



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article


May 2024 Vol.:30, Issue:5

© All rights are reserved by Karthickeyan G et al.

## Evaluation of Anti Alzheimer Activity of Ethanolic Extract of Aerial Parts of *Justicia tranquebariensis* L.F Against Scopolamine Induced Memory Impairment in Swiss Albino Mice



ISSN 2349-7203



**Karthickeyan G<sup>1\*</sup>, Sakthi Abirami M<sup>2</sup>, Mythili Priya M<sup>3</sup>**

<sup>1</sup> Department of Pharmacology, Student of pharmacy, College of Pharmacy, Madras Medical College, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, India.

<sup>2</sup> Department of Pharmacology, Faculty of pharmacy, College of Pharmacy, Madras Medical College, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, India.

<sup>3</sup> Department of Pharmacology, Student of pharmacy, College of Pharmacy, Madras Medical College, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, India.

**Submitted:** 27 April 2024  
**Accepted:** 02 May 2024  
**Published:** 30 May 2024

**Keywords:** anti-alzheimer; acetylcholinesterase; scopolamine; Donepezil; ethanolic extract of aerial parts of *Justicia tranquebariensis* L.f

### ABSTRACT

This study aims to evaluate the *Invitro* and *invivo* anti-alzheimer activity of ethanolic extract of aerial parts of *Justicia tranquebariensis* L.f (EEJT) on scopolamine induced memory impairment in Swiss albino mice. Phytochemical screening of EEJT revealed the presence of sterols, flavonoids, quinones, saponins, proteins, alkaloids, coumarins and cardiac glycosides. *Invitro* anti-alzheimer activity of EEJT was evaluated by acetylcholinesterase inhibition assay exhibited good acetylcholinesterase inhibition activity. *Invivo* anti-alzheimer activity was evaluated by scopolamine induced memory impairment model. Group I received normal saline (1 ml/kg/day, 10 days, i.p.), Group II received scopolamine (1 mg/kg/day, 10 days, i.p), Group-III received donepezil (2 mg/kg/day 10 days, oral) + scopolamine (1 mg/kg/day, 10 days, i.p.), Group IV and Group V received 200 mg/kg and 400mg/kg of EEJT, 10 days, p.o. + Scopolamine (1 mg/kg/day, 10 days, i.p) respectively. After drug treatment, the anti-alzheimer activity of EEJT was assessed by behavioural, biochemical, histopathological examination. The behavioural parameters were assessed by Morris water maze and Y maze. The Malondialdehyde (MDA) level was assessed in biochemical parameter. Escape latency time was significantly reduced in the positive control group and the EEJT treated group. Spontaneous alteration percentage was significantly increased in the positive control and the EEJT treated group when compared with negative control group. MDA was significantly decreased in the positive control and EEJT treated groups. Histopathological report showed that the sections from the hippocampus showed normal neurons, neurofilaments showing mild degeneration and normal blood vessels in the groups treated with EEJT.



[ijppr.humanjournals.com](http://ijppr.humanjournals.com)

## 1. INTRODUCTION

Alzheimer's disease is characterized by progressive impairment of memory and cognitive functions and may lead to a completely vegetative state, resulting in massive socioeconomic disruption, and early death. Prevalence increases with age and may be as high as 20% in individuals over 85. Both familial and sporadic forms have been identified. Early onset of Alzheimer's disease is associated with several gene defects, including trisomy 21 (chromosome 21), a mutation of the gene for presenilin-1 on chromosome 14, and an abnormal allele,  $\epsilon 4$ , for the lipid-associated protein, ApoE, on chromosome 19. Unlike the normal form, ApoE  $\epsilon 2$ , the  $\epsilon 4$  form facilitates the formation of amyloid  $\beta$  deposits.<sup>(1)</sup> In India, 4 million people suffered from Alzheimer's disease and the number was estimated to reach almost 7.5 million by the end of 2030.<sup>(2)</sup>

Scopolamine works by blocking muscarinic receptors. that inhibits cholinergic neurotransmission, causing memory loss in mice. Scopolamine also causes oxidative damage. Polyphenols can protect the brain by crossing the blood-brain barrier and neutralizing free radicals, thus preventing memory impairment and nervous system. The primary roles of polyphenols enhancements in memory, the immune system and heart are incorporated. Injecting Scopolamine can be used to mimic cognitive deficits seen in AD, and treatment will focus on inhibiting acetylcholinesterase to restore cholinergic system activity.<sup>(3)</sup>

Donepezil, galantamine and rivastigmine are the only anticholinesterase agents approved by the US Food and Drug Administration for AD therapy till now. These drugs have been found to ameliorate the symptoms with improvement in the performance of AD patients; however, none of these drugs proved to be successful in restricting or reversing the development of the disease.<sup>(4)</sup>

The alcoholic extract of the aerial parts of *J. tranquebariensis* yielded the lignans, such as beta-Cubebin, lariciresinol, Medioresinol, lyoniresinol, isolariciresinol. The phytosterols such as brassicasterol, campesterol, stigmasterol, sitosterol and a sterol glucoside.  $\beta$ -sitosterol 3- O glucoside.<sup>(5)</sup> Lariciresinol and Isolariciresinol were proven to be anti-inflammatory and antioxidant activity.<sup>(6)</sup> Medioresinol significantly reduced the amount of amyloid precursor protein.<sup>(7)</sup> Lyoniresinol reveals antioxidative.<sup>(6)</sup> Phytosterols such as Stigmasterol, Sitosterol which are found to play a role in management of Alzheimer disease.

(8-12) Therefore, EEJT has been selected as the drug for studying its anti-alzheimer effects according to the analysis of literature review.

## 2. MATERIALS AND METHODS

### Procurement of *Justicia tranquebariensis* L.f. and extraction process

The aerial parts of *Justicia tranquebariensis* L.f were collected from Palayamkottai, Tamil Nadu, India and authenticated by a botanist Dr. S. Mutheeswaran. The standard drug Donepezil and the inducing agent Scopolamine were procured from Sri Krishna medicals, Chennai, Tamil Nadu, India and then aerial parts of *Justicia tranquebariensis* L.f were shade dried and made into a coarse powder, then EEJT was prepared using Soxhlet extraction.

### 2.1 PHYTOCHEMICAL ANALYSIS <sup>(13)</sup>

#### 2.1.1 Test for Carbohydrate

**a) Molisch's Test:** To the 0.5ml of sample, few drops of alcoholic alpha naphthol and 0.2ml of concentrated sulfuric acid were added slowly through the sides of the test tube. A purple to violet colour ring at the junction indicates the presence of Carbohydrate.

