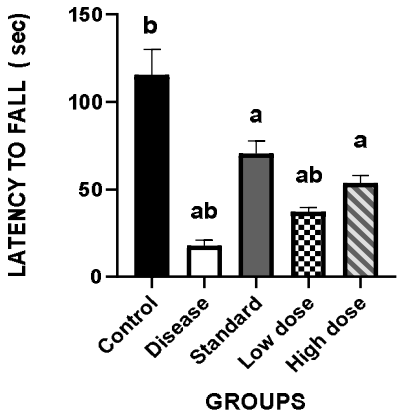

Figure 2: Effect of *Spathodea campanulata* leaf ethanolic extract on fall-off time in seconds in rota rod. The values are expressed as mean ± SEM (n=6) $p< 0.05$ is considered as statistically significant. a = $p < 0.05$ when compared to control group and b = $p < 0.05$ when compared to standard group. Here Control denotes Group I: Normal Control group, Disease denotes Group II: Disease control group, Standard denotes Group III: Standard control group, Low dose denotes Group IV: Low dose group and High dose denotes.

The rats from control group which were treated with normal saline showed higher values of fall-off time indicating there was no neuromuscular impairment in them whereas the disease group animals showed significant decrease in fall-off time when compared to normal control group indicating that the inducing agent haloperidol has impaired the dopaminergic system of the rats. The rats from the standard group when pre-treated with the standard drug Syndopa showed significant increase in the fall-off time when compared to disease group. The low dose and high dose group animals showed significant increase in fall-off time compared to the disease and standard group. *Spathodea campanulata* leaf extract showed potential in the experimental study for improving muscular coordination, suggesting that it may have regenerative or healing properties. This action could be because of neuroprotective action of this extract against the actions of haloperidol.

3.2.3. Biochemical assay

Biochemical estimations like estimation of catalase, acetylcholinesterase and dopamine were performed on the 21st day of study.

Estimation of catalase

The catalase level in the rat brain in group IV and group V exhibited dose dependent significant increase when compared with groups II. The rats of group IV were statistically significant($p<0.05$) when compared with standard control group and diseased control group and group V was statistically significant($p<0.05$) when compared with group I. The group V showed better activity compared to group IV. In the results obtained, group V and group III had similar catalase level indicating the capability of the plant extract to show antioxidant activity is similar to the standard drugs actions.

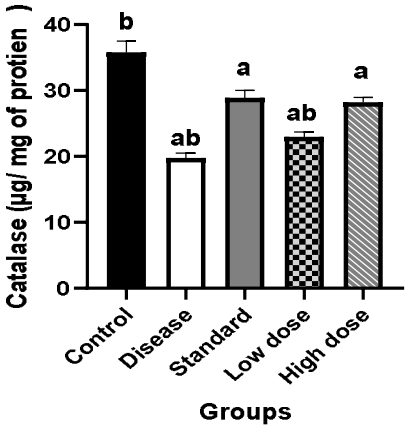

Figure 3: Effect of *S. campanulata* on level of Catalase in brain. The values are expressed as mean ± SEM (n=6) $p< 0.05$ is considered as statistically significant. a = $p < 0.05$ when compared to control group and b = $p < 0.05$ when compared to standard group. Here Control denotes Group I: Normal Control group, Disease denotes Group II: Disease control group, Standard denotes Group III: Standard control group, Low dose denotes Group IV: Low dose group and High dose denotes

On estimation of catalase, the disease group had significant decrease in the catalase level which confirms the neurodegeneration caused by haloperidol. The level of catalase was higher in low dose and high dose animal group compared to the disease group suggests the antioxidant potential of this extract.

Estimation of acetylcholinesterase

The acetylcholinesterase level in the rat brain of group IV and group V exhibited dose dependent significant decrease when compared with groups II. The group IV was statistically significant($p<0.05$) when compared with group III and group 1 and group V was statistically significant ($p < 0.05$) when compared to group I. The group V showed better inhibition activity compared to group IV. In the results, group V and group III had similar acetylcholinesterase level when compared to disease group indicating the capability of the plant extract to show decrease in acetylcholinesterase level is similar to the standard drugs actions.

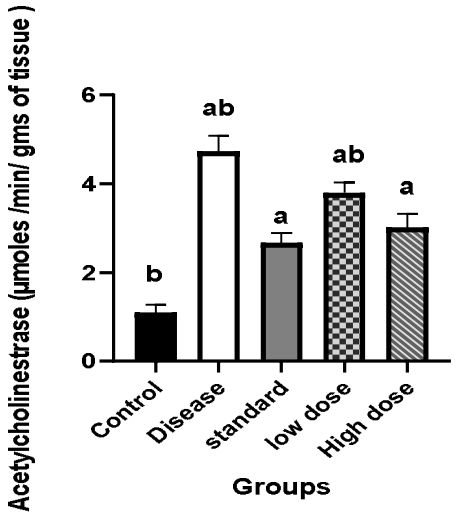

Figure 4: Effect of *S.campanulata* on level of Acetylcholinesterase in brain. The values are expressed as mean ± SEM (n=6) $p< 0.05$ is considered as statistically significant. a = $p < 0.05$ when compared to control group and b = $p < 0.05$ when compared to standard group. Here Control denotes Group I: Normal Control group, Disease denotes Group II: Disease control group, Standard denotes Group III: Standard control group, Low dose denotes Group IV: Low dose group and High dose denotes

On estimation of acetylcholinesterase level, the disease group had higher values compared to the control group. The standard, low dose and high dose group had lower values compared to the disease group. This suggests leaf extract had anti- acetylcholinesterase activity comparable to standard drug.

