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Phytotherapeutics of *Vicia faba* L. in Parkinson's Disease: An *in silico* Approach

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ABSTRACT *Vicia faba* L. has a major role in the traditional diet and medicinal system of different countries. *V. faba* is a natural source of the precursor of dopamine (Levodopa) which is used treatment of Parkinson's disease. The objective of the study is to investigate the *in vitro* antioxidant capacity, Gas Chromatography-Mass Spectrometry and docking of *V. faba* against Parkinson's disease. The values indicated that acetone and aqueous extract exhibited a higher value of total phenolic and flavonoid contents respectively. The results of the 2, 2- diphenyl-1-picrylhydrazyl free radical scavenging assay and ferric reducing antioxidant power assay displayed that aqueous extract had better radical scavenging activity. In the 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical scavenging assay, ethanolic extract showed a lower Inhibitory $Concentration_{50}$ value. *In silico* analysis indicated that the phyto-compounds present in *V. faba* has a higher binding affinity to the dopamine1 receptor. The results suggested that *V. faba* has good antioxidant properties and can be used as a dopamine agonist for the dopamine1 receptor which may be useful in combating Parkinson's disease.

INTRODUCTION

Antioxidants can safeguard human cells by counteracting free radical damage. Reduction in the defence mechanism of antioxidants and elevated generation of reactive species like oxygen and hydroxyl free radicals plays a pivotal role in the implication of neurodegenerative disorders like Parkinson's disease, Huntington's disease and Alzheimer's disorder (Niedzielska et al. 2015). In Parkinson's disease, an increase in reactive oxygen species initiates cascade reactions involving oxidative stress and dopaminergic neuronal dysfunction. The prevalence of Parkinson's is around 10 million worldwide and in India, it is around 0.58 million (Garg and Dhamija 2020). Mitochondrial dysfunction, fission and fusion imbalance, calcium overload, impaired storage of dopamine and neuroinflammation are the major cause of the production of reactive oxygen species (Guo et al. 2018).

Medicinal plants have been preferred over synthetically produced drugs because of their diverse bioactive compound and fewer side effects. The plant sources are rich in antioxidant capacity which can be useful in maintaining oxidative stress. *Vicia faba* L. is one among them, which has been cultivated in different parts of the world for culinary usage. *V. faba* is a promising functional food and nutraceutical. Commonly *V. faba* is referred to as broad beans or fava beans which belong to the family of *Fabaceae*. *V. faba* is native to Southwest Asia and North Africa. In different countries, different names have been used for *V. faba* like Bakla in Hindi and Turkish, Kara oncet in Indonesian, Jaba in Spanish, Gourgane in Canada, Ackerbohne in German, Fava-comum in Portuguese, Tuinboon in Dutch (https://www.feedipedia.org/node/4926). The edible part (beans) of *V. faba* has a major place in the traditional diets of India, South America, the Mediterranean, Chinese, African and Middle Eastern countries (Mehran and Golshani 2013). Broad beans are the oldest among legumes being cultivated for 5000 years in the Mediterranean region and Central Asia (Chitra and Kiruthiga 2018). *V. faba* has started gaining attention due to its low fat, low glycemic indexes and highly positive nutritional content (Loizzo et al. 2021). *V. faba* contains several medicinal values like anti-diabetic, diuretic and anti-hypertensive properties (Srivastava 2014; Mejri et al. 2018). *V. faba* has grabbed attention in recent times due to the presence of higher antioxidant compounds and Levodopa (pre-

cursor of dopamine). Levodopa is used in managing the symptoms of Parkinson's disease even during the later stages (Okumura et al. 2016). Abdel-Sattar et al. (2021) have investigated the anti-Parkinson's effect of *V. faba* in rotenone-induced mice. The findings reported that methanol extract of *V. faba* was efficient in improving the dopamine content, motor sign and inflammatory markers. In a study, the four different methods of cooking *V. faba* were analysed. The findings reported that the antioxidant as well as the levodopa content of *V. faba* was found to be increased after the steaming and microwaving treatments (Duan et al. 2022).

Objective

The objective of the present study is to investigate whether the phytochemicals present in *V. faba*, identified through the Gas Chromatography-Mass Spectrometry (GC-MS) has the ability to bind with dopamine 1 receptor, which can be used as a therapeutic intervention for Parkinson's disease.

MATERIAL AND METHODS

Collection and Authentication of Plant Material

The seeds of *V. faba* (Beans) were obtained from Ooty, Nilgiri district, Tamil Nadu, India. The seeds were shade dried, powdered and stored in air-tight containers for future use. The plant identification was done by a Botanist at the campus of Tamil Nadu Agriculture University, Coimbatore. The authentication voucher number is BSI/SRC/5/23/2020/Tech/666.

Chemicals

Bovine serum albumin, anthrone, Folin-Ciocalteu's reagent, sodium carbonate, quercetin, vallinic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, ferric chloride, 2,2-azinobis[3-ethylbenzothiazoline-6-sulfonate] (ABTS)and potassium persulfate were purchased from HiMedia Laboratories (Maharashtra, India).

Extract Preparation

The collected seeds were washed, shade dried, ground to powder and stored. Based on the polarity, three different solvents (Aqueous, Acetone and Ethanol) were chosen. The extraction was done using the Soxhlet apparatus; 25gms of plant material was weighed and packed tightly in the thimble and extracted with each solvent at 45- 50 °C until the siphon arm becomes colourless (Fagbemi et al. 2021). 25gms of plant material with 250ml of distilled water was taken in the flask and boiled in the water bath for 7 days at room temperature with occasional shaking. The supernatant was obtained by subjecting the mixture to centrifugation for 5 minutes at 5000rpm. And the supernatant was freeze-dried (Omar et al. 2019).

Preliminary Qualitative Analysis

Phytochemical screening tests were performed to identify the phytoconstituents present in the extracts of *V. faba*. The tests such as carbohydrates (Molisch's test), phenolic compounds (Ferric chloride test), polysterols (Libermann-Burchard Test), tannins (Lead acetate test), protein (Ninhydrin test), amino acid (Biuret test), alkaloids (Mayer's test), saponins (Foam test), terpenoids (Salkowski test), steroids (Libermann test), glycosides (Borntrager's test), coumarin (Fluorescence test) and flavonoids (Alkaline test) were performed (Sawant et al. 2013; Senguttuvan et al. 2014).

Estimation of Protein

The protein present in the extract was determined using Folin's phenol method (Lowry et al. 1951). The bovine serum albumin was used as the stock standard (2mg/ml). The sample was added with an alkaline copper reagent (5ml). After 10 minutes of incubation folin's phenol reagent (0.5ml) was added and again incubated for 30 minutes. The sample was read at an absorbance of 670nm.

Estimation of Carbohydrate

The estimation of carbohydrates was carried out using the Anthrone method by Hedge et al. (1962). The glucose was used as the stock standard (1mg/ml). The samples were hydrolysed in a boiling water bath for three hours with 2.5N HCl and centrifuged. The supernatant was taken for determining the carbohydrate content of the plant using the anthrone reagent.

