

Evaluation of Chenopodium Quinoa Extract on Diazepam Induced Memory Impairment in Animal Models

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Authors' contributions

*This work was carried out in collaboration among all authors. All authors read and approved the final
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ABSTRACT

Aim: The present study explored the pharmacological and *insilico* studies of ethanolic seed extract of *Chenopodium quinoa*.

Materials and Methods: The screening of *in vivo* anti- amnesic activity of the ethanolic seed extract of *Chenopodium quinoa* was carried out using Actophotometer and Rotarod apparatus. The extract exhibited active constituents like myristic acid, palmitic acid, eicosadienoic acid, pentadecanoic acid, tocopherols, stigmaterol, β -sitosterol, quercetin, benzoic acid, kaempferol, arachidonic acid, benzofuran and 2-bromodecanoic acid. *In silico* approaches like docking studies (Mcule software) and Ramchandran plot (procheck), online softwares were used in the study to establish mechanism of action of active constituents present in the extract.

Results: The extract treated groups at doses (200 mg/kg and 400 mg/kg, bd.wt) showed significant anti-amnesic activity. The basal activity score in actophotometer is as an indicator for impairment of learning and memory. Fall of time by rotarod is used to evaluate learning and memory in rodent models of CNS disorders as in case of amnesia. The results revealed that quercetin, kaempferol, myristic acid, palmitic acid, stigmaterol, lenolenic acid, pentadecanoic acid, tocopherols, arachidonic acid and standard donepezil have got highest glide scores against selected Protein Data Bank (PDB ID): 1EVE, 2FY4, 7CUM and 3EJ8. According to Ramchandran plot a good

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quality model would be expected to have over 90% in the most favoured region. Proteins like PDB ID: 1EVE, 2FY4, 7CUM and 3EJ8 showed almost 80-90% favoured region which clearly indicates that the selected proteins in the present study are of good quality.

Conclusion: Our research unveiled that ethanolic seed extract of *Chenopodium quinoa* possessed significant anti- amnesic activity.

Keywords: *Chenopodium quinoa*; Protein Data Bank (PDB); docking studies; Mcule software and Ramachandran plot.

1. INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia – a continuous decline in thinking, behavioural and social skills that affects a person's ability to function independently due to abnormal build-up of proteins in and around brain cells. One of the proteins involved is amyloid, which forms plaques around brain cells and tangles within them. The other protein is tau, which forms tangles within brain cells. Memory and other critical mental skills are progressively lost as a result of this disease [1]. Memory and other critical mental abilities are gradually lost as brain cell connections and cells deteriorate and die. The prevalence was higher in older age groups (75 and up) than in younger age groups (under 75). Male and female prevalence rates in rural and urban populations, however, were identical. The cholinesterase inhibitor donepezil is currently the standard of care for Alzheimer's disease [2]. Donepezil is widely used in clinical practice for Mild Cognitive Impairment – Alzheimer's disease (MCI-AD) since in routine clinical practice; it is challenging to differentiate mild AD from MCI-AD without a detailed neuropsychological evaluation including assessment of activities of daily living. The ability of the Benzodiazepines is known as “acquisition-impairing” molecules, and their effects on anterograde memory processes are well described. Diazepam has well-known amnesic properties. These effects, however, are selective for certain psychobiologically distinct memory functions and a highly differentiated unfolding of cognitive impairment in response to increasing doses of diazepam. Diazepam produced a marked deficit in episodic memory, yet despite this, dense amnesia with selectively impaired anterograde episodic memory and attention while totally sparing access to information in long-term memory (semantic or knowledge memory) [3].

The botanical name of quinoa is *Chenopodium quinoa* Willd and belongs to the Goosefoot family “*Chenopodiaceae*”. Quinoa is a dicot plant that can grow from 1 to 3m high; it is considered a

pseudo-cereal, not a true grain but rather a fruit. The seeds are round and flat, about 1.5-4.0 mm in diameter and their color varies from white to grey and black, with tones of yellow, rose, red, purple and violet. [4,5]. Quinoa has a fat content ranging from 2 to 10%. Quinoa and soya oils have similar fatty acid compositions, making quinoa a good source of essential fatty acids such linolenic (18:2n-6: 52%) and linolenic (18:2n-6: 52%). (18:3n-6: 4 percent). Quinoa has a lot of minerals in it. It has higher levels of calcium, magnesium, iron, and zinc than most cereals, with a particularly high iron content. When quinoa seeds are polished and washed, the mineral content is reduced by 12–15 percent in terms of iron, zinc, and potassium, 27 percent in terms of copper, and 3 percent in terms of magnesium. More riboflavin (B2) and -tocopherol are found in quinoa than in rice, barley, or wheat. Vitamin E can be found in quinoa seeds [6]. Quinoa seeds have antibacterial, antioxidant, anti-inflammatory, anti-tumor, and anti-carcinogenic properties. The present study aimed to evaluate the neurobehavioral and neuroprotective effect of the ethanolic seed extract of *Chenopodium quinoa* on Diazepam-induced amnesia in mice.

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, 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 subjected for extraction process.

2.2 Preparation of Ethanolic Extract of *Chenopodium quinoa* (Soxhlet)

The Soxhlet extractor is a type of continuous extraction of a component from a solid mixture.