Estimation of dopamine

The dopamine level in the rat brain of group IV and group V exhibited dose dependent significant increase when compared with groups II. The group IV was statistically significant($p<0.05$) when compared with group III and group 1 and group V was statistically significant ($p < 0.05$) when compared to group I. The group V showed higher levels of dopamine when compared to group IV. In the results group V and group III had similar dopamine level indicating the capability of the plant extract to show increase in dopamine level is similar to the standard drugs actions.

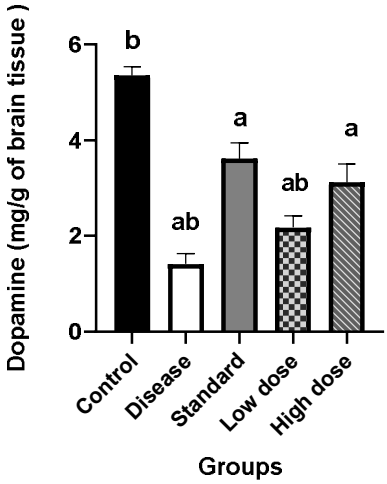

Figure 5: Effect of *S.campanulata* on level of Dopamine in brain. The values are expressed as mean ± SEM (n=6) $p< 0.05$ is considered as statistically significant. a = $p < 0.05$ when compared to control group and b = $p < 0.05$ when compared to standard group. Here Control denotes Group I: Normal Control group, Disease denotes Group II: Disease control group, Standard denotes Group III: Standard control group, Low dose denotes Group IV: Low dose group and High dose denotes

On estimation of dopamine level in the brain, the disease group had lower level of dopamine compared to the control indicating dopaminergic loss. The standard, low dose and high dose group had higher level of dopamine compared to the disease group**.** This suggests leaf extract had capacity to elevate dopamine level in the brain comparable to standard drug.

3.2.4. In-silico studies

A. Molecular docking

Phytochemicals like verminoside, apigenin, catapol, diosmethin, luteolin, spathodic acid, specioside, cerebroside and levodopa (standard) was taken for docking with the protein targets. Genes like TNF, CASP3, MME, MMP2, DRD4, MMP9, TTR and EGFR was obtained has common target genes between the phytochemicals and Parkinson's disease from disgenet and swiss target predictions. PDB ID: 1TNR, 7RN9, 7P11, 7XGJ, 6TTK, 1GKC, 3P3U and 3POV respectively was selected has protein target based on the common genes**.** When 1TNR was docked with all the selected phytochemicals and standard; catapol had the highest docking score of -6.194 and cerebroside had the lowest docking score of -1.327. When 7RN9 was docked with all the selected phytochemicals and standard; luteolin had the highest docking score of -3.783 and specioside had the lowest docking score of -2.092. When 7P11 was docked with all the selected phytochemicals and standard; catalpol had the highest docking score of -6.139 and cerebroside had the lowest docking score of -1.833. When 7XGJ was docked with all the selected phytochemicals and standard; verminoside had the highest docking score of -11.184 and cerebroside had the lowest docking score of -5.099. When 6TTK was docked with all the selected phytochemicals and standard; specioside had the highest docking score of -11.659 and spathodic acid had the lowest docking score of -6.061. When 1GKC was docked with all the selected phytochemicals and standard; cerebroside had the highest docking score of -7.952 and levodopa had the lowest docking score of -3.272. When 3P3U was docked with all the selected phytochemicals and standard; Specioside had the highest docking score of -3.727and verminoside had the lowest docking score of -2.091. When 3POV was docked with all the selected phytochemicals and standard; catalpol had the highest docking score of -6.652 and specioside had the lowest docking score of -2.091.

Table 2: Docking scores of selected phytoconstituent when docked with target proteins

Targets	**1TNR** (Human TNF Beta)	**7RN9** (Caspase-3 with inhibitor Ac-VDFVD -CHO	**7P11** (Galactin-8)	**7XGJ** (Human MMP-2)	**6TTK** (Human KLHL12 In complex with DVL1 Peptide)	**1GKC** (MMP9-inhibitor Complex)	**3P3U** (Human Transthyretin)	**3POV** (SOX-DNA Complex)
catalpol	-6.194	-	-6.139	-7.390	-10.296	-	-3.487	-6.652
luteolin	-4.857	-3.783	-3.449	-9.135	-8.257	-	-	-6.259
verminoside	-4.843	-2.554	-5.837	-11.184	-8.946	-	-2.091	-
specioside	-4.593	-2.092	-4.477	-9.291	-11.659	-	-3.727	-2.000
diosmetin	-4.153	-2.556		-	-	-	-	-

spathodic acid	-3.919	-3.120	-3.672	-	-6.061	-	-2.749	-4.421
apigenin	-3.523	-2.790	-3.403	-7.390	-6.998	-	-	-5.000
cerebroside	-1.327	-3.987	-1.833	-5.099	-	-7.952	-5.312	-5.739
diosmetin	-	-2.556	-3.045	-8.964	-9.698	-	-	-5.589
Levodopa	-3.306	-4.599	-5.875	-7.570	-8.192	-3.272	-2.569	-5.736

