Original Research Article

IN SILICO AND *IN VIVO* BIOLOGICAL EVALUATION OF *CHENOPODIUM QUINOA* ON SCOPALMINE INDUCED MEMORY IMPAIRMENT IN MICE

ABSTRACT

Aims: The present research is focused on screening *in vivo* and *insilico* anti- amnesic activity of the ethanolic extract of seeds of *Chenopodium quinoa*. To establish the exact the mechanism of action, the phytochemical constituents of the extract were subjected to *in silico* studies using schrodinger and pass software. **Methodology:** The powdered material of seeds of *Chenopodium quinoa* were dried and extracted with ethanol by soxhlation technique. *In vivo* evaluation of anti- amnesic activity of the ethanolic extract of seeds of *Chenopodium quinoa* was carried using Transfer latency by Elevated plus maze, Novel object recognition test and Passive avoidance time by cook's pole climbing apparatus. The major phytoconstituents were subjected to molecular docking using PDB ID: 1EVE, 6N33, 2FV5 and 6W2X. Prediction studies for biological activities, adverse effects, direct and possible targets and using PASS (Predictions of activity spectra for substances) online software. **Results:** The extract was administered at doses (200 mg/kg and 400 mg/kg) showed significant anti- amnesic activity. The prolongation of the transfer latency time (secs) in the elevated plus-maze test is as an indicator for impairment of learning and memory. In Novel objective recognition test the time of memorizing the familiar object with that of the novel object and time taken for each animal to recognize the novel object compare to familiar object is noted. The results revealed that Quercetin, Beta sitosterol, Kaempferol, Dimethyl sulphide, Myristic acid, Palmitic acid, Stigmasterol, Lenolenic acid, Pentadecanoic acid, Tocopherols, Arachidonic acid and standard Donepezil have got highest glide scores against PDB ID: 1EVE, 6N33, 2FV5 and 6W2X. The ADME results revealed the higher oral bioavailability. The major phytoconstituents were subjected to prediction studies for biological activities, adverse effects, direct and possible targets and using PASS (Predictions of activity spectra for substances) online software. **Conclusion:** From *in vivo* and *in silico* results it is evident that ethanolic extract seeds of *Chenopodium quinoa* possessed significant anti- amnesic activity.

KEYWORDS: Donezepil, *Chenopodium quinoa*, Docking studies, Schrodinger software, Molinspiration, ADME analysis and PASS.

1. INTRODUCTION

The term dementia is a loss of mental ability severe enough to interfere with normal activities of daily living Such as s Alzheimer's disease (AD), vascular dementia, dementia with Lewy bodies, fronto-temporal dementia, Parkinson's disease, etc. 4 million people in India are living with this disease, Which is characterized by severe disruption of memory without deficits in intelligence, attention, perception, or judgments Memory is defined as a process of registration, storage, and retrieval of information, which occurs due to changes in transmission efficacy at the synapse where nerve cells communicate with each other there are two major classes of amnesia, anterograde and retrograde.The person is unable to create new memories. He remembers everything from the past. However, he cannot keep the record of the event that occurred after the injury to the brain cause anterograde amnesia. In retrograde Amnesia patient is unable to recall memories from the past. He can form memories of the recent events that will remain intact. However, the memories stored in the brain prior to brain damage are lost. Complete or partial memory loss can be seen depending on the extent of the damage. Where, Anterograde amnesia is more severe than retrograde amnesia. Improper oxygen supply to the brain, alcohol abuse, lack of acetylcholine and certain medications that causes amnesia [1]. Donepezil binds reversibly to acetyl cholinesterase and inhibits the hydrolysis of acetylcholine, thus increasing the availability of acetylcholine at the synapses, enhancing cholinergic transmission. This means there is a higher concentration of acetylcholine in the brain, which leads to better communication between nerve cells. Scopolamine produces memory loss in brain cells of mice by competing with acetylcholine (ACh) and other muscarinic agonists for a common binding site on the muscarinic receptor. Muscarinic receptor antagonists inhibit responses to postganglionic cholinergic nerve stimulation and mainly amygdala (emotional processes). Scopolamine itself is lipid soluble and has greater lipid solubility than helps in crossing the Blood brain barrier *Chenopodium quionoa* is an endemic plant peculiar to South America Belongs to the family (*Chenopodiaceae*). Seeds of quinoa are used as antimicrobial, antioxidant, anti-inflammatory,

antitumor and anti-carcinogenic effects. The present study aimed to evaluate the neurobehavioral and neuroprotective effect of the ethanolic extract of *Chenopodium quiona* on Scopolamine – induced amnesia in mice [2].

2. MATERIALS AND METHODS

2.1 Plant collection and drying

Seeds of *Chenopodium quinoa* were collected from the local market during the month of December 2020. This Material was identified and authenticated by Botanist Dr. P. Suresh Babu, junior lecturer, New Government Degree College, kukatpally. The marketed seeds were shade dried for a week and coarsely powdered in a mixer grinder. The powdered material was Stored or taken up for extraction process.

2.2 Preparation of ethanolic extract of *Chenopodium quinoa* (*Soxhlet)*

The powdered material of seeds of *Chenopodium quinoa* were dried and extracted with ethanol by soxhlation technique. The organic extracts obtained were evaporated to dryness by keeping at room temperature. Large amounts of drug can be extracted with a much smaller quantity of solvent. This process of extraction is economical in terms of time, energy and consequently financial investments.

2.3 Preliminary phytochemical analysis of the extract

The extract was subjected to preliminary phytochemical investigations to identify various phytoconstituents present in the ethanolic extract seeds of *Chenopodium quinoa*.

2.4 Acute toxicity testing

The acute toxicity studies were carried out using OECD (Organisation for Economic Cooperation and Development) 425 guidelines. Present study was carried out in CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, and Hyderabad, India. (Reg.No. 1175/PO/ERe/S/08/CPCSEA).

2.5 Animal housing

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

2.5.1 *In vivo* methods for evaluation of anti-amnesic activity

In vivo evaluation of anti- amnesic activity of the ethanolic extract of *Chenopodium quinoa* seeds (EECQ) was carried out in following models.

A. Transfer latency by Elevated plus maze.

B. Novel object recognition test.

C. Passive avoidance time by cook's pole climbing apparatus.

2.5.1.1 Transfer latency by Elevated plus maze: (learning and memory)

30 Swiss albino mice of either sex weighing about 25-30 gm were selected for this study. They were divided into five groups with 6 animals in each group.

Group I received (control) normal saline.

Group II received (disease control) Scopolamine (1.0 mg/kg, *i.p*).

Group III received test drug EECQ at dose of 200 mg/kg, *p.o*.

Group IV received test drug EECQ at dose of 400 mg/kg, *p.o*.

Group V received (standard) Donepezil (1mg/kg, *i.p)*.

Thirty (30) minutes after the different treatments, all the groups received Scopolamine (1 mg/kg, bd.wt, *i.p*) except for the control which received distilled water to induce anterograde amnesia and transfer latency (TL) was recorded as Initial latency (L_0) which is defined as time (seconds) taken by the animal to move from an open arm into one of the closed arms with all four legs. Retention of this learned task was examined 24h after the first-day trial as Final latency (L_1) [3,4].

The following parameters can be recorded: Initial latency (L_0), Final latency (L_1) and

The Transfer latency was expressed as inflexion ratio, calculated using formula:

$$IR = (L1 - L0) \backslash L0$$

Where,

L0: Initial TL (s) on the 1st day and

L1: TL (s) on the 2nd day.

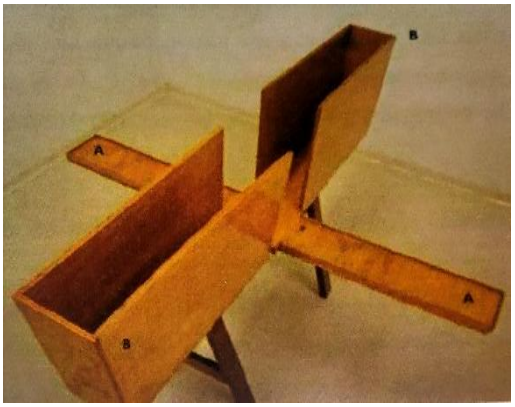


Figure 1: Elevated plus maze

2.5.1.2 Novel object recognition test: (short term memory)

30 Swiss albino mice of either sex weighing about 25-30 gm were selected for this study. They were divided into five groups with 6 animals in each group. On the day before test, mice were allowed to explore the box (without any object) for 2 minutes. In sample phase each mouse will be placed in an open field with two identical objects (plastic ball) for 5 minutes. The mouse will return to its home cage.