The powdered material of seeds of *Chenopodium quinoa* were dried and extracted with ethanol by soxhlation technique. As to get efficient extraction, this method allows a continuous extraction process; it is nothing but a series of short macerations. The larger side arm rises up with boiling solvent. A condensed drop of solvent falls into the porous cup, dissolving a solid mixture's desired component. When the smaller side-arm fills to the point of overflowing, a syphoning motion occurs. The dissolved component-containing solvent is piped into the boiler underneath remaining solvent, which then drains out of the porous cup when new solvent drops fall into it. The cycle repeats. By retaining the organic extract at room temperature, it was evaporated to dryness. With a significantly lower volume of solvent, large amounts of medication can be extracted. This process of extraction is economical in terms of time, energy and consequently financial investments [7].

2.3 Preliminary Phytochemical Analysis of the Extract

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

2.4 Identification of Phytochemical Constituents Using Gas Chromatography

The extract was subjected to GC-MS studies to identify the exact phytochemical constituents. GC-MS analysis were carried out by Agilent 6890 series GC-MS instrument coupled with mass spectroscopy as detector. Temperature was adjusted to -30°C – 280/300°C. The HP -5MS Column with dimensions 30 m x 0.32 mm x 0.25 μm were used for analysis. This Oven temperature were adjusted to 35°C and hold time 5 min, ramp 10°C/min up to 220°C Column flow is 1.2 ml. The inlet temperatures were kept at 250°C and the source temperature of 230°C and MS Quard temperature of 150°C [8].

2.4.1 GC –MS conditions during analysis

2.4.1.1 GC condition

| | |
|----------------------|----------------------|
| Column oven | : 35°C initial, hold |
| Temperature | : Time 5 min |
| Injector Temperature | : 250°C |
| Column Flow | : 1.2 ml/min |

| | |
|------------------|--------------------------|
| Carrier Gas | : Helium 99.9995% purity |
| Injection Volume | : 1 microliter |

2.4.1.2 MS condition

| | |
|-----------------|------------|
| Ion source temp | : 230°C |
| MS quard | : 150°C |
| Ionization EI | : (-70 ev) |
| Scan speed | : 2000 |

2.5 Acute Toxicity Testing

The acute toxicity studies were carried out using OECD 425 guidelines. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, and Hyderabad, India (Reg.No. 1175/PO/ERe/S/08/CPCSEA) [9].

2.6 Animal Housing

The mice were kept in poly acrylic cages with a maximum of six animals per cage and a 12 hour light/12 hour dark cycle. Animals have free access to standard diet and drinking water *ad libitum*. The animals were given a week to acclimate to the laboratory surroundings before the trial began. 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).

2.7 In vivo Methods for Evaluation of Anti-amnesic Activity

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

1. Basal activity by Actophotometer
2. Fall of time by rotaroad test

2.7.1 Basal activity by Actophotometer

30 healthy albino mice of either sex weighing 25-30 gm were selected for the study. They were divided into five groups of 6 animals each. The actophotometer consist of a square arena (30 × 30 × 25 cm) with wire mesh bottom, in which the animal moves for 5 minutes. Six lights and six photocells were placed in the outer periphery of the button in such a way that mice can block only one beam. The movement of animal interrupts a beam of light falling on a photocell during which a count was recorded and displayed.

Group I: received (control) normal saline.

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

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

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

Group V: received Donepezil 1mg/kg, *i.p.*

Respective drugs were administered to all groups 1hour before the trials. The basal activity score of each animal will be noted on 8th and 9th day. The difference in the activity will be recorded considering standard drug treatment score and extract treatment score [10].



Fig. 1. Basal activity by Actophotometer

2.7.2 Fall of Time by Rotarod Test

30 healthy albino mice of either sex weighing 25-30 gm were selected for the study. They were

divided into five groups of 6 animals each. For the training trials, the mice will be placed on the rotarod at 25 rpm for about 10 minutes per day. Respective drugs were administered to all groups 1hour before the trials.

Group I received (control) normal saline.

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

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

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

Group V: received Donepezil 1mg/kg, *i.p.*

Mice will be initially selected for the ability to remain on the rotating bar rotating at a constant speed of 25 rpm for at least two consecutive 180 seconds trial. Measure the fall time of individual animals using the same conditions and noted on 8th and 9th day of the trial [10].

2.7.3 Histopathological studies

The mice brain was collected and isolated with formalin solution 10 %. Then, the brains were routinely embedded in paraffin and stained with haematoxylin eosin. The hippocampal lesions were assessed microscopically at 40X magnification [11].



Fig. 2. Fall of time by rotarod test

2.8 Statistical Analysis

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

2.9 In silico Analysis

2.9.1 Molecular Docking Studies

2.9.1.1 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, 2FY4, 7CUM and 3EJ8.

2.9.1.2 Mcule docking results

Mcule docking software was used in the present study. The selected proteins are Acetyl choline esterase inhibitor (PDB ID: 1EVE), choline acetyltransferase inhibitor (PDB ID: 2FY4), GABA inactivator (PDB ID: 7CUM) and NOS inhibitor (PDB ID: 3EJ8).