1TNR: TNR is said to be associated in the development of parkinsons disease(Sáenz, Munhoz, et al; 2021.). When 1TNR was docked with all the selected phytochemicals and standard; catapol had the highest docking score ie; -6.194. It showed 4 hydrogen bond interactions with amino residues between hydroxy group -SER 116, hydroxy group – GLN 118, hydroxy group – GLN 118 and hydroxy group and SER 132. Verminoside showed second highest docking score of -4.843. It showed 5 hydrogen bond interactions with amino residues between hydroxyl group – SER 116, hydroxyl group- GLN 118, hydroxyl group- GLN 118, hydroxyl group- ALA 138, hydroxyl group- ALA 138. Speciocide had docking score -4.593. It showed 2 hydrogen bond interaction with amino residues between hydroxyl group – ALA 138 and hydroxyl group- GLN 118 amino residues. Diosmetin had docking score -4.153, it had hydrogen bond interaction between hydroxyl group-SER 116 amino residue and it also showed pi- pi stacking interaction between benzene ring and TYR 134 amino residue. Spathodic acid had docking score -3.919, it had 3 hydrogen bond interaction with amino residue between hydroxyl group – GLN 118, hydroxyl group-TYR 134 and hydroxyl group-TYR 134. Apigenin had docking score -3.523, It had hydrogen bond interaction between hydroxyl group- SER 116 amino residue, and it also showed pi- pi stacking interaction between benzene ring and TYR 134 amino residue. Cerebroside had docking score -1.327, it showed 5 hydrogen bond interaction with amino residues between hydroxyl group – TYR 96, hydroxyl group-SER 116, amino group- SER 116, hydroxyl group-ASP 152, hydroxyl group-ASP 152. Standard drug levodopa had docking score -3.306, it showed 3 hydrogen bond interactions with amino residue between hydroxyl group – SER 116, amino group- SER 117 and hydroxyl group – TYR 134.

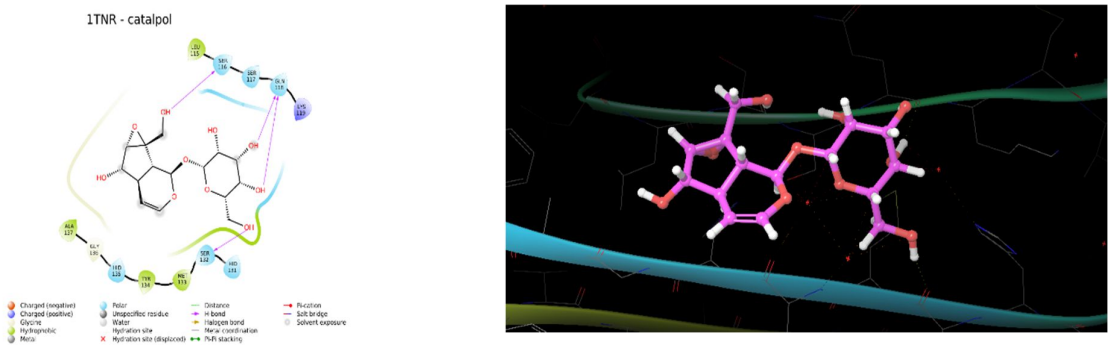

Figure 6: Interaction of catapol with 1TNR

7RN9: 7RN9 is Caspase-3 subunit p17 molecule. Inhibition of CASP3 is said to prevent the progress of PD(Liu, Guo, et al; 2013.). When 7RN9 was docked with the phytochemicals and standard drug; Cerebroside had the highest docking score of -3.987, It showed 2 hydrogen bond interactions with amino residues between hydroxyl group-ASN 141 of A chain, hydroxyl group- LYS 156 of A chain. Luteolin had the second highest docking score of-3.783, It showed hydrogen bond interaction with amino residues between Hydroxyl group-TYR 83 of A chain. Spathodic acid had docking score of -3.120, it showed hydrogen bond interaction with amino residue between hydroxyl group-TYR 83 of the A chain. Apigenin had docking score of -2.790, it showed hydrogen bond interaction with amino residues between hydroxyl group-TYR 83 of A chain and 2 pi- pi stacking interaction with amino residues between benzene ring-PHE 232 of B chain. Diosmetin has docking score of -2.556, it shows hydrogen bond interaction with amino residues between hydroxyl group-TYR 83 of A chain. verminoside has docking score of -2.554, It shows

hydrogen bond interactions with amino acid between hydroxyl group-THR 140 of A chain. Specioside has docking score of -2.092, it shows hydrogen bond interaction with amino residue between hydroxyl group-ILE 160 of A chain. Standard drug levodopa showed docking score -4.599, It showed hydrogen bond interaction with amino residue between hydroxyl group-TYR 83, of A chain, and pi-pi stacking with amino residues between benzene ring- PHE 78 of A chain.