Group I received (control) normal saline.

Group II: received (disease control) Scopolamine (1.0 mg/kg, *i.p*).

Group III received test drug EECQ at dose of 200 mg/kg, *p.o.*

Group IV received test drug EECQ at dose of 400 mg/kg, *p.o*.

Group V received (standard) Donepezil 1mg/kg, *i.p*.

Thirty (30) minutes after the different treatments, all the groups received Scopolamine (1 mg/kg, bd.wt, *i.p*) except for the control which received distilled water to induce amnesia In the test phase, each mouse will be placed again in the open field in which one of the identical objects had been replaced with a novel object (plastic square). A mouse will score as exploring when the nose was in contact with the object and time will be recorded manually using a stopwatch [5].

Percentage of discrimination index (DI) will be calculated by formula:

$$DI = N\text{-}F/\ N+F \times 100$$

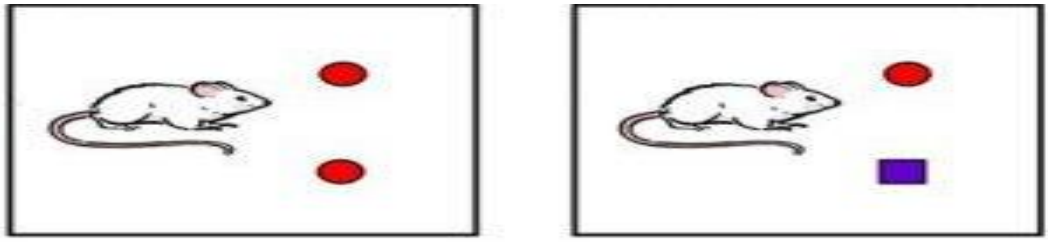


Figure 2: Novel object recognition

2.5.1.3 Passive avoidance time by Cook's pole climbing apparatus (long term memory)

30 Swiss albino mice of either sex weighing about 25-30 gm were selected for this study. They were divided into five groups with 6 animals in each group. An acclimatization period of 5 minutes in the chamber, a buzzer will be given followed by a shock through the grid floor. The rat will jump on to the pole (shock-free zone) to avoid foot shock. All the animals will be subjected to a training schedule individually by placing them inside the Perspex chamber of the apparatus. Jumping on the pole functionally terminates the shock and this will classify as an escape while such jumping prior to the onset of the shock was considered as avoidance. The session will be terminated after completion of 60 trials with an interval of 20-30 seconds between trials.

Group I received (control) normal saline.

Group II received (disease control) Scopolamine (1.0 mg/kg, *i.p*).

Group III received test drug EECQ at dose of 200 mg/kg, *p.o.*

Group IV received test drug EECQ at dose of 400 mg/kg, *p.o*.

Group V received (standard) Donepezil (1mg/kg, *i.p)*.

Thirty (30) minutes after the treatment with extract and standard drug to group III, IV and V all the groups received Scopolamine (1 mg/kg, bd.wt, *i.p*) except for the control to induce amnesia After 1 hour the shock avoidance response was noted as a parameter. This procedure will be repeated at 24 hours to evaluate which group receive 95 to 99 % avoidance by conditioned avoidance response (CAR) [6].

Figure 3: Cook's pole climbing apparatus

2.6 Insilico analysis

2.6.1 Molecular Docking Studies

2.6.1.1 Structure based drug design

Initially the protein downloaded from PDB was prepared by removing chain B. Water molecules present in both the chains are removed. Energy minimization was done. Later molecules drawn using chemdraw were converted to mol format and ligprep was created. Grid generation was done by removing crystal ligand and the structures were docked against protein 1EVE, 6N33, 2FV5 and 6W2X.

2.6.1.2 Schrodinger XP-docking results

XP docking indicates that some of our compounds have good binding ability with Acetyl choline esterase inhibitor (PDB ID: 1EVE), Aβ plaque deposition inhibitor (PDB ID: 6N33), TNF α inhibitor (PDB 2FV5) and GABA inactivator (PDB ID: 6W2X).

2.6.1.3 Ramachandran plot Ramachandran plot has been generated from PROCHECK validation server which was used to access the quality of the model by looking into the allowed and disallowed regions of the plot [7].

2.7 *In silico* ADME study using molinspiration

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

physicochemical parameter ranges (MWT ≤ 500, log P ≤ 5, H-bond donor's ≤ 5, Hbond acceptors ≤ 10) [8].

2.7.2 *In silico* Bioactivity study using molinspiration: The bioactivity score of selected "active constituents were also evaluated using the tool Molinspiration Cheminformatics server (http://www.molinspiration.com). In this computational chemistry technique large chemical databases are analyzed in order to identify possible new drug candidates. Only SMILES or SDfile structures of active molecules are sufficient for the training, no information about the active site or binding mode is necessary. This is particularly useful in projects where structure-based approach cannot be applied because information about 3D receptor structure is not available" [9].

2.7.3 PASS (Prediction of Activity Spectra for Substances).

"The new drug development is a very tedious method and is related with a high probability of negative consequences in terms of pharmacological efficacy. In such a scenario, it becomes fundamental that a device is available which ought to predict the pharmacological properties beforehand. It would allow the researchers to streamline the lookup more efficiently. Prediction of activity spectra of substances (PASS) is such a device which can predict the pharmacological homes beforehand and would help in screening pharmacological manageable leads for a particular condition" [10]. The present homework includes the use of PASS for a survey of the pharmacological credibility of selected phytoconstituents of EECQ for right targets for Anti amnesic activity.

2.7.3.1 Input and Output of PASS

PASS uses as input data a MOL- or SD-file23 representing the structural information about the molecules under study. On the basis of these data, MNA descriptors (Multilevel Neighborhoods of Atoms) are generated automatically. Based on the statistics of MNA descriptors for active and inactive compounds from the training set, two probabilities are calculated for each activity: Pa - the probability of the compound being active and Pi - the probability of being inactive. Being probabilities, the Pa and Pi values vary from 0.000 to 1.000 (with three relevant decimals being calculated), and in general Pa + Pi < 1, since these probabilities are calculated independently. Pa and Pi can be considered to be measures of the compound under study belonging to the classes of active and inactive compounds, respectively, or can be seen as estimates for the first and second kinds of errors in the prediction. All MNA descriptors influence the estimates in the activity prediction. Their influence can be either positive (if the descriptors are found in compounds with the particular

activity), or negative (if the descriptors are found in compounds without the particular activity), or even neutral (if the descriptors are found in both active and inactive compounds). In the last case, they decrease the relative impact of the "positive" and "negative" descriptors [11].

2.7.3.2 Interpretation of Predictions

"The PASS predictions can be interpreted, and used, in a flexible manner. The most probable activities, for a given compound, are characterized by Pa values close to 1, and Pi values close to 0. Let us first consider cases where the Pa value is high and is much larger than Pi. If a statistically significant set of samples with predictions obtained with the threshold Pa > 0.9 is selected from a much larger database and assayed, one has to expect to lose 90% of the active compounds, but the fraction of false-positives will be very small. For a cutoff of Pa > 0.8, only 80% of the actives will be lost, but the fraction of false positives will be a little bit higher. Finally, if one goes down to the criteria Pa > Pi, the probability of the first kind of error equals the probability of the second kind of error, i.e., one is just as likely to miss true actives as to find false positives" [12, 13, 14].

"However, maximizing Pa values for the desired activity is not the only criteria for selection of the most promising compounds. Another aspect might be the novelty of a compound. If Pa is very high, the compound might be a close analogue of known pharmaceutical agents. Thus, if one is interested in finding new leads, especially New Chemical Entities (NCE), one may want to choose compounds for which the specified activity is predicted with lower probability, say, 0.5 < Pa < 0.7. In this case, the probability of false positives is likely to be higher, but if the activity will be confirmed in the experiment, one has a higher chance of having obtained an NCE" [12, 13, 14].