**b) Benedict' Test:** To 1ml of sample, few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) were added and boiled on water bath. The formation of a reddish-brown precipitate indicates the presence of Reducing Sugars.

**c) Fehling's Test:** To 1ml of sample, Fehling's solution A and B were added and heated for few minutes. The formation of a brick red precipitate indicates the presence of Carbohydrates.

#### 2.1.2 Test for Proteins and Amino acids

**a) Millon's Test:** The sample was treated with 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid). Appearance of a white precipitate which turns red upon gentle heating indicates the presence of Proteins.

**b) Biuret Test:** The sample was treated with 1ml of 10% sodium hydroxide, 1ml of 1% copper sulphate solution. Appearance of Violet colour indicates the presence of Proteins.

**c) Xanthoprotein Test:** The sample was treated with 2ml of con. nitric acid. Appearance of Orange colour indicates the presence of Proteins.

### 2.1.3 Test for Alkaloids

a) **Mayer's Test:** To 1ml of sample, Mayer's reagent [Potassium mercuric iodide solution] was added. Formation of Cream colour precipitate indicates the presence of Alkaloids.

b) **Dragendroff's Test:** To 1ml of sample, Dragendroff's reagent [Potassium bismuth iodide solution] was added. Formation of Reddish-brown precipitate indicates the presence of Alkaloids.

c) **Hager's Test:** To 1ml of sample, Hager's reagent [Saturated solution of Picric acid] was added. Formation of yellow colour precipitate indicates the presence of Alkaloids.

### 2.1.4 Test for Glycosides

a) **Legal's Test:** To 1ml of sample, few drops of pyridine and alkaline sodium nitroprusside solution were added. Appearance of blood red colour indicates the presence of Glycosides.

b) **Borntrager Test:** 1ml of sample was added with 0.5ml of dilute sulphuric acid and boiled for few minutes, then it was filtered. The filtrated sample was treated with ether or chloroform, to the organic layer few drops ammonia solution was added. Appearance of pink or violet colour indicates presence of glycosides.

### 2.1.5 Test for Cardiac Glycosides

**Keller killani Test [Test for Deoxy sugars]:** The sample was added with 0.4ml of glacial acetic acid containing a trace amount of ferric chloride. It was transferred to a small test tube and then 0.5ml of con. sulphuric acid was added carefully by the side of the test tube. Appearance of blue colour in the acetic layer indicates the presence of Cardiac Glycosides.

### 2.1.6 Test for phenolic compounds

**Ferric chloride Test:** The sample was treated with 1ml of water and boiled for few minutes then it was filtered. The filtrate was treated with ferric chloride solution. Appearance of bluish black colour indicates the presence of Phenolic compounds.

### 2.1.7 Test for Flavonoids

a) **Shinoda Test (Magnesium Hydrochloride reduction Test):** The sample was treated with few fragments of magnesium ribbon, then few drops of concentrated hydrochloric acid was added, Appearance of magenta colour indicates the presence of flavonoids.

**b) Alkaline reagent Test:** The sample was treated with 1ml of sodium hydroxide. Appearance of yellow colour indicates the presence of Flavonoids.

**c) Mineral acid Test:** The sample was treated with few drops of concentrated sulphuric acid. Appearance of orange colour indicates the presence of flavonoids.

#### **2.1.8 Test for Saponin**

**Foam froth Test:** The sample was treated with 10ml of water and boiled for few mins then it was filtered. The filtrate was shaken well and noted for the stable froth. A 1 cm layer of foam indicates the presence of Saponins.

#### **2.1.9 Test for Tannins**

**a) Gelatin Test:** The sample was treated with 2ml of 1% gelatin and 10% sodium chloride. Formation of a white precipitate indicates the presence of Tannins.

**b) Lead acetate Test:** To 2ml of sample, few drops of lead acetate solution were added. Formation of a white precipitate indicates presence of tannins.

#### **2.1.10 Test for Sterols**

**a) Salkowski Test:** The sample was treated with 0.3ml of chloroform with few drops of concentrated Sulphuric acid was added, shaken well and allowed to stand for some time. Appearance of red colour will appear at the lower layer indicates the presence of steroids, formation of yellow coloured lower layer will indicate the presence of Triterpenoids.

**b) Libermann Burchard Test:** The sample was treated with 2ml of chloroform, small amount of acetic anhydride and 1ml of con.Sulphuric acid. The colour change from red to bluish green indicates the presence of Sterols.

#### **2.1.11 Test for Terpenoids**

**Noller's Test:** Two or three granules or tin metal were dissolved in 2ml tinonyl chloride solution and added to 1ml of the extract and warmed. The formation of pink color indicates the presence of Terpenoids.

#### **2.1.12 Test for Fats and Fixed oils**

**Stain Test:** A small quantity of sample was pressed between two filter papers. The stain on the filter paper indicates the presence of fixed oils.

## 2.2 INVITRO EVALUATION <sup>(14)</sup>

Ethanollic extract of aerial parts of *Justicia tranquebariensis* L.f was subjected to acetylcholinesterase inhibition assay.

### PROCEDURE

The reaction mixture has a volume of 4.0 ml and is composed of 1.3 ml of Tris-HCl buffer (pH 8.0; 50 mM), 0.4 ml of ethanolic extract of *J. tranquebariensis* at varying doses (2 to 256 µg/ml), and 0.1 ml of AChE (0.28 U/ml). After adding 1.9 ml of 5,5'-dithiobis-(2-nitrobenzoic acid) DTNB (3 mM) solution and 0.3 ml of acetylthiocholine iodide (0.023 mg/ml) to the mixture, it was incubated for 15 minutes. After another 30 minutes of room temperature incubation, the final reaction mixture (4 ml) was measured for absorbance at 405 nm. The appropriate solvent was used in place of the medication to prepare the control. The appropriate solvent was used in place of the medication to prepare the control. To counteract the test drug's color impact, a blank was created by substituting the solvent for all of the reagents. Every assay determination was made in triplicate, and the findings were given as the standard error of the mean.

The percentage inhibition was calculated using the formula:

$$\% \text{ Acetylcholinestrse inhibition} = (A0 - A1) / A0 \times 100$$

Where,

A0 = Absorbance of the control, A1 = Absorbance of the standard/extract.