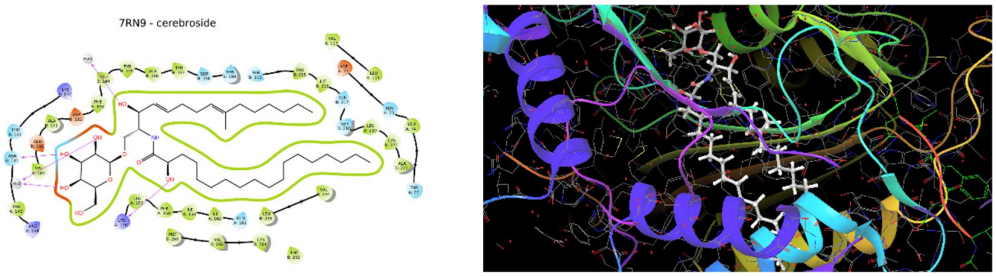

Figure 7: Interactions of cerebroside with 7RN9

7P11: When 7P11 was docked with the selected phytochemicals and standard levodopa; Catalpol had the highest docking score of -6.139, it showed 3 hydrogen bond interaction with amino residues between hydroxyl group-HID 65, hydroxyl group-GLU 89 and hydroxyl group-GLU 89. Verminoside showed second highest docking score of -5.837, it showed 2 hydrogen bond interaction between amino residues between hydroxyl group-GLU 89, hydroxyl group -GLU 89, and pi-pi interaction with amino acid residue between benzene ring -TRP 86. Specioside had docking score of -4.477, it showed 4 hydrogen bond interaction between hydroxyl group-GLU 89, hydroxyl group -GLU 89, hydroxyl group -GLU 89 and hydroxyl group - HID 65. Spathodic acid has docking score of-3.672, it showed hydrogen bond interaction with amino residue between hydroxyl group-HID 65. Luteolin had docking score -3.449, it showed 2 hydrogen bond interactions with amino residues between hydroxyl group-ASP 44, hydroxyl group-GLN 47. Apigenin had docking score of -3.403, it showed hydrogen bond interaction with amino residue between hydroxyl group-ASP 44. Diosmetin had docking score of -3.045, it showed 2 hydrogen bond interaction with amino residue with hydroxyl group-GLN 47, hydroxyl group -ASP 49 and pi- pi stacking with amino residue between benzene ring-TYR 141. Cerebroside had docking score of -1.833, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group -GLU 89, hydroxyl group -GLU 89. Levodopa had docking score of -5.875, it 2 showed hydrogen bond interaction with amino residue between amino group-TYR 141, hydroxyl group - SER 55.

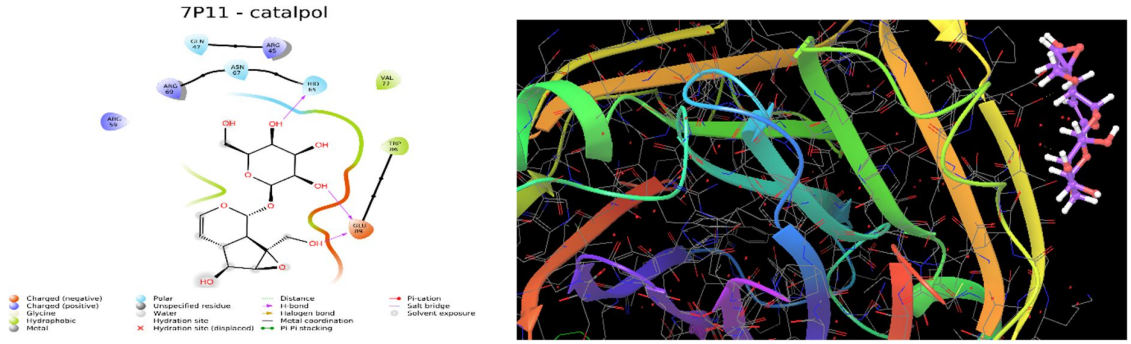

Figure 8: Interactions of catapol with 7P11

7XGJ: 7XGJ is Matrix metalloproteinase-2 (MMP2) molecule which is said to be associated in the pathogenesis of PD(Kim, Joh, et al; 2012.). When 7XGJ protein was docked with the selected phytocompounds and standard drug; Verminoside had the highest docking score of -11.184, it showed 3

hydrogen bond interaction with amino residue with hydroxyl group-ALA 86, hydroxyl group -PRO 141, hydroxyl group -THR-146. Specioside had second highest docking score of -9.291, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group -GLY 81, hydroxyl group -ALA 86. Luteolin had docking score of -9.135, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group-GLY 81, hydroxyl group -ILE 142. Diosmetin had docking score of -8.964, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group-GLY 81, hydroxyl group -ILE 142. Apigenin had docking score of -7.390, it showed hydrogen bond interaction with amino residue between hydroxyl group-GLY 81. Catalpol had docking score of -6.327, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group -GLY 81, hydroxyl group-GLY 81. Cerebroside had docking score of -5.099, it showed hydrogen bond interaction with amino residue between amine group-GLY 81. Levodopa had docking score of -7.570, it showed 5 hydrogen bond interaction with amino residue between hydroxyl group-PRO 135, amine group-ALA 140, hydroxyl group-ALA 140, amine group-ILE 142, hydroxyl group-THR 146.