2.8 Statistical Analysis

"The results are reported as the mean ± SEM (n=6) of the mean analysis of variance followed by the Dunnett's multiple comparison tests were used for comparison. Differences were considered significantly at $p< 0.05$" [15].

3. RESULTS

Ethanolic extract of was explored for its *in vivo* anti-amnesic activity using suitable rodent models. All the results obtained in the study were included below.

3.1 Preparation of ethanolic extract of seeds of *Chenopodium quinoa*

The ethanolic extract of seeds of *Chenopodium quinoa* was prepared by soxhlation technique. The percentage yield of ethanolic extract was calculated by using the following formula.

% Of yield obtained=Amount of extract obtained (gms)/ Total amount powder used × 100

% Yield of extract=46.990/140 ×100=33.5%

3.2 Preliminary phytochemical analysis

The preliminary phytochemical investigation of ethanolic extract of seeds of *Chenopodium quinoa* revealed the presence of bioactive compounds of which flavonoids, glycosides, terpenoids, phenolic compounds, sterols, tannins and Proteins were the most prominent (Table 1).

Table 1: Preliminary phytochemical analysis.

Phytoconstituents	Results
Flavonoids	+
Saponins	+
Terpenoids	+
Phenolic compounds	+
Tannins	+
Proteins	+

3.3 Acute toxicity studies

Ethanolic extract of seeds of *Chenopodium quinoa* was tested on Swiss albino mice up to a dose of 2000 mg/kg bd. wt. The animal did not exhibit any signs of toxicity or mortality up to 2000 mg/kg bd. wt. various morphological and behavioral characters were observed during the study. Hence the extract was found to be safe up to 2000 mg/kg bd. wt.

3.4 Dose selection

From toxicity studies, a dose of 2000 mg/kg bd. wt. was identified to be safe, and the working dose was considered as $1/10^{th}$ i.e., 200 mg/kg. bd. wt. In the present study pharmacological evaluations were done using 200 mg/kg. bd. wt. and 400 mg/kg. bd. wt.

***3.5 In vivo* anti-amnesic activity**

The ethanolic extract of seeds of *Chenopodium quinoa* was screened for its anti-amnesic activity using the following models.

3.5.1 Transfer latency by Elevated plus maze

Table 2: Effect of EECQ on Transfer latency time Using Elevated Plus Mazes

Groups	Initial latency (L0) (Sec)	Final latency (L1) (Sec)	Inflexion ratio $IR = (L1 - L0) \backslash L0$ (Sec)
Control	34±0.6	34.16±0.5	33.1±0.5
Disease control Scopolamine (1 mg/kg bd.wt)	47.5±0.3	61.1±0.2	60.2 ±0.1*
EECQ (200 mg/kg bd. wt)	25.5±0.3	23.3±0.3	21.6± 0.3^{*aA}
EECQ (400 mg/kg bd. wt)	18.5±0.4	17.6±0.4	16.9 ±0.1^{*aB}
Donepezil (1.0 mg/kg bd.wt)	31.6±0.3	32.5±0.1	30.1± 0.3^{*a}

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, disease control & standard. Significant values are expressed as control group (* = p< 0.0001), disease control group (a=p<0.0001) & standard (A = p<0.0001, B = p<0.001).

3.5.2 Novel object recognition test

Table 3: Effect of EECQ on Discriminative index using Novel Object Recognition task

Groups	Object Recognition Task (secs)	
	Novel Object	Familiar Object
Control	45.1±0.7	7.9±0.3
Disease control Scopolamine (1 mg/kg bd.wt)	65.8±0.7*	13.5±0.8*
EECQ (200 mg/kg bd.wt)	36.6±0.9^{*aA}	5.8±0.1^{*aA}
EECQ (400 mg/kg bd.wt)	28±0.8^{*aC}	3.1±0.3^{*aB}

Donepezil (1.0 mg/kg bd.wt)	25±0.4^{*a}	1.4±0.2^{*a}

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, disease control & standard. Significant values are expressed as control group (* = p< 0.0001), Disease control group (a=p<0.0001) & Standard (A = p<0.0001, B = p<0.0005, C = p<0.001).

3.5.3 Passive avoidance time by cook's pole climbing apparatus

Table 4: Effect of EECQ Passive Avoidance Test by cook's pole climbing apparatus

Groups	Time taken to climb the pole (Secs)	
	1hr	24hr
Control	18.6±0.3	19.5±2.0
Disease control Scopolamine (1 mg/kg bd. wt)	21.5±0.2**	23.1±6.0**
EECQ (200 mg/kg bd.wt)	15±0.2^{**aB}	13.8±3.0^{**aA}
EECQ (400 mg/kg bd.wt)	17±0.3^{***aA}	16.5±3.0^{*Aa}
Donepezil (1.0 mg/kg, bd.wt)	13.1±3.0^{**a}	11.8±5.0^{**a}

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, disease control & standard. Significant values are expressed as control group (* = p<0.0005, ** = p<0.0001, *** = p<0.005), Disease control group (a = p<0.0001) & Standard (A = p<0.0001, B = p=0.0001).

***Insilico* analysis**

3.6 Molecular docking

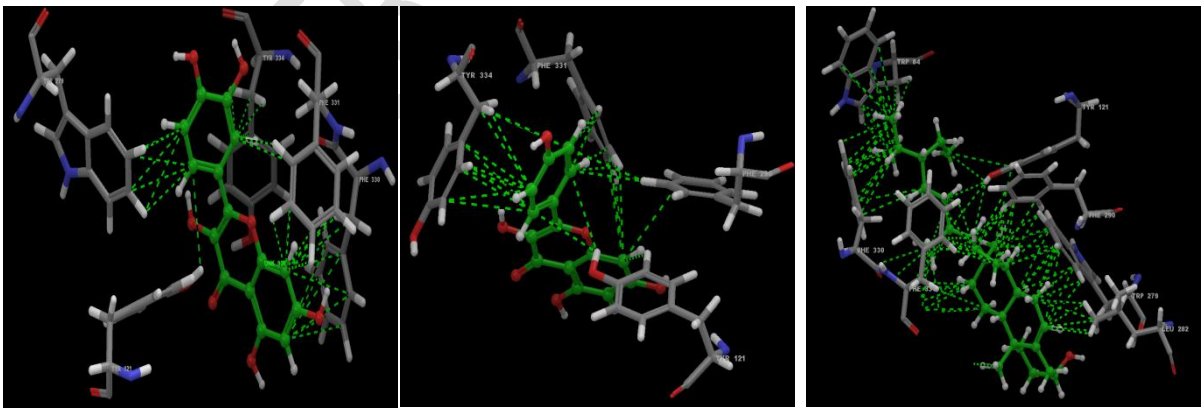
Table 5 : Schrodinger XP Docking Score

Compounds	1EVE	6N33	2FV5	6W2X
Tetradecanoic acid/ Myristic acid	-6.19	-8.25	-5.29	-6.65
Hexadecanoic acid /Palmitic acid	-9.34	-4.35	-5.87	-8.39
Alpha Lenolenic acid	-7.23	-6.85	-5.51	-6.51
9,12,15 octadecatrienol	-8.13	-5.13	-5.36	-7.14