## 2.3 ACUTE ORAL TOXICITY STUDY <sup>(15)</sup>

Acute Oral toxicity was performed as per OECD guidelines No. 423 for the ethanolic extract of aerial parts of *Justicia tranquebariensis* by Hari V et al., (2021) The results show that the ethanol extracts of *Justicia tranquebariensis* L.f. are safe at a dose of 2,000mg/kg bw and no signs of toxicity was observed.

## 2.4 IN-VIVO STUDIES

### Experimental Animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no: 1917/GO/ReBi/S/16/CPCSEA/20.09.2021 and

3/AEL/IAEC/MMC, Date: 26/12/2023. 30 Swiss albino mice used for this study were procured from Animal house, Madras Medical College, Chennai, India. During the quarantine period, the animals are kept separate from those already in the facility while the animal's health and microbiological status are evaluated. The newly acquired Swiss albino mouse was quarantined for one week to reduce the risk of pathogens being introduced into established animals and to allow for psychological, physiological, and nutritional stabilization prior to use. The animal house was well-ventilated and maintained at a steady temperature and a relative humidity of 55-60%. The mice were housed in large polypropylene cages with paddy husk as bedding material, which was changed twice-weekly. The mice were kept on standard pellets and treated with purified water. The mice were fed ad libitum, except during fasting. All animal cages used in this study had correct identification i.e. labels and each animal in the cage had a picric acid mark on the tail for proper identification.

### ***In vivo* anti-Alzheimer activity (Scopolamine induced amnesic mice)**

#### **Grouping and induction of amnesia <sup>(16)</sup>**

Animals were acclimatized for 1 week and then randomly assigned into 5 groups, each bearing six animals.

Group I (G1) Normal control -saline (1 ml/kg/ day, 10 days, i.p.)

Group II (G2) Negative control - scopolamine (1 mg/kg/day, 10 days, i.p)

Group-III (G3) Positive control- donepezil (2 mg/kg/day 10 days, oral) + scopolamine (1 mg/kg/day, 10 days, i.p. )

Group IV (G4) Low dose (200 mg/kg, 10 days, p.o. ) + Scopolamine (1 mg/kg/day, 10 days, i.p)

Group V (G5) High dose (400mg/kg, 10 days, p.o. ) + Scopolamine (1 mg/kg/day, 10 days, i.p).

At the end of the study, behavioural parameters were evaluated. Instantly after performing behavioural tests, mice were sacrificed by cervical dislocation, and brains were isolated and further used for biochemical and histopathological examination.

#### 2.4.1 BEHAVIORAL ASSESSMENT DONE BY FOLLOWING METHODS

##### **Morris water maze** <sup>(16)</sup>

The morris water maze test involved a round maze measuring 122 cm in diameter and 51 cm in height, containing water and a circular platform made of acrylic with a diameter of 10 cm and a height of 35 cm. Underneath the water's surface at a depth of 1 cm, a platform with a diameter of approximately 10 cm was positioned. During the training sessions, the mouse was allowed to find its way to the platform underwater. Mice are allowed a maximum of 2 minutes to search for the submerged platform and can stay on it for approximately 15 seconds. The time taken to reach the platform, known as escape latency, was noted in every trial, with animals unable to find the platform within 120 seconds being placed on it. Animals were subjected to four daily sessions of trials.

##### **Y maze Test** <sup>(17)</sup>

Spontaneous alternation behavior in the Y-maze task was used to evaluate short-term memory. In this study, the Y-maze utilized has three arms that are 35 cm long, 25 cm high, and 10 cm wide, along with a central area shaped like an equilateral triangle. 30 minutes post drug administration; a mouse was positioned at the end of a maze arm and given 8 minutes to freely navigate through it. An arm entry was recorded when the hind paws of the mice were entirely inside the arm. Spontaneous alternation behavior is described as the act of moving into each of the three arms in consecutive selections. The spontaneous alternation behaviors will be the total number of arms entered minus two, and the percentage of spontaneous alternation will be determined.

$$\text{Actual alternations} / \text{Maximum alternations} \times 100.$$

Before testing the next animal, the maze was wiped clean using a cloth and a solution of 10% ethanol. The behavior of spontaneous alternation is seen as a reflection of spatial working memory, a type of short-term memory.

#### 2.4.2 BIOCHEMICAL STUDIES

##### **TISSUE PREPARATION**

Immediately after the behavioral tests, the mice were euthanized by cervical dislocation and their brains were swiftly extracted and placed in containers with frozen saline to solidify for 10 minutes. The brain was placed in a graduated cylinder and then PBS (pH 7.4) was added



to create a 10% homogenate. The supernatant for biochemical analysis was collected after centrifuging each tube at 10000 rpm/min at 4° C for 15 minutes.

### ESTIMATION OF MALONDIALDEHYDE <sup>(17)</sup>

The presence of lipid peroxidation was detected through the production of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) using the technique developed by Nieshus and Samuelsson in 1986. Approximately 0.1 ml of homogenate in Tris HCl buffer at pH 7.4 was mixed with 2 ml of TBA –TCA-HCL reagent in a 1:1:1 ratio (Thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA). The mixture was then incubated in a water bath for 15 minutes, followed by cooling and centrifugation at 1000 rpm for 10 minutes at room temperature. The clear supernatant's absorbance was recorded at 535 nm compared to a reference blank. The values are reported in nanomoles of MDA per minute per milligram of protein (nmoles/min/mg of protein).

### STATISTICAL ANALYSIS

The results were presented as mean ± SEM. Statistical analysis of the data was conducted through one-way ANOVA, then followed by Dunnett’s multiple comparison test using Graphic pad prism software version 8.0.2. One method employed was One-way ANOVA to determine the statistical variance among the variables. A statistically significant P value was considered at P<0.05, P<0.01, P<0.001, or P<0.0001.