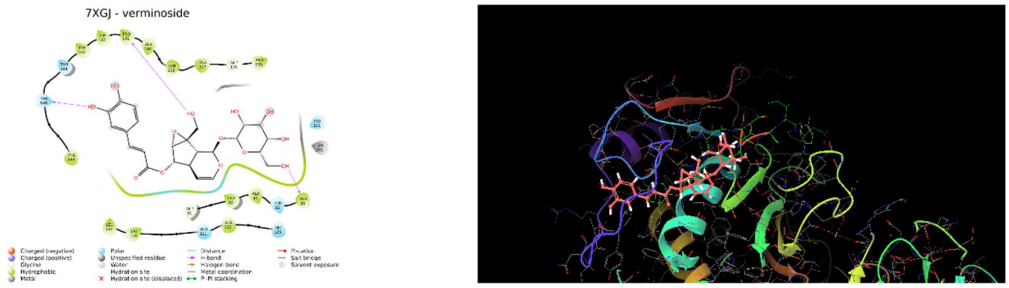

Figure 9: Interactions of verminoside with 7XGJ

6TTK: 6TTK a protein related to DDR4 which is involved in the development of PD (Kiyohara, Miyake, et al; 2011). When 6TTK protein was docked with the selected phytochemicals and standard drug; Specioside had the highest docking score of -11.659, it showed 3 hydrogen bond interaction with amino residue between hydroxyl group -SER 324, hydroxyl group-VAL-468, hydroxyl group-VAL 562.Catalpol had the second highest docking score of-10.296, it showed 5 hydrogen bond interaction with amino residue between hydroxyl group -VAL 322, hydroxyl group-LEU 371, hydroxyl group-LEU 421, hydroxyl group-ALA 560, hydroxyl group-VAL 562. Diosmetin had docking score of-9.698, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group -SER 326, hydroxyl group-ALA 374.Verminoside had docking score of-8.946, it showed 3 hydrogen bond interaction with amino residue between hydroxyl group -VAL 322, hydroxyl group-SER 326, OH-ALA 560. Luteolin had docking score of -8.257, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group-ALA 372, hydroxyl group-VAL 468. Apigenin had docking score of -6.998, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group-ALA 372, hydroxyl group-ALA 560. Spathodic acid had docking score of -6.061, it showed 3 hydrogen bond interaction with amino residue between hydroxyl group-VAL 468, hydroxyl group- VAL 562, hydroxyl group-VAL 564. Levodopa had docking score of -8.192, it showed 5 hydrogen bond interaction with amino residue between hydroxyl group -VAL 322, amine group- VAL 322, hydroxyl group-ALA 374, hydroxyl group-LEU 421, amine group-ALA 560.

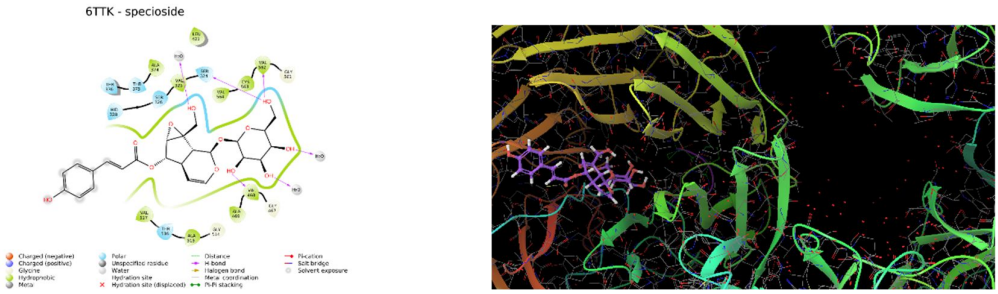

Figure 10: Interactions of Specioside with 6TTK

1GKC:1GKC protein which is related to MMP9 which is associated in the pathogeneses of PD(He X, Zhang L, et al; 2013.). When protein 1GKC was docked with selected phytocompounds and standard drug levodopa; Cerebroside was the only phytochemical to dock, which had a docking score of-7.952, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group -THR 426, hydroxyl group GLY 428. Levodopa had docking score of -3.272, it showed 3 hydrogen bond interaction with amino residue between hydroxyl group -THR-426, hydroxyl group-GLU 427, hydroxyl group- GLY 428.