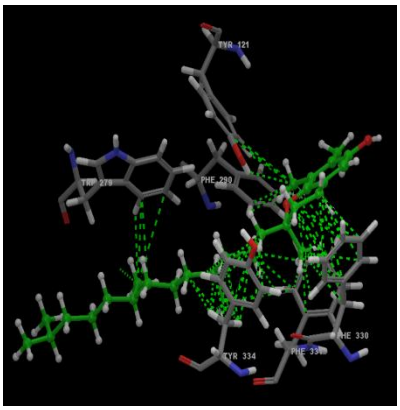
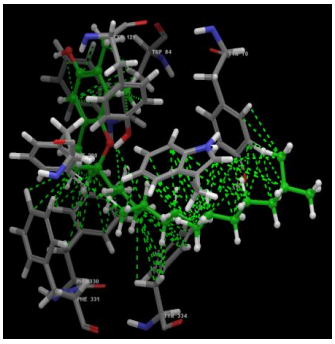
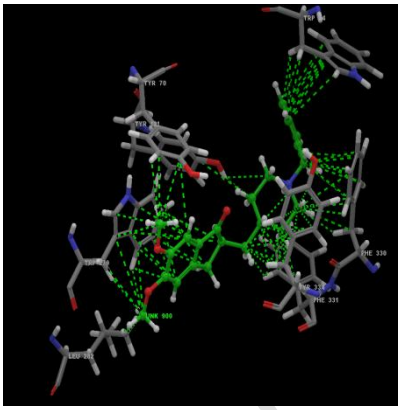
Eicosadienoic acid	-5.34	-6.58	-5.54	-6.62
Pentadecanoic acid	-6.27	-6.18	-5.19	-6.43
Tocopherols	-8.76	-4.70	-5.87	-8.53
Stigmasterol	-8.47	-5.80	-5.51	-5.39
Beta sitosterol	-9.49	-6.20	-5.60	-8.05
Quercetin	-10.67	-11.72	-10.70	-9.28
Benzoic acid	-6.57	-6.93	-5.46	-5.53
Kaempferol	-10.39	-12.23	-9.50	-8.43
Arachidonic acid	-4.96	-6.86	-0.45	-6.59
Benzofuran	-6.12	-6.85	-4.25	-5.30
1,3- bis (2-chloroethyl) urea	-5.00	-5.14	-5.87	-5.20
Methyl sulfidtiole/dimethyl sulphide	-7.38	-8.59	-5.77	-5.82
2,4 thiazolidinedione	-6.74	-6.71	-5.6	-5.13
2-Bromotetrade canoic acid/2-Bromodecanoic acid	-6.70	-9.00	-5.34	-6.15
Donepezil	-11.98	-7.58	-8.93	-6.63

G score = glide score, The more negative the Glide score, the more favorable the binding

Image 1: PDB ID: 1EVE

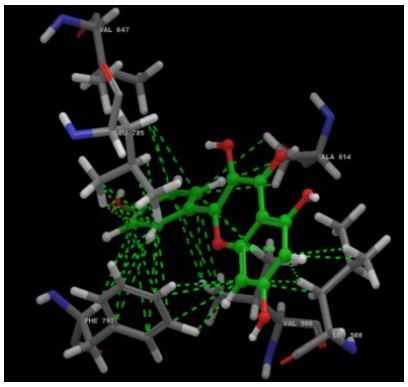
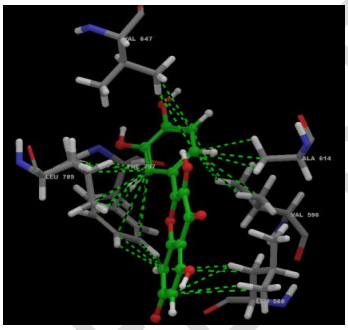
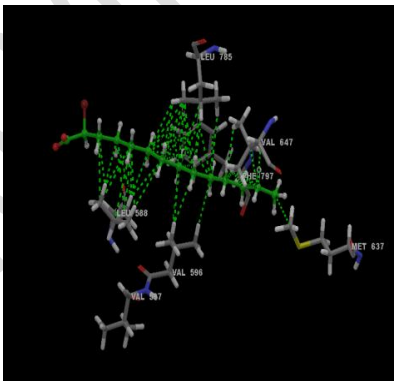


A) Quercetin -10.67 B) Kaempferol -10.39 C) Beta sitosterol -9.49

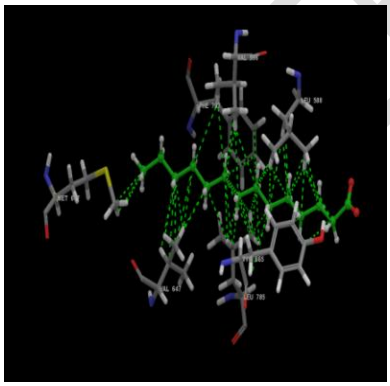
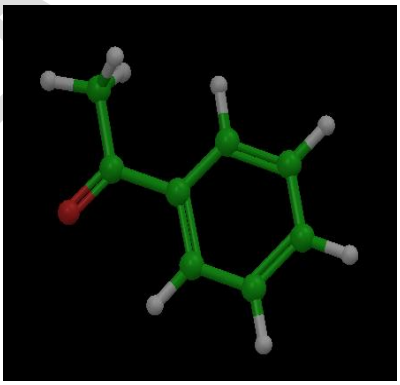
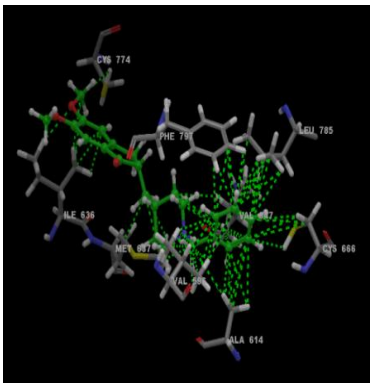