Estimation of Total Phenolic Content

The total phenolic content (TPC) of extracts was determined using Folin-Ciocalteu's method (Tanruean et al. 2021). Gallic acid was used as the stock standard (1mg/ml). The plant extracts were treated with Folin-Ciocalteu's reagent (0.5ml) followed by the addition of 20 percent sodium carbonate (0.5ml) and incubated at room temperature for 1 hour. The blue colour formed was read at 760nm. The results obtained were expressed in terms of mg of gallic acid equivalent (mg GAE/g extract).

Estimation of Total Flavonoid Content

The total flavonoid content (TFC) of extracts was determined using the Aluminium chloride method by following Woisky and Salatino (1998). Quercetin was used as the stock standard (1mg/ml). The samples were treated with 2 percent aluminium chloride (0.5ml) and incubated at room temperature for 30 minutes. The colour formed was read at 420nm. The results were expressed in terms of mg of quercetin equivalent (mg QE /g extract).

Estimation of Tannins Content

The total tannins content (TTC) was estimated using the Folin-Ciocalteu method. Tannic acid was used as the standard. The sample was added with Folin-Ciocalteu reagent (0.5ml) and treated with 35 percent of sodium carbonate (1ml) and made up to 10 ml by adding distilled water. The mixture was then incubated at room temperature for 30 minutes and read at 700nm (CI and Indira 2016).

Determination of Antioxidant Activity

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of extracts was evaluated by DPPH assay (Varalakshmi et al. 2011). 0.2mM of DPPH solution was prepared by mixing DPPH (7.8mg) in 95 percent methanol (100ml) and stored at-20 °C overnight. The methanolic solution of extracts of different concentrations (200, 400, 500, 600 and 800µg) was treated with DPPH solution (0.5ml) and incubated for 30 minutes in dark conditions. The ascorbic acid was used as standard. The colour change from purple to yellow is observed and read at 517nm.

% Radical Scavenging capacity = $[(A_0 - A_1)/A_0] \times 100$

A_0 = Control Absorbance, A_1 = Sample Absorbance.

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing activity of the plant extract was determined using the Potassium ferricyanide-ferric chloride method (Vijayalakshmi and Ruckmani 2016). Different concentrations of extract ranging from 100, 200, 300, 400 and 500µg were treated with 0.2M sodium phosphate buffer (2.5ml), 1 percent potassium ferricyanide (2.5ml) and incubated for 20 minutes at 50 C in a water bath. After incubation, 10 percent trichloroacetic acid (2.5ml) was added and centrifuged for 10 minutes at 3000rpm. The supernatant (2.5ml) collected was added with distilled water (2.5ml) and 0.1 percent ferric chloride (0.5ml) and incubated for 10 minutes at room temperature. The pale green/blue colour formed was read at 700nm.

ABTS Radical Scavenging Assay

ABTS radical scavenging activity of extracts was evaluated according to (Arnao et al. 2001). 2.4mM potassium persulfate and 7mM ABTS stock solutions were prepared. Equal quantities of both solutions were mixed to get the working solution and incubated in dark for 16 hours. 1 ml of ABTS solution was then diluted with methanol to read 0.700 ± 0.050/0.020. The plant extract with concentrations of 25, 50, 100, 150 and 200µg was treated with ABTS solution (1ml) and allowed to stand for 7 minutes. The decolourization of samples was read at 734nm.

% Radical Scavenging capacity = $[(A_0 - A_1)/A_0] \times 100$

A_0 = Control Absorbance, A_1 = Sample Absorbance.

GC-MS Profiling

The identification of secondary metabolites present in the three extracts of *V. faba* was done using GC-MS profiling. Analysis of extracts of *V. faba* was carried out using the equipment Thermo Fischer ITQ1100. Helium gas was used as carrier gas with a flow rate of 1.0 ml/min. The temperature in the oven was programmed as 60 °C for 10 minutes and then gradually increased to its maximum temperature of 250^0C at 3 minutes. The internal standard used was 2-Octanol. The compound peaks

formed were interpreted by comparing them with computer libraries and as well as their retention indices.

Molecular Docking

Preparation of Ligand

The results of GC-MS depicted that aqueous extract had 600 compounds, acetone extract had 519 compounds and ethanol extract had 179 compounds present in them. The 2D and 3D conformations of all compounds were obtained from Pubchem in sdf format (Zeb et al. 2021).

Target Protein Retrieval

Dopamine 1 was the protein selected for interaction with the compounds from *V. faba* because they convert the precursor molecule levodopa into dopamine in the brain. The 3D X-ray crystal structure of the dopamine 1 receptor was obtained from Protein Data Bank.

Compounds present in extract were scrutinized using SwissADME online tool based on their pharmacokinetics and drug-likeness properties. The AutoDock Vina (version 1.1.2) was used for docking the virtually screened molecules with proteins selected (Zeb et al. 2021).

Statistical Analysis

All the experiments were carried out in triplicates (n=3). The processed data of TPC, TFC and TTC were represented with their Mean ± Standard Error (S.E) values. For *in vitro* antioxidant results the significance was examined using the one way ANOVA in GraphPad Prism 8.

RESULTS

Phytochemical Qualitative Analysis

The preliminary qualitative analysis results have exhibited the presence of various phytochemicals like alkaloids, flavonoids, phenolic compounds, tannins, steroids, carbohydrates, terpenoids, protein, amino acid, saponins, glycosides, coumarin and polysterols in given *V. faba* extracts (aqueous, acetone and ethanol). Among the 3 extracts, the aqueous extract had shown the presence of a higher number of phytochemicals followed by ethanol and acetone. Glycosides and coumarin were absent in all the extracts. The results are given in Table 1.

Table 1: Preliminary qualitative analysis of different extracts of *V. faba*

S. No.	*Tests*	*Aqueous*	*Acetone*	*Ethanol*
1	Alkaloids	+	-	-
2	Flavonoids	+	+	-
3	Phenolic compounds	+	+	+
4	Tannins	+	+	+
5	Steroids	+	-	+
6	Carbohydrate	+	+	-
7	Terpenoids	+	-	+
8	Protein	+	-	+
9	Amino acid	+	-	+
10	Saponins	+	-	+
11	Glycosides	-	-	-
12	Coumarin	-	-	-
13	Polysterols	+	+	+

*Present= '+' Absent= '-'

Source: Data obtained from analysis are represented in the form of table by the authors

Protein and Carbohydrate Content

The protein and carbohydrate content of *V. faba* beans are estimated in fresh samples. The results highlighted that *V. faba* contains 16.2±0.26mg/g and 15.4±0.23mg/g of protein and carbohydrate respectively.

Total Phenolic, Flavonoid and Tannin Content

Among the 3 extracts, acetone extract displayed higher content of total phenolic content (TPC) with a value of 6.9±0.48mg GAE/g extract, followed by aqueous and ethanol with values of 4.9±0.13 and 2.4±0.03mg GAE/g extract respectively. The acetone extract showed a higher concentration of TFC with the value of 11.8±0.33mg QE/g extract, followed by ethanolic and aqueous with values of 7.7±0.26 and 2.5±0.13mg QE/g extract respectively (Table 2). The results of the present study indicated that a higher amount of TPC and TFC is present in acetone extract of *V. faba*. TTC was found to be higher in acetone extract (8.2±0.75mg TA/g extract) followed by ethanol and aqueous (7.6±0.66 and 5.9±0.52mg TA/g extract) extracts.