## 3. RESULTS AND DISCUSSION

### 3.1 PERCENTAGE YIELD

Aerial parts of *Justicia tranquebariensis* L.f were shade dried and made into a coarse powder, then the ethanolic extract of *Justicia tranquebariensis* L.f was prepared using Soxhlet extraction and percentage yield has mentioned below,

**TABLE 1. PERCENTAGE YIELD OF EEJT**

S.NO	TYPE OF EXTRACT	YIELD VALUE (% w/w)
1.	Ethanolic extract	6.67

### 3.2 PHYTOCHEMICAL SCREENING

From the preliminary phytochemical screening, it was found that ethanolic extract of the aerial parts of *Justicia tranquebariensis* L.f showed the presence (+) and absence (-) of different secondary metabolites. The observation was listed below,

**TABLE 2. PHYTOCHEMICAL TESTS AND RESULTS**

S.NO	TEST	RESULTS
1	TEST FOR STEROLS	+
2	TEST FOR FLAVANOIDS	+
3	TEST FOR QUINONES	+
4	TEST FOR SAPONINS	+
5	TEST FOR PROTEINS	+
6	TEST FOR ALKALOIDS	+
7	TEST FOR COUMARINS	+
8	TEST FOR GLYCOSIDES	-
	1. Anthraquinone glycosides 2. Cardiac glycosides	+
9	TEST FOR CARBOHYDRATES	-
10	TEST FOR TANNINS AND PHLOBATANNINS	-

Based on the above table all the phytochemicals were present such as sterols, flavonoids, quinones, saponins, proteins, alkaloids, coumarins and cardiac glycosides. Then the absence of certain phytochemicals are anthraquinone glycosides, carbohydrates, tanins and phlobatanins.

### 3.3 INVITRO STUDY

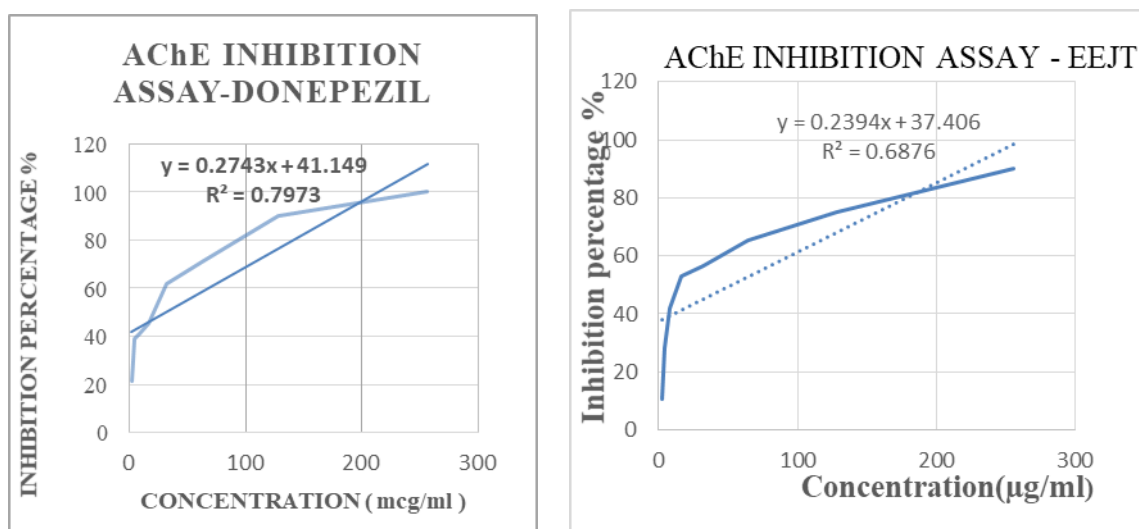
#### *Invitro* enzyme inhibitory activity of ethanolic extract of aerial parts of *Justicia tranquebariensis L.f* against Acetylcholinesterase enzyme ( AChE )

The ethanolic extract of aerial parts of *Justicia tranquebariensis L.f* was evaluated for acetylcholinesterase inhibitory activity. The absorbance of the extract decreased upon increase in the concentration in a dose dependent manner, which shows enzyme inhibition. The percentage inhibition was calculated for ethanolic extract of aerial parts of *Justicia tranquebariensis L.f* and the standard drug Donepezil are tabulated below.

The percentage inhibition of Donepezil ranges from 21.34% to 100.18% for a concentration of 2 to 256 µg/ml respectively and IC<sub>50</sub> was found to be 32.26µg/ml. The percentage inhibition of ethanolic extract of aerial parts of *Justicia tranquebariensis L.f* ranges from 10.52% to 89.98% and IC<sub>50</sub> was found to be 52.61µg/ml. From the IC<sub>50</sub> value ethanolic extract of *Justicia tranquebariensis L.f* exhibited good inhibitory activity when compared to the standard drug Donepezil which shows great inhibitory activity against AChE.

**Table 3: INVITRO ENZYME INHIBITORY ACTIVITY OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *Justicia tranquebariensis L.f* AGAINST AChE**

Concentration (µg/ml )	Percentage Inhibition ( % )	
	Standard ( Donepezil )	<i>Justicia tranquebariensis L.f</i>
2	21.34	11.51
4	38.71	30.13
8	40.43	43.12
16	45.31	55.18
32	61.74	57.6
64	71.4	66.6
128	89.97	76.3
256	100.18	89.95
IC <sub>50</sub> (µg/ml)	32.26	47.52



**FIGURE 1. GRAPHICAL REPRESENTATION OF AChE INHIBITION ASSAY FOR STANDARD DRUG – DONEPEZIL AND AERIAL PARTS OF EEJT**

**3.4 Acute toxicity study <sup>(40)</sup>**

The results show that the administration of *Justicia tranquebariensis L.f.* was safe up to a dose of 2000 mg/kg. No toxic symptoms or mortality were observed at this dose.

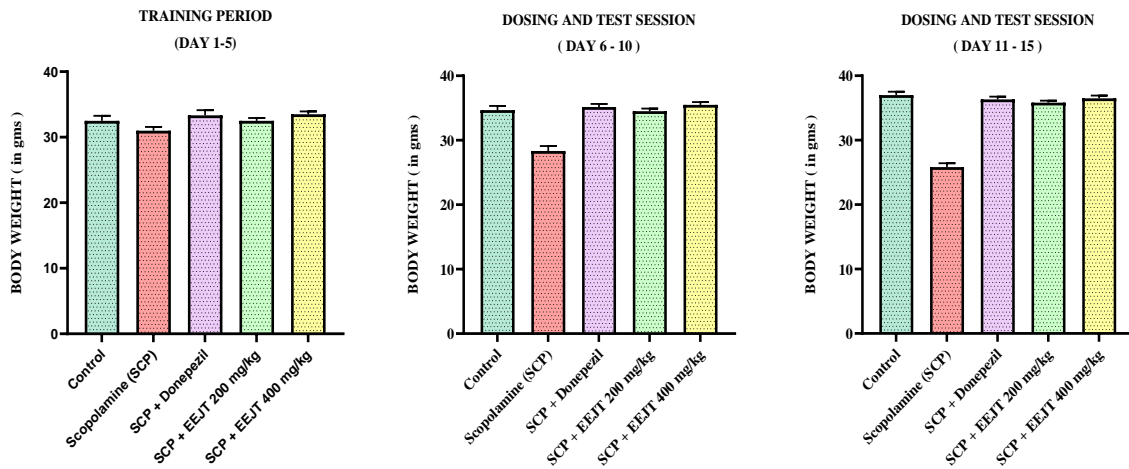
**3.5 INVIVO STUDY**

**TABLE 4. EFFECT OF AERIAL PARTS OF EEJT ON CHANGES IN BODY WEIGHT OF SWISS ALBINO MICE**

The body weight of the animals was taken for every 5 days interval throughout the experiment period.