D) Hexadecanoic acid -9.34 E) Tocopherol -8.76 F) Donepezil -11.98

Image 2: PDB ID: 6N33

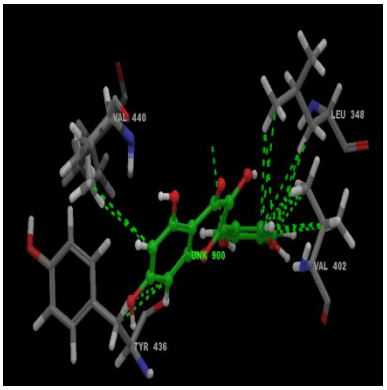




A) Kaempferol -12.23 B) Quercetin -11.72 C) 2-Bromotetradecanoic acid -9.00

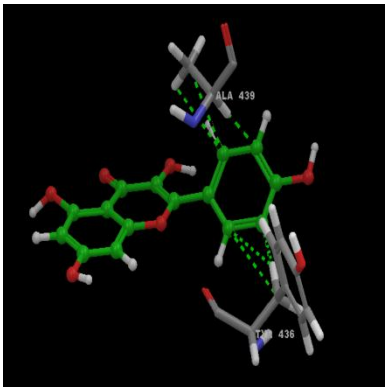




D) Tetradecanoic acid -8.25 E) Methy sulfidtiole -8.59 F) Donepezil -7.58

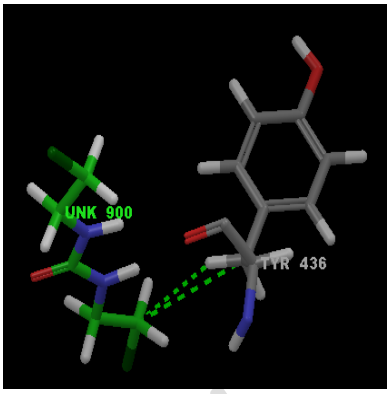
Image 3: PDB ID: 2FV5



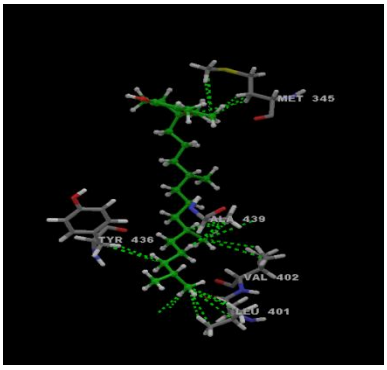
A) Quercetin - 10.70



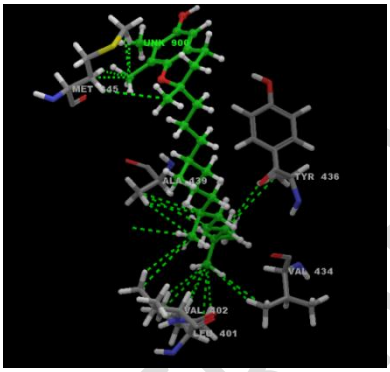
B) Kaempherol -9.50



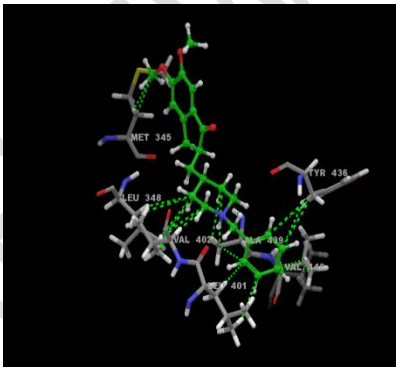
C) Tocopherol - 5.87



d) 1,3- bis(2-chloroethyl) urea - 5.87

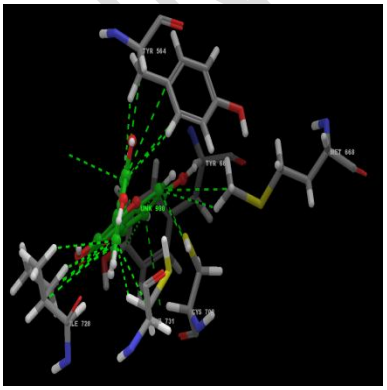


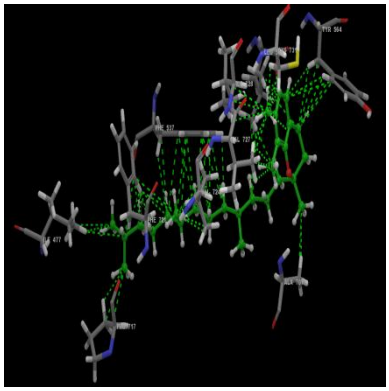
e) Hexadecanoic acid - 5.87

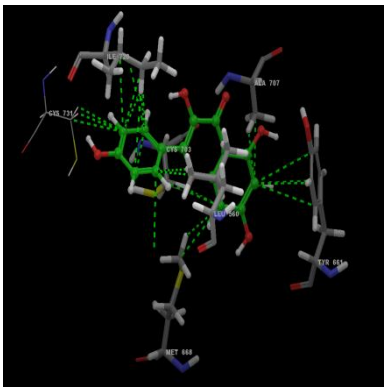


F) Donepezil -8.93

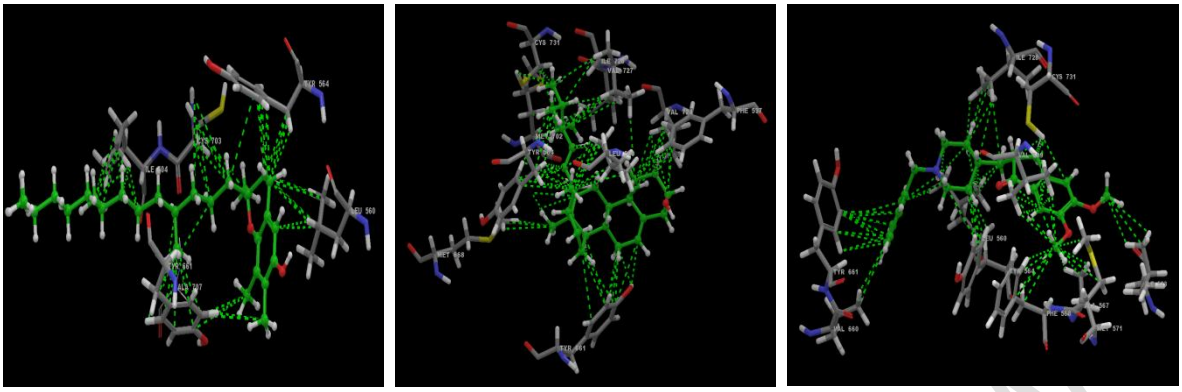
Image 4: PDB ID: 6W2X







A) Quercetin -9.28 B) Tocopherol -8.53 C) Kaempherol -8.43



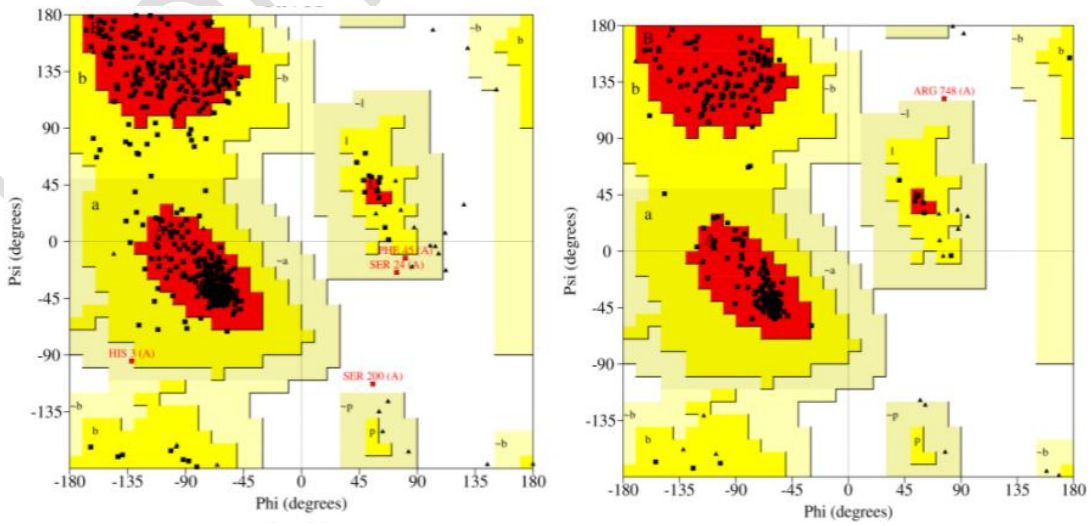
D) Hexadecanoic acid -8.39 E) Stigmasterol -8.39 F) Donepezil -6.63

3.7 Ramachandran plot Analysis

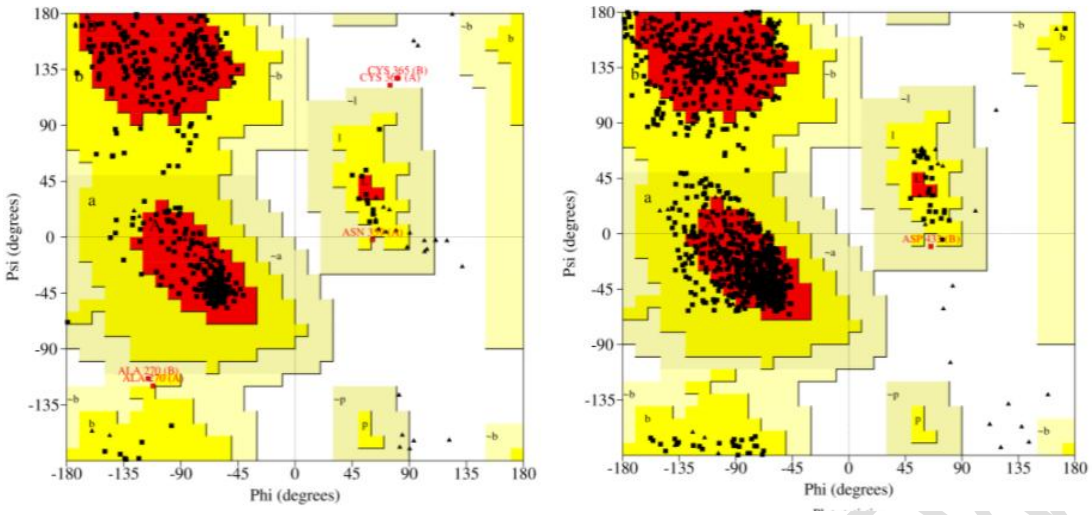
Protein 1EVE, 6N33, 2FV5 and 6W2X were analysed for Ramachandran plot to know amino acid presence in different regions of respective protein tabulated in table 2 and pictorial representation by figure below.