2.9.2 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 [10].

3. RESULTS AND DISCUSSION

Ethanol extract of *Chenopodium quinoa* was explored for its *in vivo* anti-amnesic activity using suitable rodent models and *in silico* analysis using Mcule software. All the results obtained in the study were included below.

3.1 Preparation of Ethanol Extract of Seeds of *Chenopodium quinoa*

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

$$\% \text{ of yield obtained} = \frac{\text{Amount of extract obtained (gm)}}{\text{Total amount powder used (gm)}} \times 100$$

% Yield of extract = $46.990/140 \times 100 = 33.5\%$

3.2 Preliminary Phytochemical Analysis

The preliminary phytochemical investigation of ethanol 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 | + |
| Sterols | + |

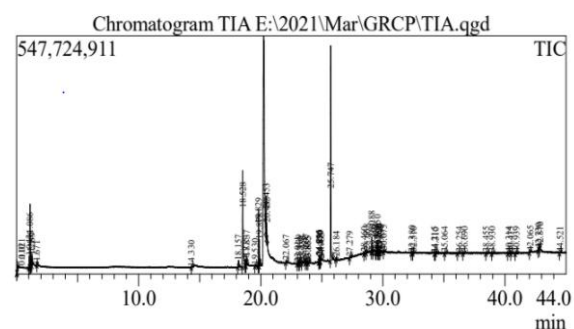


Fig. 3. Identification of phytoconstituents using gas chromatography

3.3 Acute Toxicity Studies

Ethanol 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 behavioural 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 $1/10^{\text{th}}$ 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. of the extract.

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 Basal activity score using actophotometer apparatus

The *in vivo* anti-amnesic activity of *Chenopodium quinoa* was screened using basal activity score. It was measured by using actophotometer apparatus on the 8th and 9th day. The results of basal activity score was depicted in Table 2.

3.5.2 Fall off time by Rotarod Apparatus

The *in vivo* anti-amnesic activity of *Chenopodium quinoa* was screened using fall off time. It was measured by using rotarod apparatus on the 8th and 9th day. The result of fall off time was depicted in Table 3.

The human amnesic syndrome associated with lesions of the hippocampus and amygdala is characterized by a selective impairment of recent

(explicit, episodic) memory. Benzodiazepine (BZD) treated normal subjects demonstrate similar, marked impairments in episodic memory, but in addition, BZD also induces sedation and inattention. Diazepam is well established as inhibitory modulators of memory processing. This effect is especially prominent when applied before the acquisition phase of a memory task. Explicit memory learning seems to be affected through the GABA_A receptors containing the α_1 and α_5 subunits, the role as subunits, mainly expressed in the hippocampus, in modulating distinct forms of memory gives certain memory deficit states. The phytochemical constituent identified in the ethanolic extract of *Chenopodium quinoa* seeds are saturated fatty acid, phenols, sterols, flavonoids, terpenoids, tannins, proteins and anti-oxidant [12].

α -linolenic acid increases hippocampal m-RNA levels and specific phospholipase A2 encoding-gene in brain and A β 1-42 Inhibited A β oligomerization decrease tau phosphorylation and pro-apoptotic proteins that leads in improvement in the cognitive function through the activation of extracellular signal-regulated kinases (ERK) and Akt signaling in the rat model increase the basal activity score by interrupting the beam of light in the arena [13].

Table 2. Effect of EECQ on Basal Activity Score by Actophotometer Apparatus

| Groups | 8 th Day | 9 th Day |
|---|-------------------------|-------------------------|
| Control | 473±3.0 | 474.5±3.0 |
| Disease control diazepam (1 mg/kg bd. wt) | 179±3.0 ^{**} | 180±2.0 ^{**} |
| EECQ (200 mg/kg bd.wt) | 310±4.0 ^{**aA} | 311±4.0 ^{**aA} |
| EECQ (400 mg/kg bd.wt) | 361±5.0 ^{**aA} | 383±3.0 ^{**aA} |
| Donepezil (1.0 mg/kg bd.wt) | 447±3.0 ^a | 448±3.0 ^{**a} |

Values are expressed as mean \pm 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 (** = $p < 0.0001$, * = $p < 0.0005$), disease control group (a = $p < 0.0001$) & Standard group (A = $p < 0.0001$)

Table 3. Effect of EECQ on fall off time by using Rotarod Apparatus

| Groups | Fall off time (secs) | |
|--|------------------------|------------------------|
| | 8 th day | 9 th day |
| Control | 187.3±1.2 | 188±1.2 |
| Disease control Clonazepam (1 mg/kg bd. wt) | 72.5±1.6 | 78±1.0 |
| EECQ (200 mg/kg bd.wt) | 100±1.4 ^{*aA} | 103±1.5 ^{*aA} |
| EECQ (400 mg/kg bd.wt) | 175±1.1 ^{*aA} | 176±0.8 ^{*aA} |
| Donepezil (1.0 mg/kg bd. wt) | 162±0.7 ^a | 166±0.7 ^a |