Groups	Induction/ Drug treatment	Body weight ( g )		
		Non drug treatment period ( day 1 – 5 )	Dosing & treatment period	
			( DAY 6 – 10 )	( DAY 11 – 15 )
Group 1	Normal control Saline (1 ml/kg)	32.5 ± 0.76	34.7 ± 0.67	37 ± 0.51
Group 2	Negative control Scopolamine (SCP) (1 mg/kg)	31 ± 0.58	28.3 ± 0.76	25.8 ± 0.60
Group 3	Positive control SCP + Donepezil (2 mg/kg)	33.3 ± 0.80	35.1 ± 0.48	36.33 ± 0.42
Group 4	Low dose group SCP + EEJT (200 mg/kg)	32.5±0.42	34.5±0.42	35.83 ± 0.30
Group 5	High dose group SCP + EEJT (400 mg/kg)	33.5±0.42	35.5±0.42	36.5 ± 0.42

All values are expressed in Mean  $\pm$  SEM, n = 6



**FIGURE 2. GRAPHICAL REPRESENTATION OF EFFECT OF AERIAL PARTS OF EEJT ON BODY WEIGHT OF MICE**

As shown in **table 4**, Body weight changes were observed on Non – drug treatment period ( Day 1 – 5 ) and of dosing and treatment session (Day 6 - 15). There was a marginal change in body weight throughout the experiment for Normal control group. There was decrease in body weight of negative control group mice. Animals treated with EEJT 200mg/kg and 400 mg/kg showed that body weight was maintained for low dose group and there is slight increase in body weight of mice in high dose group.

**BEHAVIORAL PARAMETERS**

**TABLE 5: EFFECT OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *JUSTICIA TRANQUEBARIENSIS L.F* ON ESCAPE LATENCY TIME (SEC) IN MORRIS WATER MAZE**

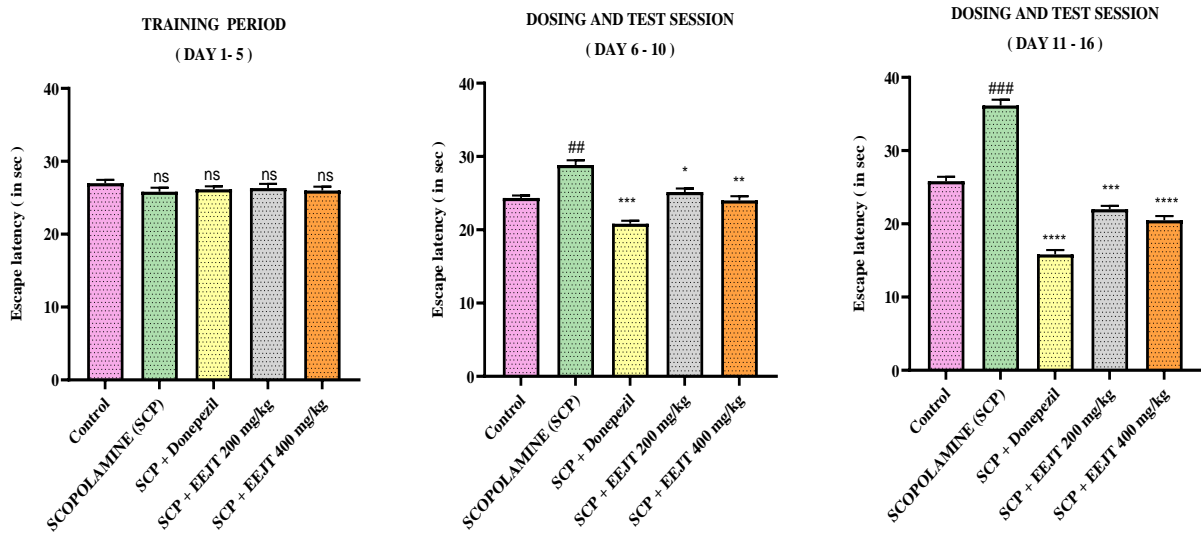
Groups	Induction/ Drug Treatment	Escape Latency Time ( Seconds )		
		Training Period ( Day 1 – 5 )	Dosing & Treatment Period	
			( DAY 6 – 10 )	( DAY 11 – 15 )
Group 1	Normal control Saline (1 ml/kg)	27 ± 0.45 <sup>ns</sup>	24.33 ± 0.33	25.83±0.60
Group 2	Negative control Scopolamine (SCP) (1 mg/kg)	25.83 ± 0.54 <sup>ns</sup>	28.83 ± 0.65 <sup>##</sup>	36.16±0.79 <sup>###</sup>
Group 3	Positive control SCP + Donepezil (2 mg/kg)	26.17 ± 0.40 <sup>ns</sup>	20.83 ± 0.40 <sup>***</sup>	15.83±0.60 <sup>****</sup>
Group 4	Low dose SCP + EEJT (200 mg/kg)	26.33±0.55 <sup>ns</sup>	24.33±0.67 <sup>*</sup>	22±0.44 <sup>***</sup>
Group 5	High dose SCP + EEJT (400 mg/kg)	26±0.51 <sup>ns</sup>	22.66±0.61 <sup>**</sup>	20.5±0.56 <sup>****</sup>

All values are expressed in Mean ± SEM, n = 6

Data were analysed by one-way ANOVA followed by Dunnett’s multiple comparison test.

\*\*\*\*p<0.0001, \*\*\*p<0.001, \*\*p<0.01, \*p>0.05 compared with Negative control.

###p<0.001, ##p<0.01 compared with Normal control and <sup>ns</sup>p>0.05 denotes non significant.