Table 6: Ramachandran plot status with protein with 1EVE, 6N33, 2FV5 and 6W2X

Residues	1EVE	6N33	2FV5	6W2X
Most favourable region (%)	87.3	94.1	87.1	82.3
Additional allowed regions (%)	11.8	5.5	11.8	17.6
Generously allowed regions (%)	0.7	0.0	0.7	0.1
Disallowed regions (%)	0.2	0.4	0.4	0.0



a) 1EVE b) 6N33



c) 2FV5 d) 6W2X

Figure 4 : Ramachandran plot of protein 1EVE, 6N33, 2FV5 and 1NFI

Table 7 : ADME properties of compounds from *Chenopodium quinoa* by molinspiration

S.No	Compound	MW	nON	nOHNH	nV	nrotb	TPSA	miLogP
1	Tetradecanoic acid/ Myristic acid	228.38	2	1	1	12	37.30	6.05
2	Hexadecanoic acid /Palmitic acid	256.43	2	1	1	14	37.30	7.06
3	Alpha Lenolenic acid	278.44	2	1	1	13	37.30	5.84
4	9,12,15 octadecatrienol	264.45	1	1	1	13	20.23	5.96
5	Eicosadienoic acid	308.51	2	1	1	1	16	7.87
6	Pentadecanoic acid	242.40	2	1	1	13	37.30	6.55
7	Tocopherols	416.69	2	1	1	12	29.46	8.98

8	Stigmasterol	412.70	1	1	1	5	20.23	7.87
9	Beta sitosterol	414.72	1	1	1	6	20.23	8.62
10	Quercetin	302.24	7	5	0	1	131.35	1.68
11	Benzoic acid	122.12	2	1	0	1	37.30	1.85
12	Kaempferol	286.24	6	4	0	1	111.12	2.17
13	Arachidonic acid	304.47	2	1	1	14	37.30	6.42
14	Benzofuran	118.14	1	0	0	0	13.14	2.26
15	1,3- bis (2-chloroethyl) urea	185.05	3	2	0	4	41.12	0.96
16	Methyl sulfidtiole/dimethyl sulphide	62.14	0	0	0	0	0	0.84
17	2,4 thiazolidinedione	117.13	3	1	1	0	46.17	-0.33
18	2-Bromotetradecanoic acid/2-Bromodecanoic acid	251.16	2	1	0	8	37.30	4.25
19	Donepezil	379.50	4	0	0	6	38.78	4.10

MW = Molecular weight, nON = number of hydrogen bond acceptors, nOH = number of hydrogen bond donors, nV = number of violation of Lipinski's rule of five, nrotb = number

of rotatable bonds, TPSA = Total Polar Surface Area and miLogP = Octanol-water partition coefficient logP.

Table 8: Bioactive score of compounds from *Chenopodium quinoa by* molinspiration

S.No	Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	Tetradecanoic acid/ myristic acid	-0.11	0.03	-0.51	-0.06	-0.19	0.13
2	Hexadecanoic acid /Palmitic acid	0.02	0.06	-0.33	0.08	-0.04	0.18
3	Alpha Lenolenic acid	0.33	0.23	-0.19	0.35	0.13	0.02
4	9,12,15 octadecatrienol	0.20	0.21	-0.11	0.18	-0.02	0.34
5	Eicosadienoic acid	0.32	0.16	-0.09	0.35	0.19	0.35
6	Pentadecanoic acid	-0.04	0.05	-0.42	0.01	-0.11	0.16
7	Tocopherols	0.17	0.06	-0.17	0.40	0.22	0.17
8	Stigmasterol	0.12	-0.08	-0.48	0.74	-0.02	0.53
9	Beta sitosterol	0.14	0.04	-0.51	0.73	0.07	0.51
10	Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28
11	Benzoic acid	-2.21	-1.57	-2.49	-2.05	-2.31	-1.60
12	Kaempferol	-0.10	-0.21	0.21	0.32	-0.27	0.26
13	Arachidonic acid	0.32	0.16	-0.09	0.35	0.19	0.35
14	Benzofuran	-2.12	-1.39	-2.33	-2.48	-2.61	-1.76

15	1,3- bis (2-chloroethyl) urea	-1.19	-0.84	-1.18	-1.58	-1.22	-0.69
16	Methyl sulfidtiole/dimethyl sulphide	-3.87	-3.86	-3.97	-3.87	-3.77	-3.76
17	2,4 thiazolidinedione	-3.68	-3.86	-4.08	-3.74	-3.89	-3.71
18	2-Bromotetradecanoic acid/2-Bromodecanoic acid	-0.62	-0.52	-1.31	0.01	-0.72	-0.13
19	Donepezil	0.22	-0.14	-0.16	0.03	0.03	0.25

Table 9: Anti Amnesic Activity predicted for the active constituents of *Chenopodium quinoa* using PASS (prediction of activity spectra for substances).

Sl.No	**Compound**	**Probable Activity (Pa)**	**Probable Activity (Pi)**	**Biological Activity**
1	Tetradecanoic acid/ myristic acid	0,841	0,008	Prostaglandin-E2 9-reductase inhibitor
		0,702	0,002	Choline dehydrogenase inhibitor
		0,610	0,002	Cyclooxygenase 1 substrate
		0,307	0,032	Vascular dementia treatment
2	Hexadecanoic acid /Palmitic acid	0,702	0,002	Choline dehydrogenase inhibitor

		0,515	0,052	Antiinflammatory
3	Alpha Lenolenic acid	0,800	0,001	Cyclooxygenase 1 substrate
		0,804	0,006	Antiinflammatory
		0,713	0,006	TNF expression inhibitor
4	9,12,15 octadecatrienol	0,761	0,001	Cyclooxygenase 1 substrate
		0,610	0,012	TNF expression inhibitor
5	Eicosadienoic acid	0,737	0,001	Cyclooxygenase 1 substrate
		0,751	0,005	TNF expression inhibitor
6	Pentadecanoic acid	0,702	0,002	Choline dehydrogenase inhibitor
7	Tocopherols	0,927	0,003	Antioxidant
		0,757	0,003	Free radical scavenger
		0,775	0,008	Anti inflammatory
8	Stigmasterol	0,702	0,041	Nootropic
		0,344	0,078	TNF expression inhibitor
9	Beta sitosterol	0,317	0,092	TNF expression inhibitor
		0,467	0,067	Antiinflammatory
10	Quercetin	0,872	0,003	Antioxidant
		0,811	0,003	Free radical scavenger
11	Benzoic acid	0,323	0,036	Non-steroidal antiinflammatory agent
12	Kaempferol	0,856	0,003	Antioxidant
		0,771	0,003	Free radical scavenger
		0,348	0,273	Nootropic
13	Arachidonic acid	0,751	0,005	TNF expression inhibitor
		0,715	0,001	Cyclooxygenase substrate
14	Benzofuran	0,732	0,006	Neurodegenerative diseases treatment

15	1,3- bis (2-chloroethyl) urea	-	-	-
16	Methyl sulfidtiole/dimethyl sulphide	--	-	-
17	2,4 thiazolidinedione	0,435	0,183	Nootropic
		0,166	0,007	Acetylcholine M3 receptor agonist
		0,293	0,152	Dementia treatment
		0,133	0,041	Choline dehydrogenase inhibitor
18	2-Bromotetradecanoic acid/2-Bromodecanoic acid	0,429	0.004	Choline dehydrogenase inhibitor
19	Donepezil	0,788	0,003	Cholinergic
		0,553	0,099	Nootropic

Table 10: Adverse Effects predicted for the active constituents of *Chenopodium quinoa* using PASS (prediction of activity spectra for substances).