Table 2: Total phenolic, total flavonoid and tannin content of *V. faba*

Extract	*Aqueous (mg/g)*	*Acetone (mg/g)*	*Ethanol (mg/g)*
TPC	4.9±0.13	6.9±0.48	2.4±0.03
TFC	2.5±0.13	11.8±0.33	7.7±0.26
TTC	5.9±0.52	8.2±0.75	7.6±0.66

*The values given are the representation of Mean ± S.E (n=3). TPC: Total Phenolic content, TFC: Total Flavonoid Content, TTC: Total Tannin Content.
Source: Data obtained from analysis are represented in the form of table by the authors.

In Vitro Antioxidant Activity

The antioxidant capacity of the *V. faba* was determined using three different assays such as DPPH free radical scavenging activity, Ferric reducing antioxidant power assay and ABTS radical scavenging assay.

DPPH Free Radical Scavenging Activity

The colour changes were measured spectrometrically at 517nm. The results of DPPH radical scavenging activity of *V. faba* revealed that aqueous extract had an IC_{50} value of 223.7µg/ml, acetone extract exhibited an IC_{50} value of 242.17µg/ml and ethanolic extract showed an IC_{50} value of 244.40µg/ml. DPPH radical scavenging capacity of *V. faba* extracts can be arranged in the following order: Aqueous > Acetone > Ethanol. The results indicated that the aqueous extract of *V. faba* has a lower IC_{50} value. The results suggested that the aqueous extract has a good DPPH radical scavenging activity when compared with the other two extracts because the IC_{50} value is inversely proportional to antioxidant activity. But almost all the three extracts showed the same level of activity (Table 3). The aqueous extract at 200µg showed 45±15.16 inhibition percent and at 800µg inhibition percent was 82.4±0.75. Acetone extract at 200µg showed 49.2±8.71 inhibition percent and at 800µg inhibition percent was 79.8±2.88. Ethanolic extract at 200µg showed 45.3±3.16 inhibition percent and at 800µg showed inhibition percent was 77.1±4.10.

Ferric Reducing Antioxidant Power Assay

The pale green colour formed was measured at 700nm. The intensity of colour formation depends upon the number of ferric ions reduced. FRAP assay results indicated that aqueous extract has higher ferric ions reducing capacity, followed by the acetone and ethanol extracts. The results indicated that the activity of the extract increased with an increase in concentration. The results of the FRAP assay are tabulated in Table 4.

Table 3: DPPH free radical scavenging activity of different extracts of *V. faba*

Concentration (µg/ml)	*Aqueous extract*	*Acetone extract*	*Ethanol extract*	*Standard % inhibition*
200	36.2±0.37	36.3±0.46	38.8±0.35	57.3±1.01
400	48.6±0.51	49.9±0.84	45.9±0.77	68.4±2.76
500	57.9±0.45	52.3±0.38	52.7±0.15	79.6±0.54
600	69.9±0.29	65.2±0.63	64.8±0.31	86.5±2.05
800	75.5±0.52	70.5±0.31	70.9±0.55	95.1±1.41
IC_{50}	223.7	242.17	244.4	83.5

Source: Data obtained from analysis are represented in the form of table by the authors.
Footnote: The data in the table represents mean ± standard error and values have shown significant value of P<0.05

Table 4: Ferric reducing capacity of different extracts of *V. faba*

Concentration (µmolfe/g)	*Aqueous*	*Acetone*	*Ethanol*	*Standard % inhibition*
100	0.10±0.01	0.07±0.003	0.013±0.003	0.45±0.005
200	0.13±0.005	0.09±0.005	0.023±0.003	0.56±.0.017
300	0.15±0.003	0.10±0.005	0.026±0.003	0.69±0.022
400	0.20±0.011	0.11±0.003	0.036±0.003	0.78±0.02
500	0.23±0.013	0.12±0.003	0.043±0.003	0.85±0.09

*The values given are the representation of Mean ± S.E (n=3).
Source: Data obtained from analysis are represented in the form of table by the authors
Footnote: The data in the table represents mean ± standard error and values have shown significant value of P<0.05

ABTS Radical Scavenging Assay

The decolourisation of samples was spectrometrically measured at 735nm. ABTS radical scavenging assay of *V. faba* extracts exhibited that aqueous extract showed an IC_{50} value of 130.39µg/ml, acetone extract showed an IC_{50} value of 83.0µg/ml and ethanol extract showed an IC_{50} value of 30.77µg/ml. Among three extracts, the ethanolic extract exhibited a lower IC_{50} value (Table 5). The aqueous extract at 25µg showed inhibition percent of 26.5±0.96 and at 200µg inhibition percent was 65.4±1.73. Acetone extract at 25µg showed inhibition percent of 34.9±0.08 and at 200µg inhibition percent was 72.4±0.46. Ethanolic extract at 25µg showed inhibition percent of 46.3±0.83 and at 200µg inhibition percent was 90.3±0.96.

GC-MS Analysis

GC-MS results of *V. faba* extracts revealed the presence of phenolic compounds, amino acids, fatty acids, alkaloids, terpenoids and steroids. The running time of aqueous extract in GC-MS was 59.98 minutes, running time of acetone and ethanolic extracts was 59.97 and 24.10 minutes respectively. The identification of compounds was done by matching the spectra of the unknown compounds with Wiley, Replib, Tutorial, Mainlib and NIST libraries. The GC-MS analysis reported that ethanolic extract had a higher number of peaks with 30 known compounds followed by acetone and aqueous extracts with 19 and 13 known compounds respectively. 3 unknown peaks were found in the aqueous extract.

The detected compound and peak area percent of the aqueous extract are given in Table 6 along with a chromatogram plot (Fig. 1). The phytochemicals present in acetone extract detected by GC-MS analysis are given in Table 7 followed by their Chromatogram plot (Fig. 2). The phytoconstituents identified in ethanol extract by GC-MS analysis is displayed in Table 7 with their Chromatogram plot (Fig. 3).