**FIGURE 3. GRAPHICAL REPRESENTATION OF EFFECT OF AERIAL PARTS OF EEJT ON ESCAPE LATENCY (SEC)**

As shown in **table 5**, Escape latency time (in seconds) was taken as a parameter in Morris water maze during a period of training session (day 1 -5) and dosing and treatment session (day 6 – 15). There was no significant ( $^{ns}p>0.05$ ) difference in escape latency time among all groups during training period (day1 –5). The escape latency time was significantly ( $^{###}p<0.001$ ) increased for negative control group when compared to Normal group. Positive control group also showed significant ( $^{****}p<0.0001$ ) decrease in escape latency time when compared with Negative control group. The animals treated with EEJT 200mg/kg and 400 mg/kg showed significant ( $^{***}p<0.001$ ,  $^{****}p<0.0001$ ) decrease in escape latency time when compared with Negative control group.

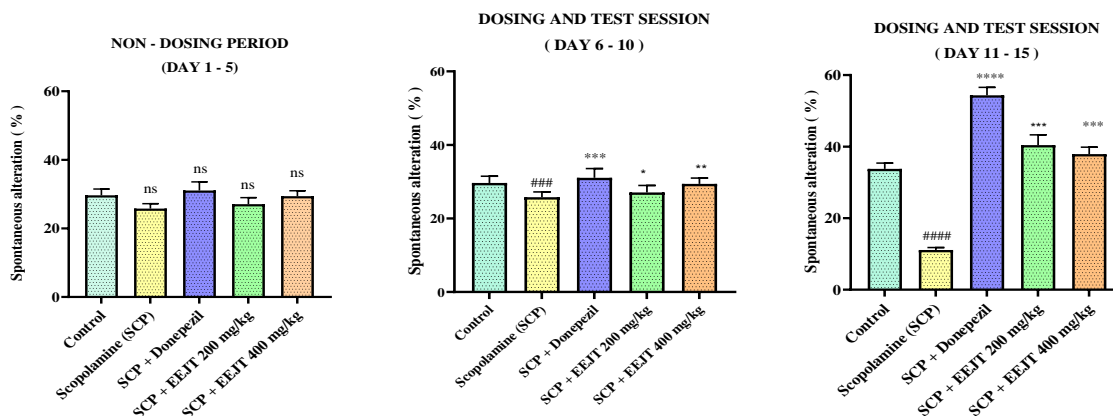
**TABLE 6: EFFECT OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *J. TRANQUEBARIENSIS* L.F ON SPONTANEOUS ALTERATION ( % ) IN Y MAZE**

Groups	Induction/ Drug treatment	Spontaneous alteration ( % )		
		Non Drug Treatment Period ( Day 1 – 5 )	Dosing & Treatment Period	
			( Day 6 – 10 )	( Day 11 – 15 )
Group 1	Normal control Saline (1 ml/kg)	29.67 ± 1.81 <sup>ns</sup>	35.14 ± 1.86	33.79 ± 1.61
Group 2	Negative control Scopolamine (SCP) (1 mg/kg)	25.77 ± 1.45 <sup>ns</sup>	16.56 ± 0.76 <sup>###</sup>	11.09 ± 0.69 <sup>####</sup>
Group 3	Positive control SCP + Donepezil (2 mg/kg)	31.06 ± 2.48 <sup>ns</sup>	41.21 ± 2.12 <sup>***</sup>	54.35 ± 2.22 <sup>****</sup>
Group 4	Low dose group SCP + EEJT (200 mg/kg)	27.08 ± 1.88 <sup>ns</sup>	31.87 ± 2.73 <sup>*</sup>	40.43 ± 2.87 <sup>***</sup>
Group 5	High dose group SCP + EEJT (400 mg/kg)	29.42 ± 1.54 <sup>ns</sup>	34.13 ± 1.53 <sup>**</sup>	37.90 ± 1.94 <sup>***</sup>

All values are expressed in Mean ± SEM, n = 6

Data were analysed by one-way ANOVA followed by Dunnett’s multiple comparison test. \*\*\*\*p<0.0001, \*\*\*p<0.001, \*\*p<0.01, \*p>0.05 compared with Negative control. ###p<0.001, ####p<0.0001 compared with Normal control and <sup>ns</sup>p>0.05 denotes non significant.





**FIGURE 4. GRAPHICAL REPRESENTATION OF EFFECT OF EEJT ON SPONTANEOUS ALTERATION ( % ) IN Y MAZE**

As shown in **table 6**, Spontaneous alternation in Y maze was observed on Non- drug treatment period ( Day 1 -5 ), Dosing and test session ( Day 6 – 15 ). There was no significant ( $^{ns}P>0.05$ ) difference in spontaneous alternation percentage among all the groups during non- drug treatment period. The spontaneous alternation percentage was significantly ( $^{####}p<0.00001$ ) decreased in Negative control group when compared to normal control group. Spontaneous alternation percentage was also significantly ( $^{****}p<0.0001$ ) increased in positive control group when compared with negative control group. The animals treated with EEJT 200mg/kg and 400mg/kg showed significant ( $^{***}p<0.001$ ,  $^{***}p<0.001$ ) increase in spontaneous alternation percentage when compared with Negative control group.

**BIOCHEMICAL ESTIMATION**

Effect of ethanolic extract of aerial parts of *Justicia tranquebariensis* L.f on malondialdehyde level.

**TABLE 7: THE BIOCHEMICAL ESTIMATION OF MALONDIALDEHYDE IN HOMOGENISED MICE BRAIN**

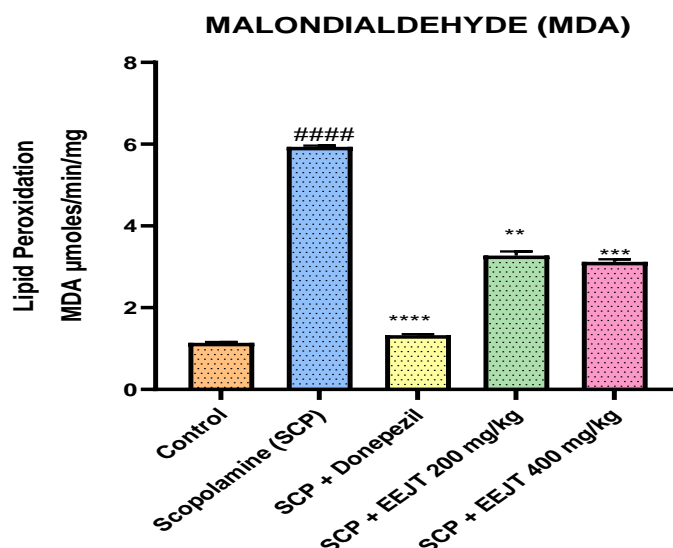
Groups	Induction/ Drug treatment	Malondialdehyde (nmoles/min/mg)
Group 1	Normal control Saline (1 ml/kg)	1.14 ± 0.20
Group 2	Negative control Scopolamine (SCP) (1 mg/kg)	5.94 ± 0.03####
Group 3	Positive control SCP + Donepezil (2 mg/kg)	1.33 ± 0.020****
Group 4	Low dose group SCP + EEJT (200 mg/kg)	3.27 ± 0.099**
Group 5	High dose group SCP + EEJT (400 mg/kg)	3.12 ± 0.060***

All values are expressed in Mean ± SEM, n = 6

Data were analysed by one-way ANOVA followed by Dunnett's multiple comparison test.