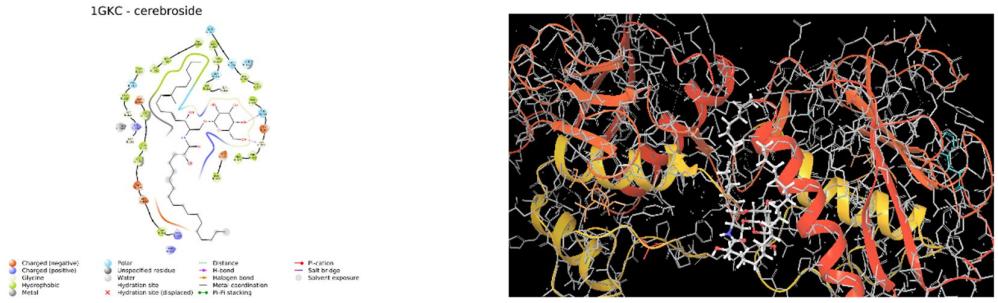

Figure 11: Interactions of cerebroside with 1GKC

3P3U

3P3U which is related to TTR which is associated to the pathogenesis of PD(Maetzler, Tian, et al; 2012.).When 3P3U protein target was docked with the selected phytochemicals and the standard drug; Cerebroside had the highest docking score of -5.312. Specioside had docking score of -3.727, it showed hydrogen bond interaction with amino residue between hydroxyl group -GLU 63 and pi- pi interaction with amino residue between benzene ring-HID 31. Catalpol had docking score of-3.487, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group-SER 46 and hydroxyl group-GLU 63. Spathodic acid had the docking score of -2.749, it showed 3 hydrogen bond interaction with amino residue between hydroxyl group-SER 46, hydroxyl group-LYS 48 and hydroxyl group- GLY 57. Verminoside had the docking score of -2.091, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group-THR 60, hydroxyl group-GLU 63. Levodopa had docking score of -2.569, it showed 4 hydrogen bond interaction with amino residue between keto group-SER 46, amine group-SER 46, hydroxy group-GLU 63 and hydroxyl group-GLU 63.

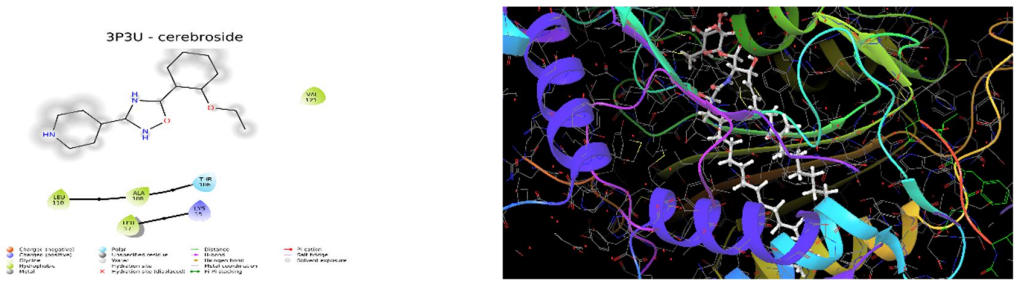

Figure 12: Interactions of Cerebroside with 3P3U

3POV

3POV which is related protein of EGFR, which was found to be decreased in the early stages of PD(Jiang Q, Wang C, et al; 2015.). When 3POV protein was docked with the phytocompounds and the standard drug; Catalpol had the highest docking score of-6.652, it showed hydrogen bond interaction with amino residue between hydroxy group-GLU 184 of A chain. Luteolin had the docking score of -6.259, it showed 4 hydrogen bond interaction with amino residue between hydroxyl group -SER 219 of A chain and pi – cation interaction between benzene ring- LYS 246 of A chain and benzene ring- MG 487 of A chain. Cerebroside had docking score of -5.739, it showed 2 hydrogen bond interaction with amino residue between hydroxy group- ASP A 136, hydroxyl group- FMT 489 of A chain. Verminoside has docking score of-5.631, it showed 2 hydrogen bond interaction with amino residue between hydroxyl group-GLU -184 of A chain, hydroxyl group-CYS 247 of A chain. Diosmetin had docking score of -5.589, it showed 3 hydrogen bond interaction with amino residue between hydroxy group-SER 219 of A chain, hydroxyl group-ASP 221 of A chain and hydroxyl group-GLU 184 of A chain, and also showed 3 pi- cation interaction with amino residues between benzene ring-MG 487 of A chain, benzene ring-MG 487 of A chain, benzene ring- LYS 46 of A chain. Apigenin showed docking score of -5.000, it showed hydrogen bond interaction with amino residue between hydroxy group- GLN 380 and pi cation interaction with amino residues benzene ring- MG 487. Spathodic acid showed docking score of -4.421, it showed hydrogen bond interaction with amino residue between hydroxy group - SER 219 of A chain. Specioside showed docking score of -2.000, it showed hydrogen bond interaction with amino residue between hydroxy group- GLU 184 of A chain. Levodopa showed docking score of -5.736, it showed hydrogen bond interaction with amino residue between hydroxy group-SER 219, amine group-GLU 184, amine group-LYN 246.

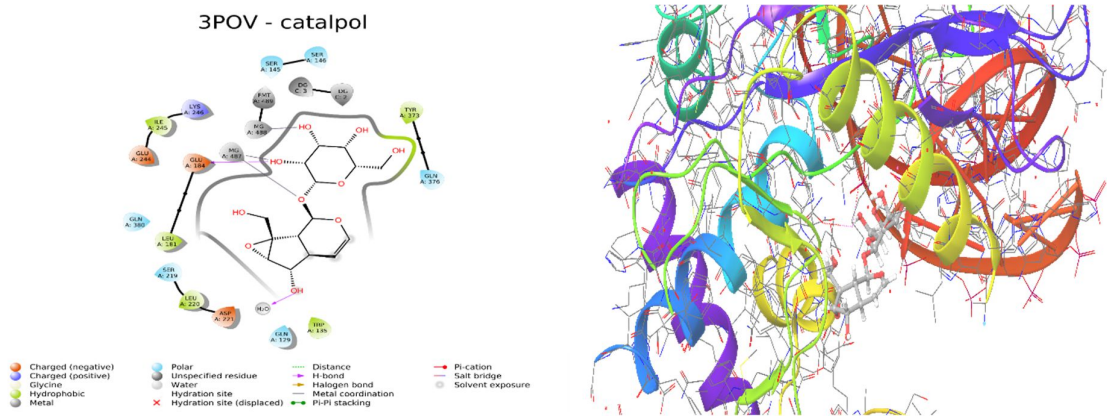

Figure 13: Interactions of Catapol with 3POV

B. ADMET properties prediction

The selected phytochemicals were subjected to evaluation of their ADMET and other physicochemical properties using quikprop schrodinger.