S. No	Compound	Probable Activity (Pa)	Probable in Activity (Pi)	Adverse effect
1	Tetradecanoic acid/ myristic acid	0.500	0.050	Nephrotoxicity
		0.474	0.209	Hepatotoxicity
2	Hexadecanoic acid /Palmitic acid	0.500	0.050	Nephrotoxicity
		0.474	0.209	Hepatotoxicity
3	Alpha Lenolenic acid	0.252	0.216	Nephrotoxicity
4	9,12,15 octadecatrienol	-	-	No Adverse Effects Predicted
5	Eicosadienoic acid	0.304	0.152	Nephrotoxicity
6	Pentadecanoic acid	0.500	0.050	Nephrotoxicity

		0.474	0.209	Hepatotoxicity
7	Tocopherols	0.389	0.267	Hepatotoxicity
8	Stigmasterol	-	-	No Adverse Effects Predicted
9	Beta sitosterol	0.258	0.205	Nephrotoxicity
10	Quercetin	0.559	0.165	Hepatotoxicity
11	Benzoic acid	0.516	0.187	Hepatotoxicity
		0.477	0.057	Nephrotoxicity
		0.359	0.102	Myocardial infarction
12	Kaempferol	0.525	0.182	Hepatotoxicity
13	Arachidonic acid	0.304	0.152	Nephrotoxicity
14	Benzofuran	0.304	0.152	Nephrotoxicity
15	1,3- bis (2-chloroethyl) urea	-	-	No Adverse Effects Predicted
16	Methyl sulfidtiole/dimethyl sulphide	-	-	-
17	2,4 thiazolidinedione	0.971	0.003	Cardiac failure
		0.553	0.168	Hepatotoxicity
		0.517	0.040	Myocardial Infarction
18	2-Bromotetrade canoic acid/2-Bromodecanoic acid	0.599	0.144	Hepatotoxicity
		0.419	0.077	Nephrotoxicity
		0.293	0.217	Myocardial Infarction
		0.279	0.194	Cardial failure
19	Donepezil	0.761	0.018	Arrthymia

Table 11: Direct and possible target Prediction for the active constituents of *Chenopodium quinoa* using PASS (prediction of activity spectra for substances).

S. No	Compound	Direct Target	Confidence	Possible Target	Confidence
1	Tetradecanoic acid/ myristic acid	Cyclooxygenase-1	0.0779	Nuclear factor NF-kappa-B complex	0.1370
		TNF-alpha	0.0311		0.1303
				Cyclooxygenase-1	
2	Hexadecanoic acid /Palmitic acid	Cyclooxygenase-1	0.0779	Cyclooxygenase-1	0.1303
		TNF-alpha	0.0311	Nuclear factor NF-kappa-B p65 subunit	0.0704
				Beta amyloid A4 protein	0.0177
3	Alpha Lenolenic acid	Cyclooxygenase-1	0.1455	Cyclooxygenase-1	0.1298
		TNF-alpha	0.0337	Nuclear factor NF-kappa-B p65 subunit	0.0372
4	9,12,15 octadecatrienol	Cyclooxygenase-1	0.1043	Cyclooxygenase-1	0.0316
		TNF-alpha	0.0168	Nuclear factor NF-kappa-B p65 subunit	0.0250
5	Eicosadienoic acid	TNF-alpha	0.0337	Cyclooxygenase-1	0.1298
6	Pentadecanoic acid	Cyclooxygenase-1	0.0779	GABA A receptor alpha-3/beta-2/gamma-2	0.2160
		TNF-alpha	0.0311		
				Nuclear factor NF-kappa-B complex	0.1043
				Cyclooxygenase-1	0.1303
7	Tocopherols	-	-	Nuclear factor NF-	0.0329

				kappa-B p65 subunit	
8	Stigmasterol	TNF-alpha	0.1476	GABA-A receptor-alpha-1/beta-2/gamma-2	0.1272
9	Beta sitosterol	TNF-alpha	0.2066	Nuclear factor NF-kappa-B complex	0.1944
10	Quercetin	Cyclooxygenase-2	0.0078	GABA-A receptor-alpha-1/beta-2/gamma-2	0.2034
11	Benzoic acid	Cyclooxygenase-1	0.0916	Cyclooxygenase-1 GABA-A receptor-alpha-1/beta-2/gamma-2	0.2159
		Cyclooxygenase-2	0.0569	Nuclear factor NF-kappa-B complex	0.2040
12	Kaempferol	Acetyylcholinesterase	0.2142	Cyclooxygenase-1	0.0973
		Cyclooxygenase-2	0.0074	Nuclear factor NF-kappa-B complex	0.0086
13	Arachidonic acid	Cyclooxygenase-1	0.0074	Cyclooxygenase-1	0.1298
		TNF-alpha	0.0337	Nuclear factor NF-kappa-B p65 subunit	0.0512
14	Benzofuran	Cyclooxygenase-2	0.0818	Nuclear factor NF-kappa-B complex	0.0323
		TNF-alpha	0.0387		
		Cyclooxygenase-1	0.0273		
15	1,3- bis (2-chloroethyl)	-	-	Acetylcholinesterase	0.1655

	urea			Nuclear factor NF-kappa-B complex	0.0708
16	Methyl sulfidtiole/dimethyl sulphide	-	-	-	-
17	2,4 thiazolidinedione	-	-	Nuclear factor NF-kappa-B p65 subunit	0.0195
				GABA-A receptor-anion channel	0.0178
18	2-Bromotetradecanoic acid/2-Bromodecanoic acid	Cyclooxygenase-1	0.0211	Cyclooxygenase-1	0.0867
		TNF-alpha	0.0171	Acetylcholine receptor-alpha1/beta1/delta/gamma	0.3953
				Nuclear factor NF-kappa-B complex	0.0539
				Acetylcholinesterase	0.0114
19	Donepezil	Acetyylcholinesterase	0.6576	Acetyylcholinesterase	0.3953
		Muscarinic acetylcholine receptor M5	0.1183	Nuclear factor NF-kappa-B p65 subunit	0.0120
		Neuronal Acetylcholine receptor protein alpha-7 subunit	0.0805		

4. DISCUSSION

Amnesia is one of the major complications associated with memory loss in the patents of neurodegenerative disease like Alzheimer's disease (AD). Memory enhancement index screening was conducted using elevated plus maze. Every animal individually was placed in

the elevated plus maze apparatus at the end of an open arm. The time required for each mice to enter into the closed arm was deemed to be transfer latency (TL). The prolongation of the transfer latency time (secs) in the elevated plus-maze test is as an indicator for impairment of learning and memory [15]. In Novel objective recognition test the time of memorizing the familiar object with that of the novel object and time taken for each animal to recognize the novel object compare to familiar object is noted. Passive avoidance test is a fear-aggravated test used to evaluate learning and memory in rodent models of CNS disorders as in case of amnesia by buzzer followed by shock when animals are individually placed. Phenolic compounds (Benzoic acid) display potent antioxidant effects by scavenging free radicals due to the presence of hydroxyl groups that show impact on memory and learning which lead to decreases in the transfer latency time showing great effect of anti-amnesic activity [16]. The neuroprotective and improved cognitive could be due to the synergistic effect of Quercetin by decreasing the AchE level, β amyloid$_{1-42}$ level, and antioxidant action in rat brain. It helps in memorising the way from open end to close end in elevated plus maze and differencing between novel and familiar object by spending more time by rearing, sniffing around the novel object [17].

"Benzofurazan cause decline in oxidative stress and up regulation of the AMPA receptor GluR2 subunit gene expression in the hippocampus which causes recover the spatial memory impairment which improve learning amd memory showing less time to reach the close arm" [18]. "Beta–sitosterol maintain the acetylcholine levels for prolong time in synaptic cleft and stimulate the cholinergic receptors. The enhanced cholinergic transmission by β-sitosterol is likely to offer useful effects for the restoration of memory in AD and in the prevention of free radicals induced neurodegeneration in aging brain this help in memorizing the familiar object and differentiate the novel object" [19]. "Arachidonic acid help in decreasing the neuroinflamation and synaptic function regulation of neuromediators. Increased the degree of oxidative stress both in the plasma and corticohippocampal brain tissues. Arachidonic acid deposition in the corticohippocampal tissues of senescent rodents caused a faster performance activity, which is reminiscent to hyperactive behavior of animals, the spatial learning ability-related memory of the rodents was found to be improved and showed a greater effect in percentage of avoidance by shock" [20]. Hexadecanoic acid is a saturated fatty acid which helps in formation of new memories related to fear and strong emotion which is beneficial for learning and memory and it is known as modulators of neurotransmission and synaptic plasticity. It reduces the risk of amnesia in the cook's pole model as the animal react to buzzer

first to avoid, The shock and increasing the time of avoidance by memorizing the training [21].The animals might have shown improvement in learning and memory which might be due to the presence of phytochemical constituents in the extract.