\*\*\*\*p<0.0001, \*\*\*p<0.001, \*\*p<0.01 compared with Negative control.

####p<0.0001 compared with Normal control and <sup>ns</sup> p>0.05 denotes non significant.



**FIGURE 5. GRAPHICAL REPRESENTATION OF EFFECT OF EEJT ON MALONDIALDEHYDE LEVEL**

As shown in **table 7**. Malondialdehyde amount was significantly increased (#### $p < 0.0001$ ) in Negative control group (Scopolamine 1 mg/kg). Groups treated with ethanolic extract of aerial parts of *Justicia tranquebariensis* L.f 200mg/kg and 100mg/kg showed significant (\*\* $p < 0.001$ , \*\*\* $p < 0.0001$ ) decrease in malondialdehyde levels compared to negative control group. The malondialdehyde was also significantly (\*\*\*\* $p < 0.0001$ ) decreased in Positive control (Donepezil 2 mg/kg) group when compared to negative control group.

### HISTOPATHOLOGICAL STUDIES

Effect of ethanolic extract of aerial parts of *Justicia tranquebariensis* L.f on histopathology of hippocampus. Sections from hippocampus showed normal neurons(N), neurofilaments(NF) with glial cells and normal blood vessels(BV) to the normal control (**fig no. 6**). Sections from hippocampus showed degenerative changes in the neurons (N\*) and reduction in neuronal count, neurofilament (NF) is highly fibrillary and there is mild vascular proliferation (BV) in negative control (**fig no.7**). Sections from hippocampus showed normal neurons(N), neurofilaments (NF) with glial cells and normal blood vessels (BV) in positive control (**fig no. 8**). Sections from hippocampus showed normal neurons(N), neurofilaments (NF) with glial cells and normal blood vessels (BV) in low dose of EEJT (**fig no. 9**). Sections from hippocampus showed normal neurons(N), neurofilaments(NF) with glial cells and normal blood vessels(BV) in high dose of EEJT (**fig no. 10**).

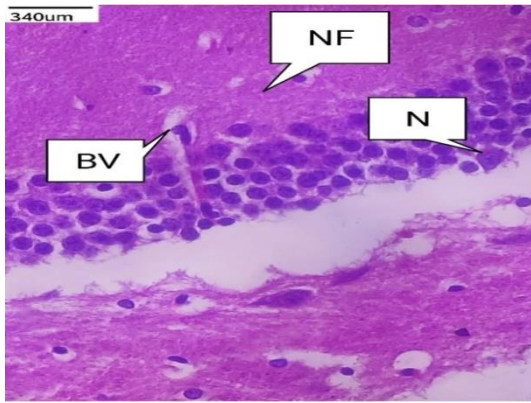


FIGURE 6. NORMAL CONTROL

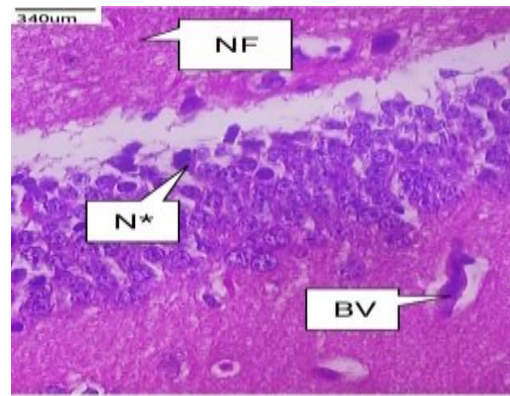


FIGURE 7. NEGATIVE CONTROL

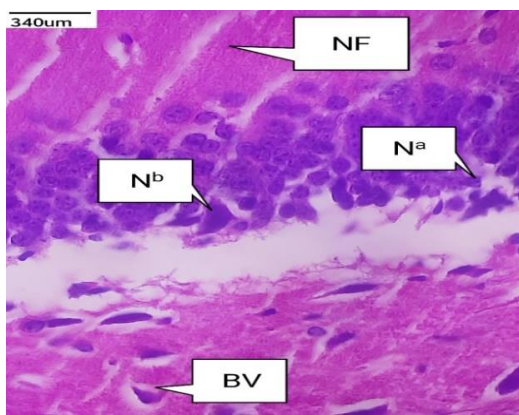


FIGURE 8. POSITIVE CONTROL

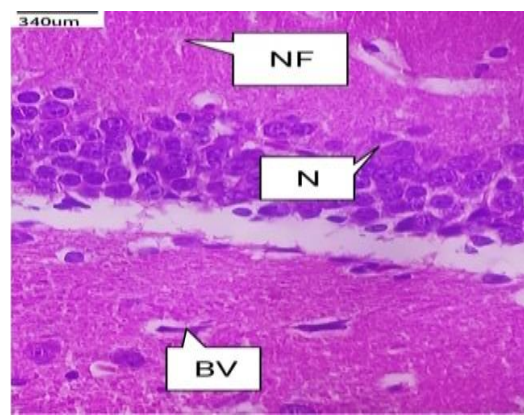


FIGURE 9. LOW DOSE GROUP OF  
EEJT

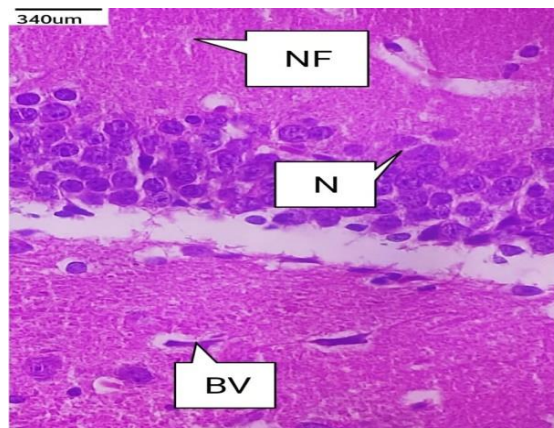


FIGURE 10. HIGH DOSE GROUP OF EEJT

## CONCLUSION

*Justicia tranquebariensis* L.f has been chosen as the plant to perform the research on its anti-alzheimer activity based on the literature review. Aerial parts of *Justicia tranquebariensis* L.f has been dried, powdered and major sterols extracted with ethanol on basis of literature