Table 5: ABTS radical scavenging assay of different extracts of *V. faba*

Concentration (µg/ml)	*Aqueous extract*	*Acetone extract*	*Ethanol extract*	*Standard % inhibition*
25	26.5±0.86	34.9±0.08	46.3±0.83	50.6±0.79
50	33.7±2.09	44.5±0.35	57.4±0.46	54.8±1.9
100	44.8±1.46	55.8±0.31	66.6±3.0	76.5±0.35
150	52.6±0.5	65.5±0.40	78.2±2.51	89.7±0.29
200	65.6±1.73	72.4±0.46	90.3±0.96	98.9±1.87
IC_{50}	130.39	83.0	30.77	22.4

Source: Data obtained from analysis are represented in the form of table by the authors
Footnote: The data in the table represents mean ± standard error and values have shown significant value of P<0.05

Table 6: Phytoconstituets of *V. faba* (aqueous extract) identified by GC MS analysis

Peaks	*RT (min)*	*Area %*	*Compound name*	*Molecular formula*	*Molecular weight*
1	31.697	2.403	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo [4.3.0]nonane	$C_{11}H_{18}N_2O_2$	210
2	32.927	2.935	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	270.5
3	33.696	13.677	Hexadecanoic acid (CAS)	$C_{16}H_{32}O_2$	256.4
4	33.923	0.657	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	372.5
5	35.428	3.472	Ethyl linoleate	$C_{20}H_{36}O_2$	308.5
6	35.523	7.868	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.5
7	36.395	8.451	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.4
8	36.569	8.296	9-Octadecenoic acid (Z)-	$C_{36}H_{68}O_4$	564.9
9	36.998	7.353	Heptadecanoic acid, 15-methyl-, ethyl ester (CAS)	$C_{20}H_{40}O_2$	312.5
10	38.291	4.239	2,2-Diethyl-N-ethyl piperidine	$C_{11}H_{23}N$	169.3
11	41.567	4.243	Bis(oct-3-yl) phthalate	$C_{24}H_{38}O_4$	390.6
12	53.773	19.252	Stigmast-5-en-3-ol, (3.beta.)- (CAS)	$C_{29}H_{50}O$	414.7

Source: Data obtained from analysis are represented in the form of table by the authors