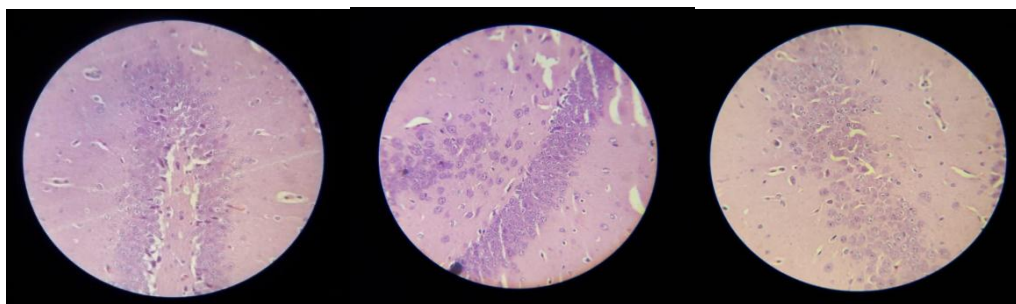
Values are expressed as mean \pm SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control, disease control & standard. Significant values are expressed as control group (* = $p < 0.0001$), Disease control group (a = $p < 0.0001$) & Standard group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

P-Coumarin activates the GABA-A receptor as it is a phenolic compound and it shows high anti-oxidant property which help in decreasing the oxidative stress and holding the rod for longer time reducing the fall of time [14]. Kaempferol act as anti-oxidant and decreases the aggregation of beta amyloid and also reduces the age-related memory impairment and increases protective responses to oxidative stress and mitochondrial dysfunction. It is potential of protection of neurons against injuries induced by neurotoxins and promotion of learning and memory leading to extend the fall of time [15].

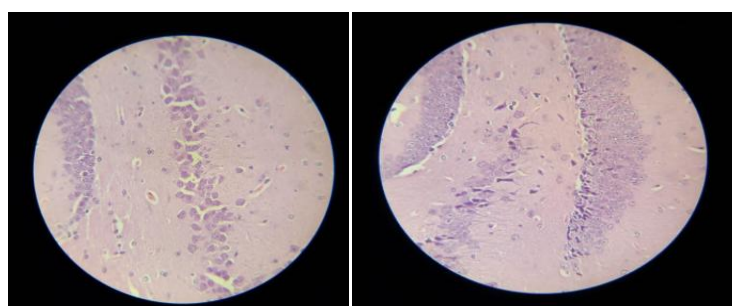
Vitamine-E (Tocopherol) prevent $A\beta_{1-42}$ induced protein oxidation, ROS production in hippocampus and frontal cortex leads to increase in the memory and neurotoxicity in primary rat embryonic hippocampal neuronal culture. Memory deficits and apoptosis which are likely to be seen in amnesia were prevented by vitamin E [16].

Donepezil binds reversibly to acetylcholinesterase and inhibits the hydrolysis of acetylcholine, thus increasing the availability of acetylcholine at the synapses, enhancing cholinergic transmission [17].

3.6 Histopathology Studies



GROUP I: Normal control GROUP II: Disease control GROUP III: EECQ (200 mg/kg, bd. wt, p.o)



GROUP IV: EECQ (400 mg/kg, bd. wt, p.o) GROUP V: Donepezil (1 mg/kg, bd. wt, p.o)

Fig. 4. Histopathology studies showing arrangement of different layers of pyramidal cells in all groups except in group II where apoptic cells are observed in hippocampus region

Group I: Control group – A compactly arranged 7-8 layer of pyramidal cells with prominent nucleus was observed in Hippocampus

Group II: Disease control group – Absence of pyramidal cells and presence of apoptotic cells was observed in Hippocampus

Group III: EECQ (200 mg/kg) - Irregular arrangement of 2-3 layers of pyramidal cells with scattered pattern and mild appearance of apoptotic cells was observed in Hippocampus

Group IV: EECQ (400 mg/kg) – Neuronal cells are well organized with 4-5 layers of pyramidal cells was observed in Hippocampus

Group V: Donepezil (1 mg/kg) - Neuronal cells are well organized with 6-7 layers of pyramidal cells and absence of apoptotic cells was observed in Hippocampus

3.7 Insilico Analysis

i) Molecular docking

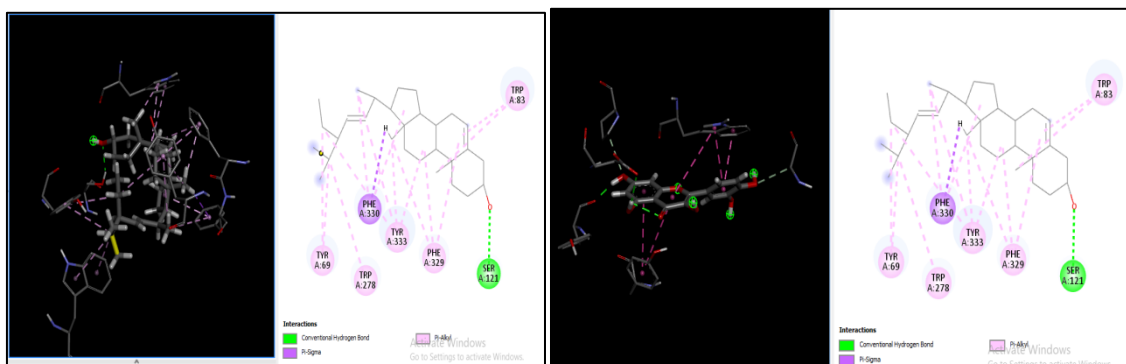
Molecular docking studies of isolated compounds obtained from GC-MS spectra of ethanolic extract of *Chenopodium quinoa* using Mcule software. The results of docking studies were given below in the Table 4.