review. All the phytochemicals were present such as sterols, flavonoids, quinones, saponins, proteins, alkaloids, coumarins and cardiac glycosides. Presence of flavonoid has good antioxidant property thus aids in scavenging reactive oxygen species and sterols play a role in management of Alzheimer disease. The *In vivo* study was performed with five groups of Male Swiss albino mice (six in each group) to assess the anti-alzheimer activity of EEJT. Donepezil was used as standard. Scopolamine was used as an inducing agent for all groups except control group. Various parameters were evaluated such as behavioural assessment, biochemical estimation and histopathological examination. Behavioural parameters were assayed using Morris water maze and Y maze which shows significant decrease in the escape latency time and increase in the spontaneous alteration percentage respectively when compared with negative control group. Biochemical estimation of malondialdehyde showed that the homogenized mice brain of low (200 mg/kg) and high dose group (400 mg/kg) was significantly decreased when compared with negative control group. Histopathological examination of hippocampal section of mice brain of Low dose group showed mild degeneration when compared to high dose (400 mg/kg) group which shows normal neurons, neurofilaments(NF) with glial cells and normal blood vessels(BV). Results have shown that the treatment with EEJT has been effective in improving memory impairment.

#### **ACKNOWLEDGEMENT**

I would like to express my heartfelt gratitude to God for guiding and supporting me throughout the completion of my project. I am immensely grateful to my parents, Mr. Ganesan A and Mrs. Valarmathi G for their unwavering love, unwavering support and unwavering belief in me. I owe my deep gratitude to my project guide Dr. M. Sakthi Abirami, M. Pharm., Ph.D., Assistant Professor, Department of Pharmacology, Madras Medical College, Chennai 03. I would like to express my heartfelt gratitude to my guide for the unwavering support and guidance provided throughout my project. I wholeheartedly extend my gratitude to my friends for their support and kind help during my project work at every stage.

**Conflict of interest statement:** The authors declared no conflict of interest.

#### **REFERENCES**

1. H. Gerhard Vogel Drug discovery and evaluation- Pharmacological assays. 2<sup>nd</sup> edition. Pg. 877-879.
2. Bennett PN, Brown MJ, Pankaj Sharma. Clinical pharmacology. 2012. Pg. 344

3. Islam MA, Zaman S, Biswas K, Al-Amin MY, Hasan MK, Alam AH, Tanaka T, Sadik G. Evaluation of cholinesterase inhibitory and antioxidant activity of *Wedelia chinensis* and isolation of apigenin as an active compound. *BMC complementary medicine and therapies*. 2021 Dec;21:1-2.
4. Yadang FSA, Ngezeye Y, Kom CW, Betote PHD, Mamat A, Tchokouaha LRY, Taiwé GS, Agbor GA, Bum EN. Scopolamine-Induced Memory Impairment in Mice: Neuroprotective Effects of *Carissa edulis* (Forssk.) Valh (Apocynaceae) Aqueous Extract. *Int J Alzheimers Dis*. 2020 Aug 31;2020:6372059.
5. The wealth of India – A dictionary of Indian Raw Material and Industrial products, Volume IV.
6. Saritha B, Brindha P. Review on Ethnopharmacognosy of *Justicia tranquebariensis* L.–A Traditional Siddha Drug. *International Journal of PharmTech Research*. 2013;5(2):467-74.
7. Kang YJ, Seo DG, Park SY. Phenylpropanoids from cinnamon bark reduced  $\beta$ -amyloid production by the inhibition of  $\beta$ -secretase in Chinese hamster ovarian cells stably expressing amyloid precursor protein. *Nutrition Research*. 2016 Nov 1;36(11):1277-84.
8. Ayaz M, Junaid M, Ullah F, Subhan F, Sadiq A, Ali G, Ovais M, Shahid M, Ahmad A, Wadood A, El-Shazly M. Anti-Alzheimer's studies on  $\beta$ -sitosterol isolated from *Polygonum hydropiper* L. *Frontiers in pharmacology*. 2017 Oct 6;8:697.
9. Pratiwi R, Nantasenamat C, Ruankham W, Suwanjang W, Prachayasittikul V, Prachayasittikul S, Phopin K. Mechanisms and neuroprotective activities of stigmasterol against oxidative stress-induced neuronal cell death via sirtuin family. *Frontiers in Nutrition*. 2021 May 12;8:648995.
10. Saeidnia S, Manayi A, Gohari AR, Abdollahi M. The story of beta-sitosterol-a review. *European journal of medicinal plants*. 2014 May 1;4(5):590.
11. Sharma N, Tan MA, An SS. Phytosterols: Potential metabolic modulators in neurodegenerative diseases. *International Journal of Molecular Sciences*. 2021 Nov 12;22(22):12255.
12. Shuang R, Rui X, Wenfang L. Phytosterols and dementia. *Plant foods for human nutrition*. 2016 Dec;71:347-54.
13. C.K. Kokate., *Pharmacognosy*, 55<sup>th</sup> edition., Nirali Prakash Publication., 2021
14. Ferreira J, Santos S, Pereira H. In vitro screening for acetylcholinesterase inhibition and antioxidant activity of *Quercus suber* cork and corkback extracts. *Evidence-Based Complementary and Alternative Medicine*. 2020 Jul 20;2020.
15. HARI V, JOTHIESWARI D, MAHESWARAMMA KS. Evaluation Of Antitumor Activity Of *Justicia Tranquebariensis* Lf. *Nveo-Natural Volatiles & Essential Oils Journal| NVEO*. 2021:5038-48.
16. Patel C, Pande S, Acharya S. Potentiation of anti-Alzheimer activity of curcumin by probiotic *Lactobacillus rhamnosus* UBLR-58 against scopolamine-induced memory impairment in mice. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2020 Oct;393(10):1955-62.
17. Lalitha V. Anti-Alzheimer's And Anti-Oxidant Activity of *Cyamopsis tetragonoloba* Fruit Extract in Mice. *Sch Acad J Pharm*. 2021 Oct;10:154-62.