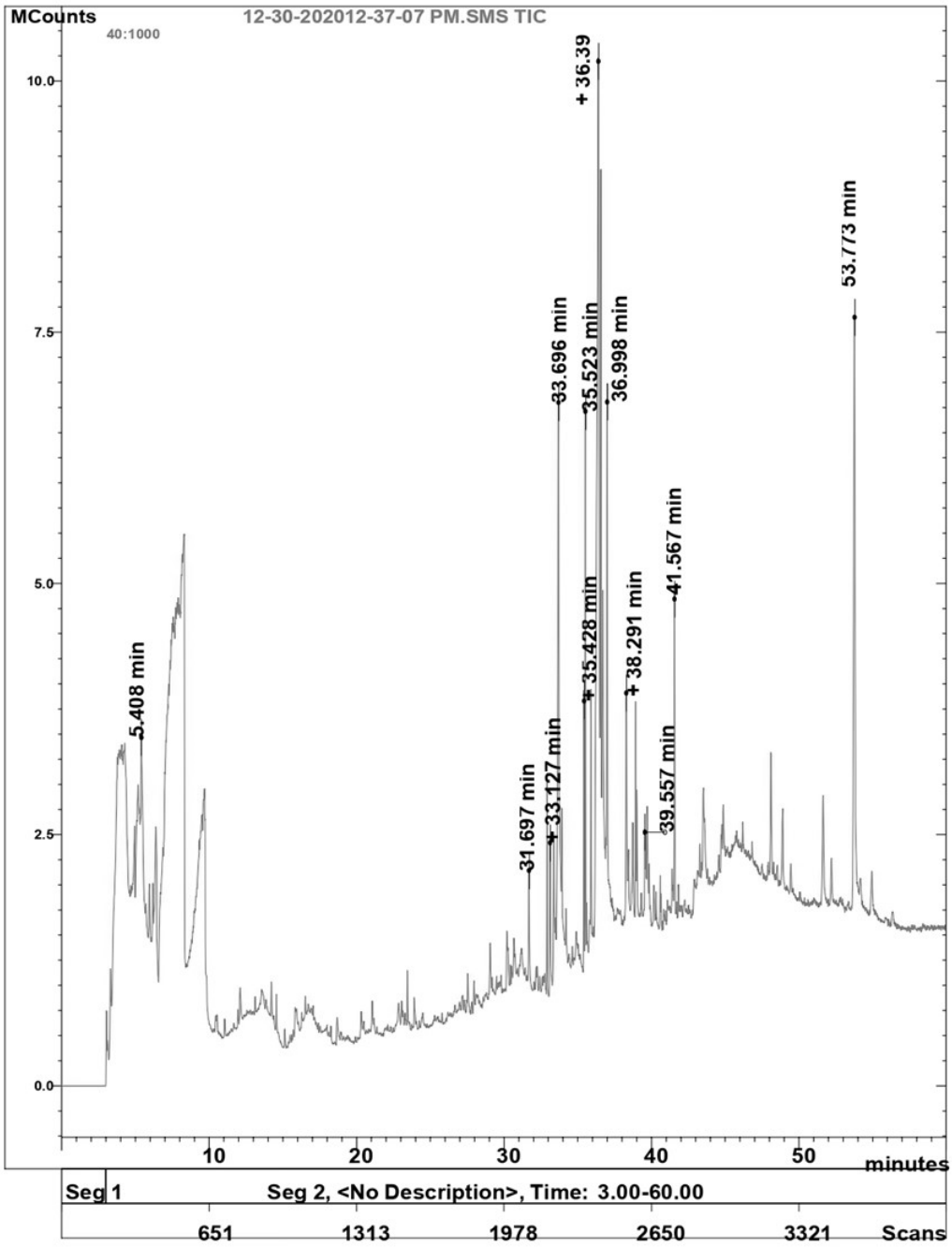

Fig. 1. GC-MS Chromatogram of aqueous extract of *V. faba*
Source: Authors

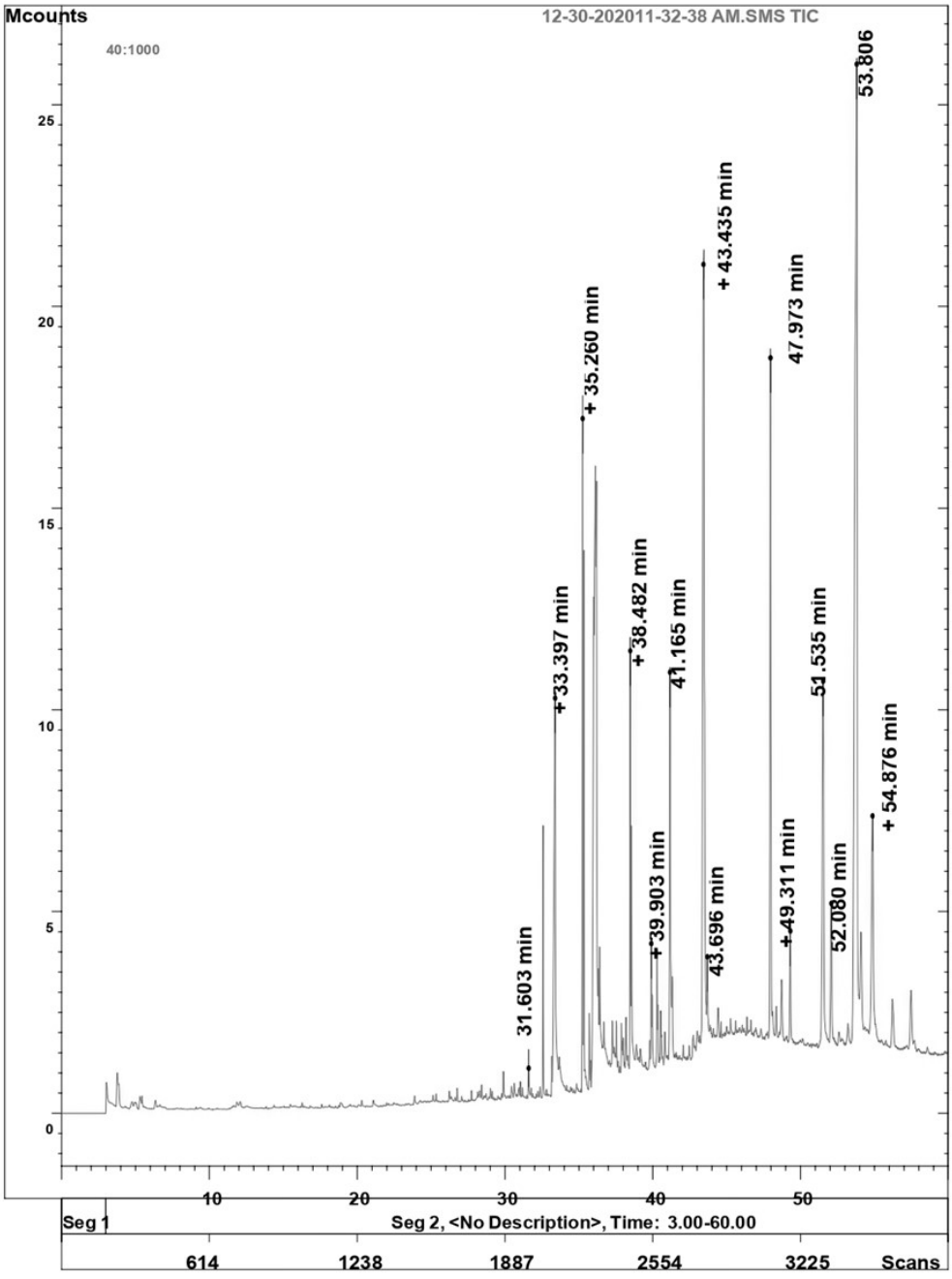

Fig. 2. GC-MS Chromatogram of acetone extract of *V. faba*
Source: Authors

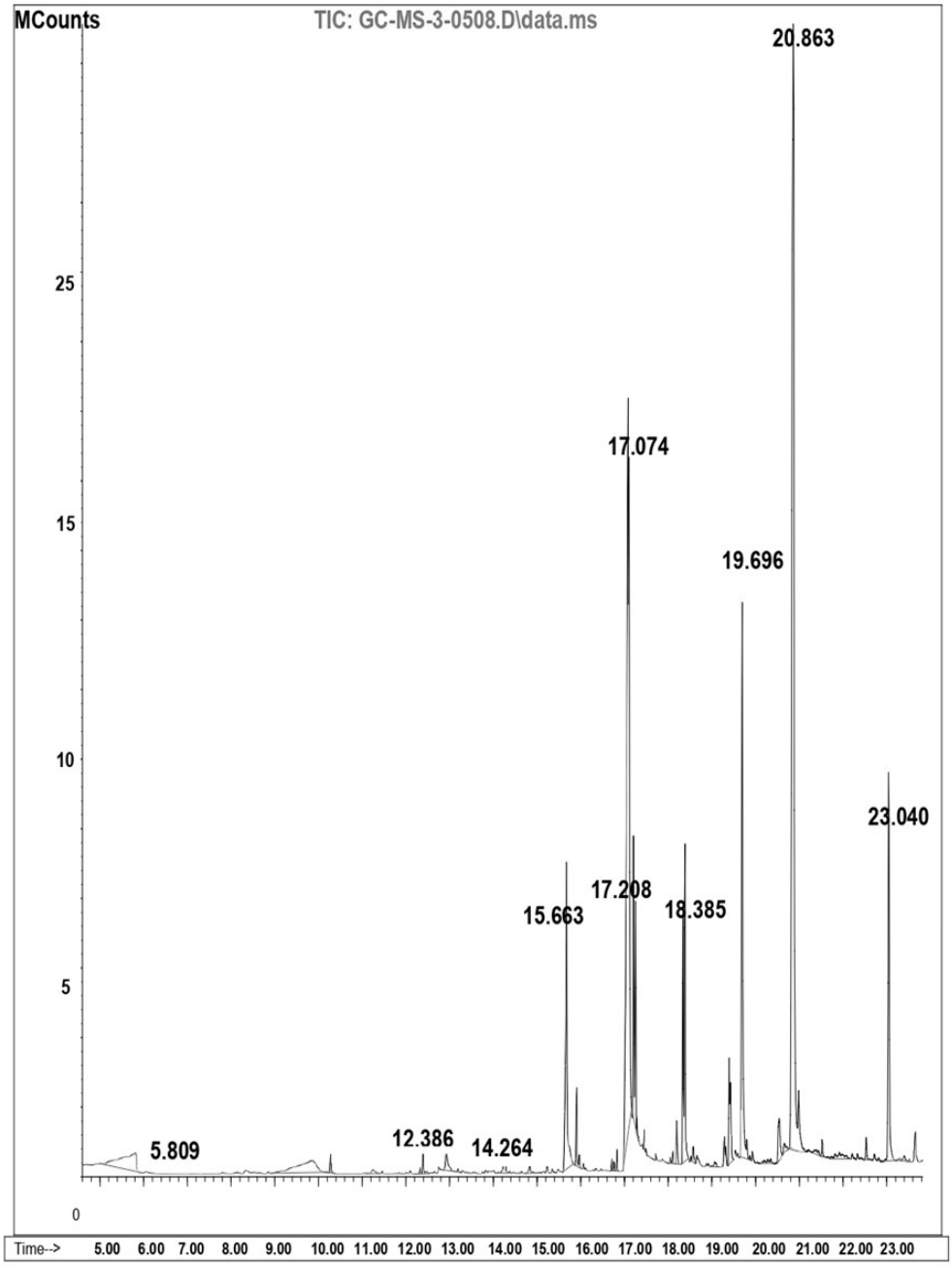

Fig. 3. GC-MS Chromatogram of ethanolic extract of *V. faba*
Source: Authors

Table 7: Phytoconstituets of *V. faba* (acetone extract) identified by GC MS analysis