Table 4. Docking scores of isolated compounds from *Chenopodium quinoa* using Mcule software

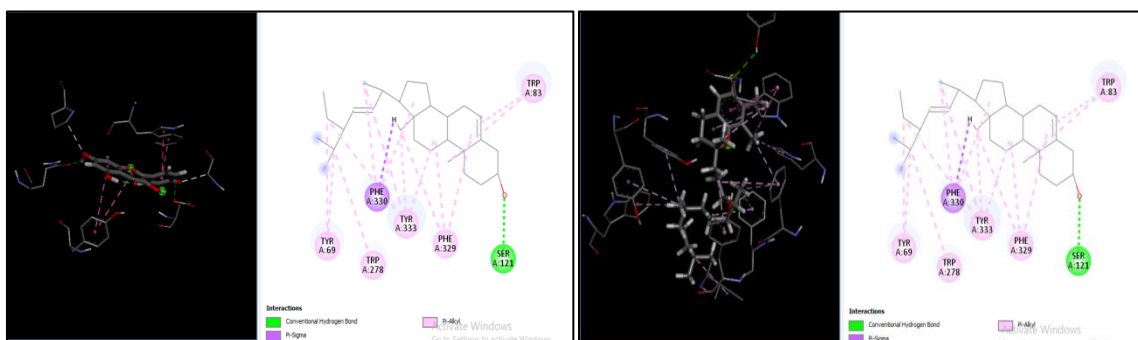
| Sl. No | Compounds | 1EVE | 2FY4 | 7CUM | 3EJ8 |
|--------|---|-------|------|------|------|
| 1 | Tetradecanoic acid/ Myristic acid | -6.5 | -4.6 | -5.2 | -5.0 |
| 2 | Hexadecanoic acid /Palmitic acid | -6.9 | -4.9 | -5.5 | -5.8 |
| 3 | Eicosadienoic acid | -7.5 | -5.1 | -6.3 | -7.0 |
| 4 | Pentadecanoic acid | -7.3 | -4.7 | -5.2 | -6.2 |
| 5 | Tocopherols | -9.9 | -6.5 | -7.5 | -8.1 |
| 6 | Stigmasterol | -11.9 | -6.4 | -8.4 | -9.6 |
| 7 | β -sitosterol | -9.1 | -- | -6.2 | -5.6 |
| 8 | Quercetin | -10.4 | -7.3 | -9.3 | -7.8 |
| 9 | Benzoic acid | -6.3 | -4.7 | -4.5 | -5.2 |
| 10 | Kaempferol | -10.2 | -4.6 | -8.5 | -7.5 |
| 11 | Arachidonic acid | -7.7 | -5.3 | -6.8 | -6.0 |
| 12 | 2-Bromotetrade canoic acid/2-Bromodecanoic acid | -6.5 | -4.6 | -5.2 | -8.1 |
| 13 | Donepezil | -10.9 | -7.6 | -8.8 | -8.7 |