Table 3: ADMET profile of phytoconstituents of *S. campanulata*

Molecule	cerebroside	specioside	spathodic acid	luteolin	diosmetin	Normal range
#stars	12	1	1	0	0	0 – 5
#amine	0	0	0	0	0	0 -1
#rtvFG	1	4	0	0	0	0 -2
CNS	-2	-2	-2	-2	-2	-2 - 2
SASA	1416.326	789.021	699.019	501.354	530.686	300 – 1000
Donor HB	7	5	4	3	2	0 -6
Accpt HB	16.1	17.35	7.1	4.5	4.5	2 - 20.0
Q Plog Po/w	5.447	-0.797	4.371	0.954	1.77	-2 - 6.5
Q Plog HERG	-5.931	-6.312	-1.731	-4.985	-5.091	not below- 5
QPP Caco	40.209	16.842	96.831	42.001	116.539	<25 poor >500 grea
Q Plog BB	-5.439	-3.469	-1.025	-1.93	-1.559	-3 - +1.2
QPPMDCK	22.1	5.988	50.442	16.079	48.453	<25 poor >500 grea
Q Plog Kp	-2.287	-4.639	-3.754	-4.862	-4.059	-8 to -1.0
#metab	10	7	5	4	4	1 -'8
Human Oral Absorption	1	1	3	3	3	
Percent Human Oral Absorption	48.678	5.353	88.085	61.583	74.297	>80% high <25% po
Rule Of Five	3	3	0	0	0	max 4
Rule Of Three	2	2	0	0	0	max3

Molecule	Catalpol	apigenin	verminoside	Levodopa	Normal range
#stars	2	0	2	1	0 – 5
#amine	0	0	0	1	0 -1
#rtvFG	3	0	4	0	0 -2
CNS	-2	-2	-2	-2	-2 - 2
SASA	546.334	490.334	776.079	407.852	300 – 1000
Donor HB	5	2	6	5	0 -6

Accpt HB	16.3	3.75	18.1	4.5	2 - 20.0
Q P log Po/w	-2.174	1.636	-1.308	-2.516	-2 - 6.5
Q P log HERG	-3.835	-5.084	-5.826	-2.739	not below- 5
QPP Caco	73.603	116.767	8.278	2.736	<25 poor >500 great
Q Plog BB	-1.97	-1.429	-3.732	-1.483	-3 - +1.2
QPPMDCK	29.485	48.555	2.779	1.182	<25 poor >500 great
Q P log Kp	-4.424	-3.969	-5.295	-7.188	-8 to -1.0
#metab	7	3	8	6	1 -'8
Human Oral Absorption	2	3	1	1	
Percent Human Oral Absorption	34.67	73.528	0	20.039	>80% high <25% poor
Rule Of Five	1	0	3	0	max 4
Rule Of Three	1	0	2	1	max3

Where: #Stars indicates the drug likeness of the compound. # Amine value indicates the number of non-conjugated amine present. #rtvFG value indicates the number of reactive functional group present in the molecule. CNS value indicates the predicted CNS activity. SASA value indicates total solvent accessible surface area. Donor HB value indicates the estimated number of hydrogen bonds that can be donated by solute to water molecule in aqueous solution. AccptHB value indicates the estimated number of hydrogen bonds that can be accepted by solute to water molecule in aqueous solution. QPlogPo/w value indicates the predicted octanol/water partition coefficient. QPlogHERG value indicates the predicted IC-50 value for the blockage of HERG K+ channel. QPPCaco value indicates the predicted CaCo-2 permiability. QPlogHERG value indicates the predicted IC-50 value for the blockage of HERG K+ channel. QPlogBB value gives predicted brain/ blood partition coefficient. QPPMDCK value gives predicted apparent MDCK cell permeability in nm/ sec. QPLogkp value gives predicted skin permeability. #Metab value indicates number of likely metabolic reactions. Human oral absorption value gives prediction on human oral absorption. Percent human oral absorption tells the predicted human oral absorption in 0 -100 scale. Rule of five value indicates the number of violations of Lipinski's rule of five. Rule of three value indicates the number of violations of Jorgensen's rule of three.

#Stars indicates the drug likeness of the compound. The compounds that have higher stars indicate that molecule is less drug – like. Phytochemicals like specioside, spathodic acid, luteolin, diosmetin, catalpol, apigenin, verminoside and Levodopa have # stars in the recommended range ie, 0 -5 whereas cerebroside have # star value 12 which indicates the molecule is less drug like. # Amine value indicates the number of non-conjugated amine present. All the phytochemicals evaluated have # amine value in the normal range ie, 0 -1. #rtvFG value indicates the number of reactive functional group present in the molecule. Large value of #rtvFG can lead to false positive results in HTS assay, decomposition, reactivity and toxicity problem in-vivo. In the evaluated phytochemicals, specioside and verminoside had #rtvFG value 4 which was higher than the recommended range whereas the rest of the phytochemical had #rtvFG in normal range ie, 0 -2. CNS value indicates the predicted CNS activity. All the evaluated phytochemicals have the value -2 which is in the normal range ie, -2 - +2. SASA value indicates total solvent accessible surface area.