Peaks	RT (min)	Area %	Compound name	Molecular formula	Molecular weight
1	31.603	0.242	Phthalic acid, 6-ethyl-3-octyl heptyl ester	$C_{25}H_{40}O_4$	404.6
2	32.587	1.705	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	270.5
3	33.397	6.300	Hexadecanoic acid (CAS)	$C_{16}H_{32}O_2$	256.4
4	35.260	5.041	Ethyl linoleate	$C_{20}H_{36}O_2$	308.5
5	35.350	3.475	9-Octadecenoic acid (Z)-, methyl ester (CAS)	$C_{19}H_{36}O_2$	296.5
6	36.011	0.593	Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	252.3
7	39.903	0.870	9,12-Octadecadienoyl chloride	$C_{18}H_{31}C_{10}$	298.8
8	39.965	0.494	Oleoyl chloride	$C_{18}H_{33}C_{10}$	300.9
9	40.362	0.412	Ethanamine, 2,2'-oxybis[N,N-dimethyl-	$C_8H_{20}N_{2O}$	160.2
10	41.165	3.649	Hexadecanoic acid, 2-hydroxyethyl ester	$C_{18}H_{36}O_3$	300.5
11	43.468	8.699	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356.5
12	43.696	0.703	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382.7
13	47.973	6.945	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416.7
14	48.729	0.647	.beta.-Sitosterol acetate	$C_{31}H_{52}O_2$	456.7
15	51.535	6.454	Ergost-5-en-3-ol, (3.beta.,24R)- (CAS)	$C_{28}H_{48}O$	400.7
16	52.080	2.102	Stigmasterol	$C_{29}H_{48}O$	412.7
17	53.806	28.851	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.7
18	54.099	2.288	Cholest-5-en-3-ol, 24-propyl	$C_{27}H_{46}O$	386.7
19	54.876	4.779	.beta.-Amyrin	$C_{30}H_{50}O$	426.7

Source: Data obtained from analysis are represented in the form of table by the authors

Molecular Docking

A total of 1,298 compounds were present in all extracts together. After the virtual screening, only 8.6 percent of compounds (ligand) were scrutinized and docked with dopamine 1 (receptor) in AutoDock Vina for assessing their binding affinity. Levodopa, a commercially available drug for Parkinson's disease was used as a control. In aqueous extract phthalic acid, 4-nitrophenyl octyl ester had a good binding affinity of -8.2kcal/mol and the interacting amino acid was tryptophan (TRP 400) (Fig. 4). In acetone extract, 2-(2-Benzofuranyl) benzothiazole had a binding affinity of -7.9 kcal/mol and the interacting amino acid is aspartic acid (ASP 80) (Fig. 5). In the ethanolic extract, N,N'-Bis(3-methoxyfluoren-9-ylidene)hydrazine) had higher binding affinity -11.7 kcal/mol and the interacting amino acid was tryptophan (TRP 400) (Fig. 6). The levodopa displayed -6.9 binding affinity with serine (SER 159 and SER 163), aspartic acid (ASP 80) and threonine (THR 85) as the interacting amino acids (Fig. 7).

DISCUSSION

Oxidative stress has become a vital risk factor for the development and advancement of fatal diseases like cardiovascular and neurodegenerative diseases. Reactive oxygen species appear to have a crucial role in Parkinson's pathogenesis; it elevates the loss of dopaminergic neuronal cells in the brain by promoting oxidative stress (Guo et al. 2018). Antioxidant supplements from plants have a promising defence mechanism against the oxidative stress induced by the reactive species (Kasote et al. 2015). Plant-based supplements like ginseng, carvacrol, levodopa, resveratrol, carbidopa and curcumin are proven to have neuroprotective effects against Parkinson's disease (Fu et al. 2015; Khazdair et al. 2021). Rabey et al. (1992) conducted a study on 6 Parkinson's patients and 5 healthy individuals. All the 11 were given cooked *V. faba* for breakfast and they were monitored for 4 hours. The results exhibited that plasma concentration of levodopa was found to be increased in Parkinson's patients and they also showed improvement in their motor skills. The improvement in Parkinson's patients may be due to levodopa present in their diet.

The present study relies on exploring the phytoconstituents and antioxidant capacity of *V. faba*. The antioxidant capacity depends on the presence of polyphenols and flavonoids present in *V. faba*. Previously Loizzo et al. (2020) tested ethanolic ex-

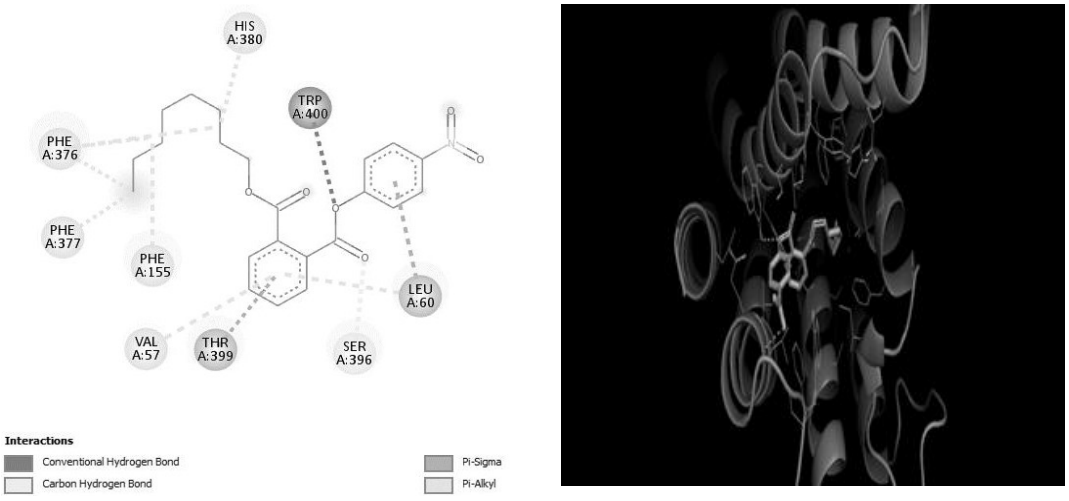

Fig. 4. 2D and 3D representation of Phthalic acid, 4-nitrophenyl octyl ester compound interacting with dopamine 1
HIS: Histidine, LEU: Leucine, VAL: Valine, TRP: Tryptophan, THR: Threonine, PHE: Phenylalanine and SER: Serine.
Source: Authors

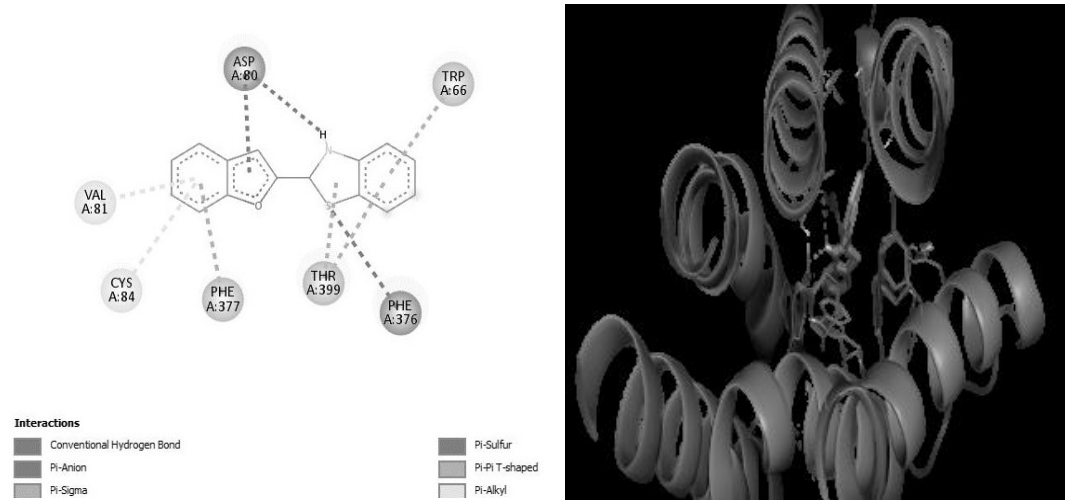

Fig. 5. 2D and 3D representation of 2-(2-Benzofuranyl)benzothiazole compound interacting with dopamine 1
ASP: Aspartic acid, TRP: Tryptophan, THR: Threonine, PHE: Phenylalanine, CYS: Cysteine and VAl: Valine
Source: Authors