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

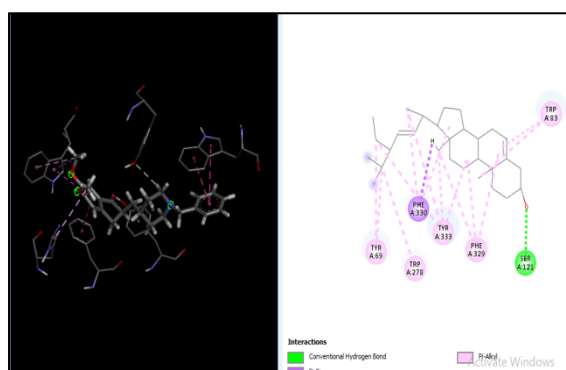
PDB ID: 1EVE



a) Stigmasterol – 11.9 b) Quercetin – 10.4

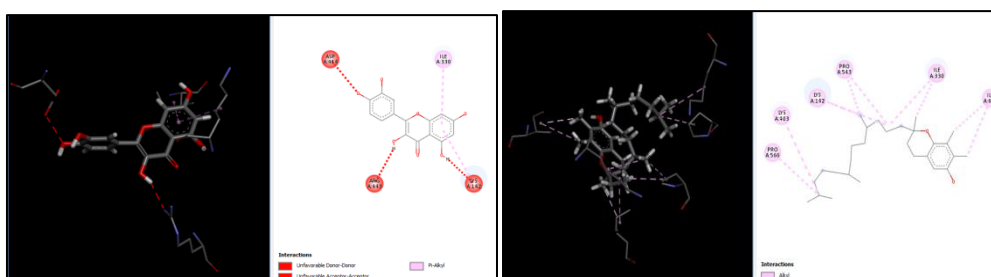


c) Kaempferol- 10.2 d) Tocopherol – 9.9

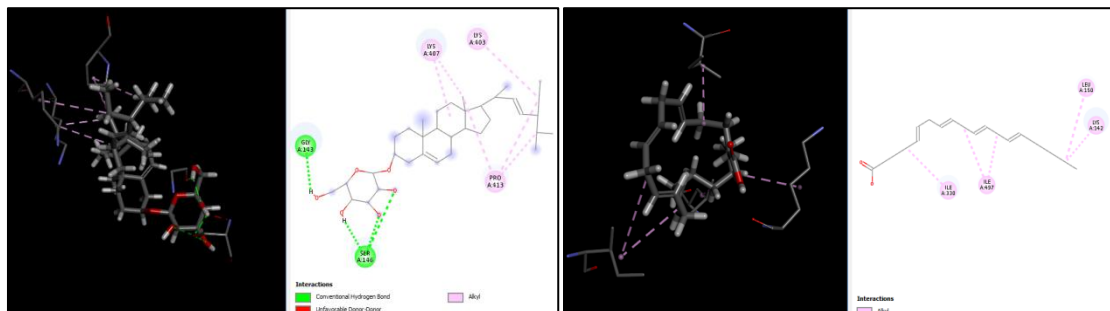


e) Donepezil-10.9

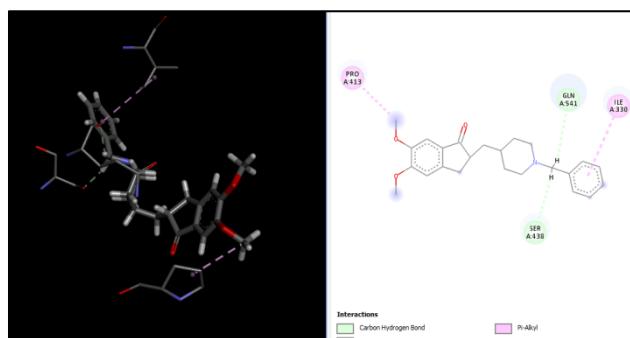
PDB ID: 2FY4



a) Quercetin -7.3 b) Tocopherol -6.5

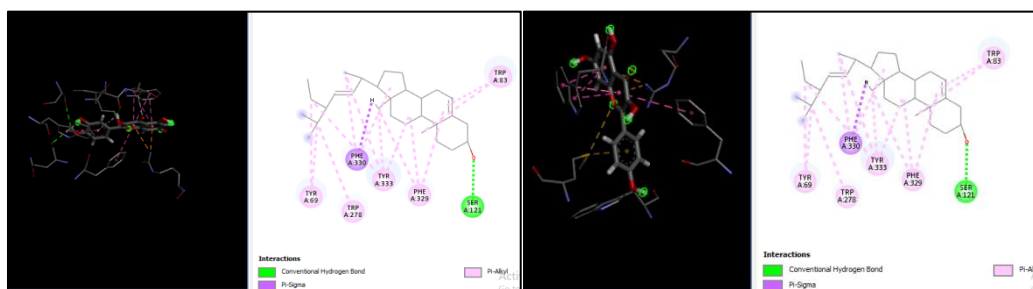


c) Stigmasterol -6.4 d) Arachidonic acid -5.3

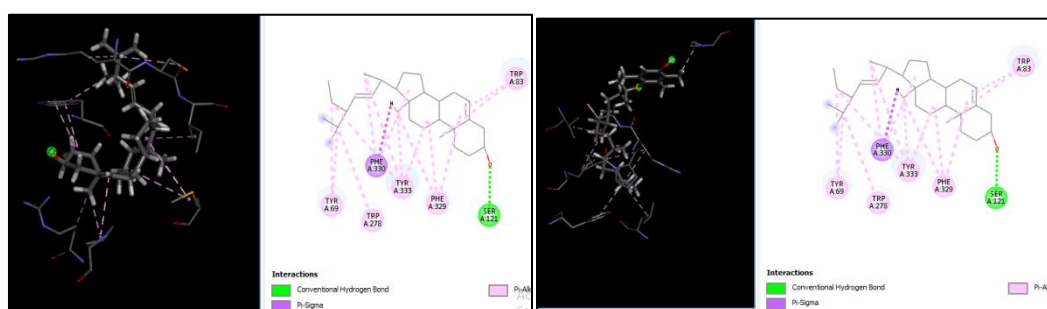


e) Donepezil -7.6

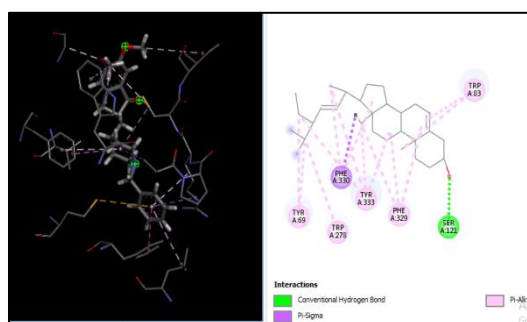
PDB ID:7CUM