Except cerebroside all the other evaluated phytochemical has SASA value in the recommended range 300 – 1000. Cerebroside have SASA value 1416.326 which is higher than the recommended range. Donor HB value indicates the estimated number of hydrogen bonds that can be donated by solute to water molecule in aqueous solution. Except cerebroside all the other evaluated phytochemical has donorHB value in the normal range 0 -6. Cerebroside has value 7 which is higher than the recommended value. AccptHB value indicates the estimated number of hydrogen bonds that can be accepted by solute to water molecule in aqueous solution. All the evaluated phytochemicals have accptHB value in the normal range 2 – 20. QPlogPo/w value indicates the predicted octanol/water partition coefficient. All the evaluated phytochemicals have QPlogPo/w value in the normal range -2 to 6.5. QPlogHERG value indicates the predicted IC-50 value for the blockage of HERG K+ channel. It is recommended to have the QPlogHERG value not below -5 but in the evaluated phytochemicals, Cerebroside has -5.931, specioside has -6.312, diosmethin has -5.09, apigenin has -5.084 and verminoside has -5.826 which is all below -5 whereas spathodic acid has -1.731, luteolin has -4.985, catapol has -3.835 and levodopa has -2.739 which are all in the normal range. QPPCaco value indicates the predicted CaCo-2 permiability. CaCO 2 is related to the gut – blood barrier system. Phytochemical like specioside, verminoside and levodopa have poor permeability and the rest of the evaluated phytochemical have the value above 25 and below 500 indicating good permeability. QPlogBB value gives predicted brain/ blood partition coefficient. The recommended range is -3 to + 1.2. Cerebroside, specioside and verminoside have values below -3 indicating poor poor brain-blood partition coefficient. The rest of the evaluate phytochemicals have value in the recommended value. QPPMDCK value gives predicted apparent MDCK cell permeability in nm/ sec. MDCK cells are a good mimic of blood-brain barrier. Cerebroside has 22.1, Specioside has 5.988, luteolin has 16.079, verminoside has 2.779 and levodopa has 1.182 which are all indicating that these phytochemicals have poor blood brain permeability. Phytochemicals like spathodic acid has 50.442, diosmethin has 48.453, catapol has 29.485 and apigenin has 48.555 which indicate that they have blood brain permeability. QPLogkp value gives predicted skin permeability. All the evaluated phytochemicals had values in the recommended range ie, -8 to -1. #Metab value indicates number of likely metabolic reactions. Except Cerebroside all the other evaluated phytochemicals have #metab value in the recommended range 1 -8. Human oral absorption value gives prediction on human oral absorption. Cerebroside, specioside, verminoside and levodopa has value 1 indicating low human oral absorption. Catapol has 2 indicating medium human oral absorption. Percent human oral absorption tells the predicted human oral absorption in 0 -100 scale. Spathodic acid has 88.085 which is above 80% indicating high human oral absorption. Specioside has 5.353, verminoside has 0 and levodopa has 20.039 which is below 25% indicating they can have low human oral absorption. Rule of five value indicates the number of violations of Lipinski's rule of five. No phytochemical has violated more than 3 rule of five where cerebroside, specioside and verminoside has violated 3 rule and catapol has violated 1 and Spathodic acid, luteolin, diosmethin apigenin and levodopa has 0 violation making them drug like compound. Rule of three value indicates the number of violations of Jorgensen's rule of three. Cerebroside, Specioside and Verminoside has 2 violations. Catapol and levodopa has 1 violation. Spathodic acid, Luteolin, Diosmethin and Apigenin has 0 violations indicating they are more likely to be orally available.

4. Conclusion

The anti-Parkinson activity of this extract was evaluated in rats using behavioural studies like haloperidol induced catalepsy and rota rod and estimation of biochemical parameters like catalase, acetylcholinesterase and dopamine.

In the haloperidol induced catalepsy and rota rod test, both low dose; high dose of this leaf extract showed significant anti-Parkinson's activity in a dose-dependent manner. On estimation of biochemical parameters, low dose and high dose showed significant increase in the catalase enzyme activity, decrease in acetylcholinesterase enzyme activity and increase in dopamine levels in the brain in a dose dependent manner. Molecular docking studies revealed that phytochemicals like catapol, cerebroside and specioside had good binding affinity to the target protein compared to other selected phytochemicals. ADMET profile studies revealed that the selected phytochemicals have good potential to be anti-Parkinson's drug.

Based on the results obtained from behavioural studies, biochemical studies and in-silico we can conclude that *Spathodea campanulata* leaf extract had potential to show anti-Parkinson's activity and showed comparable anti-Parkinson's activity with L-dopa. This leaf extract could be used as an alternative to conventional treatments for Parkinson disease which only gives symptomatic relief; however comprehensive study is required to create a nutraceutical medication from *Spathodea campanulata* leaves for neuroprotection by investigating their molecular, clinical, toxicological and pharmacological mechanism.

Conflict of interest

The authors declare that they have no conflict of interest.

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