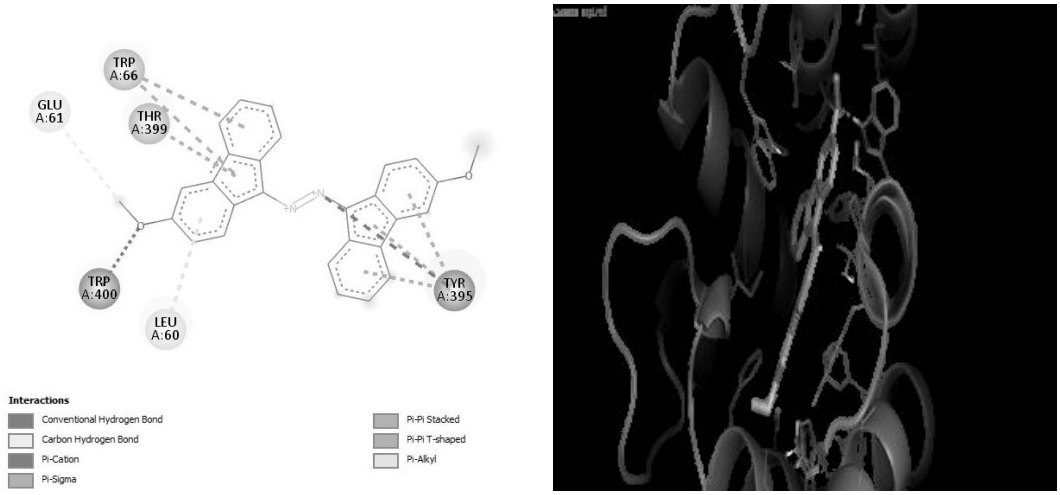

Fig. 6. 2D and 3D representation of N,N'-Bis(3-methoxyfluoren-9-ylidene)hydrazine) compound interacting with dopamine 1
TRP: Tryptophan,THR: Threonine, GLU: Glutamic acid, LEU: Leucine and TYR: Tyrosine
Source: Authors

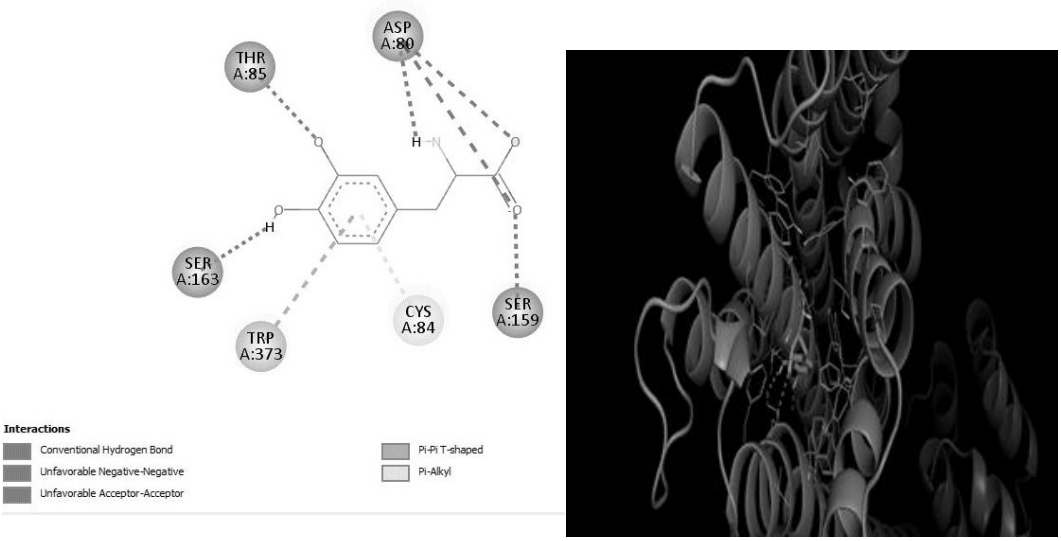

Fig. 7. 2D and 3D representation of levodopa compound interacting with dopamine 1
THR: Threonine, ASP: Aspartic acid, SER: Serine, CYS: Cysteine and TRP: Tryptophan
Source: Authors

tract of the seed pod and edible part of the *V. faba* for TPC and TFC with a sample concentration of 1.5 mg/ml. The results suggested that the ethanolic extract of the seed pod had a higher content of TPC and TFC than the edible part. The edible part had 2.06 mg GAE/g extract and 1.77 mg QE/g extract of TPC and TFC respectively. In the present study, ethanolic extract showed a higher amount of TPC and TFC. The TPC and TFC values of acetone extract of mature seeds were found to be 30.22 mg GAE/g DW and 1.613 mg QE/g DW respectively (Boukhanouf et al. 2016). Silva et al. (2018) reported that methanolic extract had 3.44 and 2.15 mg/g of phenolic and tannin content in raw *Phaseolus vulgaris* (common beans) which is comparatively lower than the phenolic and tannin content of *V. faba* extracts.

The result of the present study is found to have a higher IC_{50} value for DPPH and ABTS assays (ethanolic extract) than Loizzo et al. (2020). FRAP test of ethanolic extract has also displayed less ferric ions reducing power when compared with the results of Loizzo et al. (2020). *Mucuna pruriens* (fabaceae) is other natural of levodopa. Sreekala et al. (2020) have studied the antioxidant capacity of ethanolic extract of *M. pruriens*. The findings indicated that the *M. pruriens* had good antioxidant activity of higher concentration. Sonpetkar et al. (2012) reported that ethanolic extract of *M. pruriens* had a good radical scavenging at

Table 8: Phytoconstituets of V. faba (ethanolic extract) identified by GC MS analysis