a) Quercetin – 9.3 b) Kaempferol- 8.5

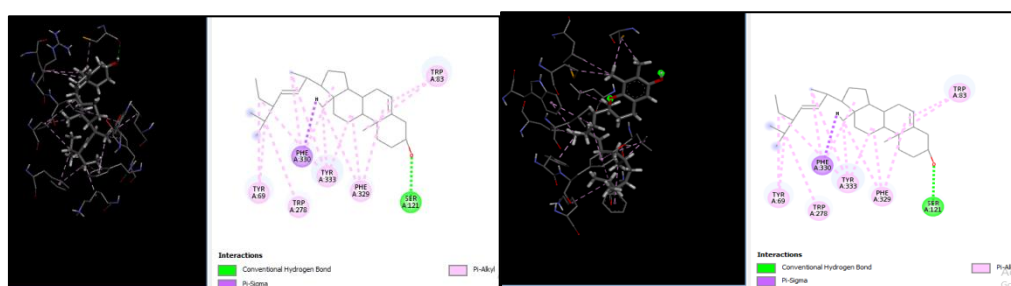


c) Stigmasterol – 8.4 d) Tocopherol – 7.5

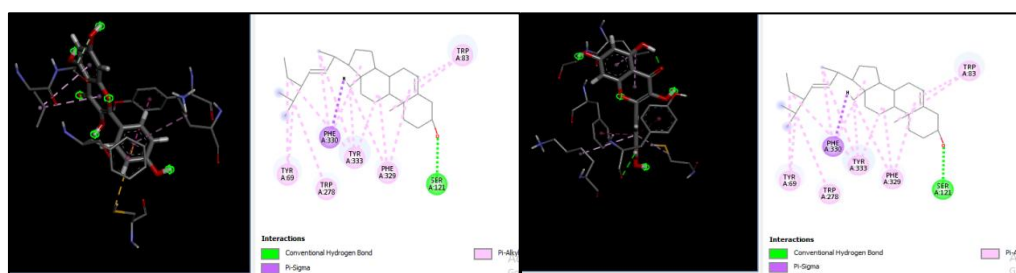


e) Donepezil-8.8

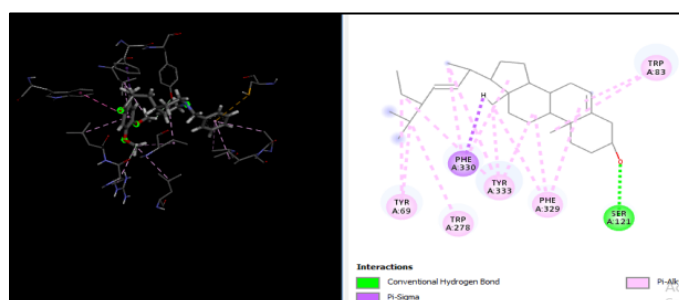
PDB ID:3EJ8



a) Stigmasterol – 9.6 b) Tocopherol – 8.1



c) Kaempferol- 7.5 d) Quercetin – 7.8



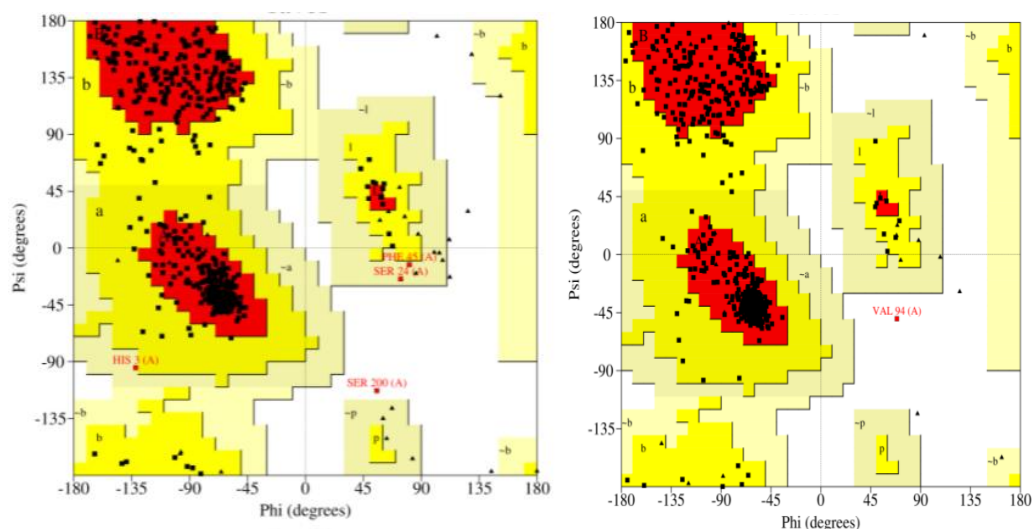
e) Donepezil- 8.7

ii) Ramachandran plot Analysis

Protein 1EVE, 2FY4, 7CUM and 3EJ8 were analysed for Ramachandran plot to know amino acid presence in different regions of respective protein tabulated in Table 5 and pictorial representation which is given in Fig. 5.