Molecular docking continues to holds great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. The docking analysis of isolated compounds from Ethanolic extract of seeds of *Chenopodium quinoa and* standard drug like donepezil were carried out using Schrödinger software. The various constituents identified in the plant extract are Myristic acid, Palmitic acid, Lenolenic acid, 9, 12, 15 octadecatrienol, Eicosadienoic acid, Pentadecanoic acid, Tocopherols, Stigmasterol, Beta sitosterol, Quercetin, Benzoic acid, Kaempferol, Arachidonic acid, Benzofuran, 1,3- bis (2-chloroethyl) urea, Methyl sulfidtiole/dimethyl sulphide, 2,4 thiazolidinedione, 2-Bromodecanoic acid and standard drug donepezil were subjected to docking against PDB ID: 1EVE, 6N33, 2FV5 and 6W2X. The highest glide scores were observed with Quercetin, Beta sitosterol, Kaempferol, dimethyl sulphide, Myristic acid, Palmitic acid, Stigmastero, Lenolenic acid, Pentadecanoic acid, Tocopherols, Arachidonic acid and standard donepezil against PDB ID: 1EVE, 6N33, 2FV5 and 6W2X. The glide scores of the quercetin, and kaempferol, were found to be more than the glide score of standard drug donepezil against all selected proteins stating that the compounds might have same affinity to bind to the proteins. These results clearly indicate that the chemical constituents mentioned above might have shown similar mechanism to that of the standard drug donepezil as anti-amnesic. “The proteins identified namely PDB ID: 1EVE, 6N33, 2FV5 and 6W2X are modeled and the qualities of the 3D model were evaluated using the PROCHECK program and assessed using the Ramachandran plot. It is evident from the Ramachandran plot that predicted models have most favorable regions, additionally allowed regions, generally allowed regions and disallowed regions. Such a percentage distribution of the protein residues determined by Ramachandran plot shows that the predicted models are of good quality. According to Ramachandran plot a good quality model would be expected to have over 90% in the most favoured region. Proteins like PDB ID: 1EVE, 6N33, 2FV5 and 6W2X showed almost 90% favoured region which clearly indicates that the selected models in the present study are of good quality” [22].

Molinspiration molecular properties were calculated on the bases of Lipinski's rule and its components. Lipinski’s rule of five is to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and

physical properties that would make it an orally active drug in humans. In the present study, all the compounds that are docked have lower molecular weight so that they are easily absorbed, diffused and transported. The various constituents identified in the seed extract are Myristic acid, Palmitic acid, Lenolenic acid, 9,12,15 octadecatrienol, Eicosadienoic acid, Pentadecanoic acid, Tocopherols, Stigmasterol, Beta sitosterol, Quercetin, Benzoic acid, Kaempferol, Arachidonic acid, Benzofuran, 1,3- bis (2-chloroethyl) urea, Methyl sulfidtiole/dimethyl sulphide, 2,4 thiazolidinedione, 2-Bromodecanoic acid. These compounds and the standard donepezil are subjected to ADME analysis using Molinspiration software. "The selected active constituents like quercetin, kaempferol, Benzoic acid, Benzofuran, dimethyl sulphide, and standard drug donezepil has zero violations and the remaining compounds which have one or two violations out of five. Any compound with zero violation clearly indicates the probability of its higher oral bioavailability. Topological polar surface area (TPSA) allows the prediction of transport properties of drug candidates in the intestines and blood-brain barrier. The TPSA score in all the selected active constituents of the extract and standard drug donezepil was found to be less than 140 which clearly indicated better permeability into the tissues. Molinspiration ADME enables the computation of key physicochemical, pharmacokinetic, drug-like and related parameters for one or multiple molecules" [23]. "Number of H-bond acceptors should be in a range of 0-10 and number of H-bond donors should be 0-5. All the selected active constituents in the present study were found to be within the range. A negative value for ilogP means the compound has a higher affinity for the aqueous phase (it is more hydrophilic); when ilogP equals 0 the compound is equally partitioned between the lipid and aqueous phases; a positive value for ilogP denotes a higher concentration in the lipid phase (i.e., the compound is more lipophilic). In the present study almost all the active constituents and standard donezepil have shown a positive ilogP value clearly indicating a higher concen-tration in the lipid phase except 2,4 thiazolidinedione which has shown a negative ilogP value indicating a higher concentration in the aqueous phase" [23].

The various constituents identified in the seed extract are Myristic acid, Palmitic acid, Lenolenic acid, 9,12,15 octadecatrienol, Eicosadienoic acid, Pentadecanoic acid, Tocopherols, Stigmasterol, Beta sitosterol, Quercetin, Benzoic acid, Kaempferol, Arachidonic acid, Benzofuran, 1,3- bis (2-chloroethyl) urea, Methyl sulfidtiole/dimethyl sulphide, 2,4 thiazolidinedione, 2-Bromodecanoic acid are subjected to score using mol inspiration. The scores for the selected compounds can be interpreted as Active (bioactivity score > 0), moderately active (bioactivity score: -5.0-0.0) and inactive (bioactivity score < -5.0). Most of the compounds including standard donezepil are active against all the receptors and enzyme

inhibitors except a few like 2,4 thiazolidinedione, Benzoic acid and dimethyl sulphide. The result of bioactivity score of these compounds against GPCR ligand, ion channel modulator, and nuclear receptor ligand, inhibitor activities towards kinase, protease and enzymes indicated that the compounds exhibit active to moderate score towards all the receptors.

"Prediction of activity spectra of substances (PASS) is a valuable interface that should be adopted as an archetypal tool for predicting the potential molecules and to predict the biological activity of certain phytoconstituents for their anti-amnesic effects. The anti-amnesic activity of selected phytoconstituents were predicted by engaging the canonical simplified molecular-input line-entry system obtained from PubChem.com followed by using PASS online" [10,11]. "Several phytoconstituents were predicted to have effects better than marketed drugs under some or the other out of the chosen areas of pharmacological mediation. On the other hand, several new paths were predicted in which the *in vitro* and *in vivo* evaluation of the phytoconstituents can be made on the basis of PASS predicted activities. It would allow the researchers to streamline the lookup more efficiently. Prediction of activity spectra of substances (PASS) is such a device which can predict the pharmacological homes beforehand and would help in screening pharmacological manageable leads for a particular condition" [10,11].

It predicts the spectra of organic things to do for a molecule in terms of probable activity (Pa) and probable inactiveness (Pi) [24]. Selected active phytochemical constituents of *Chenopodium quinoa* were subjected to pass software for anti-amnesic activity. The results of these active constituents like probable activity (Pa) and probable inactiveness (Pi) and biological activity were given in table 8. The possible interventions of selected active constituents of *Chenopodium quinoa* were found to be cholinergic, Prostaglandin-E2 9-reductase inhibitor, Choline dehydrogenase inhibitor, Cyclooxygenase 1 substrate, Antiinflammatory, TNF expression inhibitor, Nootropic, antioxidant, free radical scavenger, Selected active phytochemical constituents of *Chenopodium quinoa* were subjected to pass software for adverse effects. From the results, the constituents like 9, 12, 15 octadecatrienol, Stigmasterol, 1, 3- bis (2-chloroethyl) urea were found to be free from any adverse effects. The remaining compounds were predicted with hepatotoxicity, nephrotoxicity and myocardial infarction. The standard donezepil was predicted with arrhythmia. Selected active phytochemical constituents of *Chenopodium quinoa* were subjected to pass software for direct and possible targets. All the constituents were found to have interventions as direct targets with TNF-alpha, Cyclooxygenase1 and 2, Acetylcholinesterase, Muscarinic acetylcholine receptor

M5, and Neuronal acetylcholine receptor protein alpha- 7 subunit. And interventions as possible indirect targets with nuclear factor NF-kappa-B complex, Cyclooxygenase 1 and 2, Nuclear factor NF-kappa-B p65 subunit, GABA-A receptor -alpha-1/beta-2/gamma-2 Acetylcholinesterase, and Acetylcholine receptor- alpha1/beta1/delta/gamma [25].

From the above PASS is an important tool for effectively showing the compounds of interest for the biological actions of interest. This helps the researchers to rationalize the research.

5. CONCLUSION

The ethanolic extract of seeds of *Chenopodium quinoa* possesses anti-amnesia in rodent models revealed by *invivo* and *insilico* analysis. Further studies are needed to be carried out to isolate individual phytochemical constituents of the extract and to establish the exact mechanism for its antiamnesic activity.

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