Peaks	*RT (min)*	*Area %*	*Compound name*	*Molecular formula*	*Molecular weight*
1	5.809	3.09	L-Arabinitol	$C_5H_{12}O_5$	152.1
2	9.853	2.71	2(R),3(S)-1,2,3,4-Butanetetrol	$C_4H_{10}O_4$	122.1
3	10.275	0.25	Tetradecane	$C_{14}H_{30}$	198.3
4	11.242	0.16	1,4-Anhydro-d-galactitol	$C_6H_{12}O_5$	164.1
5	12.386	0.50	Hexadecane	$C_{16}H_{34}$	226.4
6	12.919	0.62	Ethyl .alpha.-d-glucopyranoside	$C_8H_{16}O_6$	208.2
7	14.264	0.50	Octadecane	$C_{18}H_{38}$	254.5
8	14.819	0.17	Phthalic acid, isobutyl octadecyl ester	$C_{30}H_{50}O_{14}$	474.7
9	15.663	5.61	Hexadecanoic acid (CAS)	$C_{16}H_{32}O_2$	256.4
10	15.897	0.89	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4
11	16.697	0.21	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.5
12	16.819	0.20	Phytol	$C_{20}H_{40}O$	296.5
13	17.074	25.56	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4
14	17.208	4.57	Ethyl linoleate	$C_{20}H_{36}O_2$	308.5
15	18.196	0.61	9-Methyl-Z-10-pentadecen-1-ol acetate	$C_{18}H_{34}O_2$	282.5
16	18.385	5.93	1H-1,2,3,4-Tetrazole-1-propanamide, N-(5-methyl-3-isoxazolyl)-	$C_8H_{10}N_6O_2$	222.2
17	18.563	0.18	Octanamide, N-(2-hydroxyethyl)-	$C_{10}H_{21}NO_2$	187.2
18	19.063	0.17	E,Z-1,3,12-Nonadecatriene	$C_{19}H_{34}$	262.5
19	19.396	1.93	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336.6
20	19.696	8.05	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5
21	20.341	0.24	7-Pentadecyne	$C_{15}H_{30}$	210.4
22	20.540	0.94	Cyclopropane, 2-bromo-1-methyl-1-phenyl	$C_{10}H_{11}B_r$	211.1
23	20.663	0.18	2-Ethylbutyric acid, eicosyl ester	$C_{26}H_{52}O_2$	396.7
24	20.863	29.74	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356.5
25	21.396	0.18	2-Butenenitrile, 2-chloro-3-(4-methoxyphenyl)-	$C_{11}H_{10}C_1NO$	207.6
26	21.518	0.19	Squalene	$C_{24}O$	208.3
27	21.918	0.53	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alp ha.,7.beta.,8a.beta.)-	$C_{14}H_{24}O$	208.3
28	22.529	0.26	4-Methoxy-2-octadecylphenol	$C_{25}H_{44}O_2$	376.6
29	23.040	5.59	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416.7
30	23.396	0.26	Propiophenone, 2'-(trimethylsiloxy)-	$C_{12}H_{18}O_{12}S_i$	222.3

Source: Data obtained from analysis are represented in the form of table by the authors.

different concentrations ranging from 40 to 600µg. The present study also showed a similar range of antioxidant activity in ethanolic extract.

The compounds like Stigmasterol (Sterol), Heptadecanoic acid, 15-methyl-, ethyl ester (Saturated fatty acid ester), Phytol (Diterpenoid) and Squalene (Triterpene) have good antioxidant activity (Karthikeyan et al. 2016). Steroid (Ergost-5-en-3-ol, (3.beta., 24R)- (CAS)) and Triterpenoid (.beta.-Amyrin) present in extracts displayed anti-inflammatory activity (Mujeeb et al. 2014; Okoye et al. 2014). Fatty acids like hexadecanoic acid and ethyl linoleate were found in all three extracts to have anti-inflammatory activity (Aparna et al. 2012; Park et al. 2014).

Forty five compounds had been scrutinized from the aqueous extract with binding affinity ranging from -8.2 to -7 kcal/mol. 43 compounds had been selected with binding affinity ranging from -7.9 to -7.1 kcal/mol. Totally 21 compounds have been selected in ethanolic extract, with binding affinity ranging from -11.7 to -7.1kcal/mol. In a previous study conducted by Mirza et al. (2015) food and drug administration-approved drug apomorphine (-8.3 kcal/mol), bromocriptine (-8.7 kcal/mol) and ropinirole (-8 kcal/mol) has been docked with Dopamine 3 receptor.

Choudhary et al. (2019) studied the anti-diabetic activity of catechin and gallic acid extracted from ethanolic derived for *V. faba*. The results reported that the compound had better binding capacity with six interacting amino acids. In a recent study, the furanoflavonoid was docked with four different receptors of Parkinson's. The outcome of the study revealed that the flavonoid had a higher binding capacity to the receptors than the dopamine agonists (Gnanaraj et al. 2022). Comparing the results of former studies with current results showed compounds of *V. faba* had higher binding capacity. Méndez-López et al. (2022) studied the binding capacity of 13 phytoconstituents of *V. faba* to aryl hydrocarbon receptors. The results showed that phytochemicals of *V. faba* have a higher affinity towards aryl hydrocarbon receptor which can be useful in the treatment of autoimmune disease.

The results of the present study suggested that standard control drugs have lower binding affinity than the compounds present in *V. faba*. On comparing the binding affinities of levodopa and the phytocompounds of the *V. faba* extracts, it is found that most of the selected compounds showed a higher binding affinity with the target protein than the levodopa. N,N'-Bis (3-methoxy-fluoren-9-ylidene) hydrazine) compound from ethanolic extract *V. faba* had the highest binding affinity (-11.7 kcal/mol) than the control (levodopa -6.7 kcal/mol). All the top 3 compounds scrutinized from each extract obeyed all Lipinski rules. Thus the phytocompounds from *V. faba* can be used as dopamine agonists from stimulating the dopamine receptor.

CONCLUSION

In the present study, the edible part (beans) of the *V. faba* is chosen for the analysis. Among the three extracts, the aqueous extract exhibited better antioxidant activity than acetone and ethanol. But the higher content of phenolic compounds, flavonoids and tannins was displayed by acetone followed by aqueous and ethanol. The GC-MS results suggested that ethanolic extract has shown a higher number of compounds with 30 peaks. But, among these three extracts tested acetone extract had a higher number of bioactive compounds. Most of the compounds present in these three extracts like stigmasterol, gamma tocopherol, hexadecanoic acid, ethyl linoleate and ergost-5-en-3-ol, (3.beta.,24R) are proven to have a considerable effect against inflammation and oxidative stress. The phytol and squalene also had good antioxidant properties. The results highlighted that beans of the *V. faba* have a good antioxidant activity which can be useful in managing oxidative stress-induced diseases. The results of *in silico* studies, also displayed that the phytochemicals found in *V. faba* have a higher affinity towards the target protein, suggesting that *V. faba* phytocompounds can be possibly used as dopamine agonist candidate in the therapeutic approach against Parkinson's disease.

RECOMMENDATIONS

The results of *in vitro* antioxidant assays and *in silico* analysis of *V. faba* have shown that plant has good antioxidant capacity and better binding affinity to the dopamine receptor. This suggests that *V. faba* may have neuroprotective effect. So further the neuroprotective effect of the plant can be conformed using an animal model.

ABBREVIATION

GC-MS: Gas Chromatography-Mass Spectrometry
DPPH: 2, 2- Diphenyl-1-Picrylhydrazyl

ABTS: 2,2-Azinobis[3-Ethylbenzothiazoline-6-Sulfonate]
GAE: Gallic Acid
QE: Quercetin
FRAP: Ferric Reducing Antioxidant Power
SE: Standard Error
TPC: Total Phenolic Content
TFC: Total Flavonoid Content
TTC: Total Tannins Content
TRP: Tryptophan
ASP: Aspartic Acid
THR: Threonine

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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