Table 5. Ramachandran plot status with protein with 1EVE, 2FY4, 7CUM and 3EJ8

| Residues | 1EVE | 2FY4 | 7CUM | 3EJ8 |
|--------------------------------|------|------|------|------|
| Most favourable region (%) | 87.3 | 91 | 83.3 | 87.4 |
| Additional allowed regions (%) | 11.8 | 8.8 | 15.9 | 12.2 |
| Generously allowed regions (%) | 0.7 | 0.0 | 0.3 | 0.4 |
| Disallowed regions (%) | 0.2 | 0.2 | 0.0 | 0.0 |



1EVE 2FY4

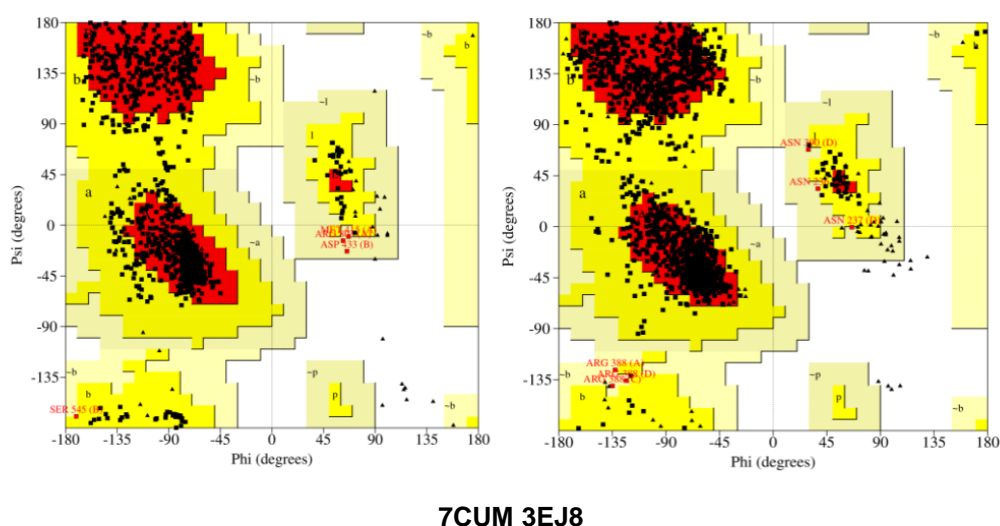


Fig. 5. Ramachandran plot of protein 1EVE, 2FY4, 7CUM and 3EJ8

A computational molecular-docking study is an essential method for the prediction of the binding capacity of active biological constituents against selected proteins. It continues to hold 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 donepezil were carried out using Mcule software. The various constituents identified in the plant extract are myristic acid, palmitic acid, eicosadienoic acid, pentadecanoic acid, tocopherols, stigmasterol, β -sitosterol, quercetin, benzoic acid, kaempferol, arachidonic acid, benzofuran, 2-bromodecanoic acid and standard donepezil were subjected to docking against PDB ID: 1EVE, 2FY4, 7CUM and 3EJ8. The order of rank for binding scores for PDB ID 1EVE: stigmasterol, donepezil, quercetin, kaempferol, tocopherols, β -sitosterol, eicosadienoic acid, arachidonic acid, pentadecanoic acid, palmitic acid, myristic acid, bromodecanoic acid, benzoic acid. The order of rank for binding scores for PDB ID 2FY4: donepezil, quercetin, tocopherol, stigmasterol, arachidonic acid, eicosadienoic acid, palmitic acid, benzoic acid, pentadecanoic acid, myristic acid, kaempferol, 2-bromodecanoic acid, and did not show any binding score with β -sitosterol. The order of rank for binding scores for PDB ID 7CUM: quercetin, donepezil, kaempferol, stigmasterol, tocopherol, arachidonic acid, eicosadienoic acid, β -sitosterol, palmitic acid, myristic acid, 2-bromodecanoic acid, pentadecanoic acid and benzoic acid. The order of rank for binding

scores for PDB ID 3EJ8: stigmasterol, donepezil, tocopherol, 2-bromodecanoic acid, quercetin, kaempferol, eicosadienoic acid, pentadecanoic acid, arachidonic acid, palmitic acid, β -sitosterol, benzoic acid and myristic acid. Overall the highest glide scores were observed with quercetin, kaempferol, myristic acid, palmitic acid, stigmasterol, pentadecanoic acid, tocopherols, arachidonic acid and standard donepezil against PDB ID: 1EVE, 2FY4, 7CUM and 3EJ8. 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 an anti-amnesic. The proteins identified namely PDB ID: 1EVE, 2FY4, 7CUM and 3EJ8 are modelled 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, 2FY4, 7CUM and 3EJ8 showed almost 80-90% favoured region which clearly indicates that the selected models in the present study are of good quality [18].

4. CONCLUSIONS

The interpretation of the present scientific research results reflect that the constituents of seed extract of *Chenopodium quinoa* possesses biologically active potential compounds with anti-amnesic activity. In addition, the outcome of the present research of the extract showed potential anti-amnesic activity that may be due to inhibition of Acetyl choline esterase, choline acetyltransferase, GABA and NOS (nitric oxide synthase). Thus, this contemporary research can offer pharmacological evidence for quinoa seeds and reveals strongly that the ethanolic seed extract of *Chenopodium quinoa* contains some active biomarkers that may be responsible for anti-amnesic activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical approval for the study was taken as per the IAEC and the approval number is GRCP/COL/170219887010/2020.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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