IJPSR (2022), Volume 13, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 July 2021; received in revised form, 06 August 2021; accepted, 07 August 2021; published 01 April 2022

EVALUATION OF ANTI-ALZHEIMER'S ACTIVITY OF ALCOHOLIC EXTRACT OF *CISSUS QUADRANGULARIS* STEMS IN WISTAR ALBINO RATS

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Keywords:

Alzheimer's disease, Dementia, *Cissus quadrangularis*, Scopolamine, Anti-oxidants.

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ABSTRACT: Alzheimer's disease is the most frequent cause of dementia worldwide and its incidence is increasing due to the world's aging population. Alzheimer's disease (AD) is one of the greatest medical care challenges of our century and is the main cause of dementia and neurodegenerative diseases. Dementia is defined as an acquired loss of cognition in many cognitive domains. Herbal medicine has evolved as the greatest option for the treatment of Alzheimer's disease due to its wide availability, low cost, high patient compliance, ease of formulation and lack of harmful side effects. Novel techniques can be used for the development of herbal medicine. In these research article animal studies, has been carried out to known Alzheimer disease (AD) caused by chemical compounds may be helpful in deeper understanding the mechanism of disease and AD treatment. In the present study, the efficacy of an ethanolic extract of *Cissus quadrangularis* stems (EECQ) was evaluated against scopolamine-induced Alzheimer's in the Wistar albino rat. As *Cissus quadrangularis* stems contain many active chemical constituents, including phenolic compounds, flavonoids, phytosterols, triterpenoids, Vitamin C, Tannins, and alkaloids, *etc*., serve as useful antioxidants are present in this plant. The *Cissus quadrangularis* stems were reported to have antioxidant properties. The stem of the *Cissus quadrangularis* contains carotene mainly β-carotene, which is responsible for showing the anti-Alzheimer's activity. The antioxidant potential is the best supplement for the diseases associated with oxidative stress. Hence, an attempt was made to evaluate the anti-Alzheimer activity from *Cissus quadrangularis* stems to extract.

INTRODUCTION:

Alzheimer's Disease: Dr. Alois Alzheimer, in 1906 he recognized the intellect of a woman who misplaced her life due to certain peculiar symptoms such as memory loss, unpredictable actions, cognitive disability and then concluded that the loss of life was once due to the presence of neuritic plaques and neurofibrillary tangles [1]. In the aged population, it has evolved into a predominant neurodegenerative sickness.

The amyloid precursor protein by-product β-amyloid protein, an aspect of healthy nerve cells, deteriorates and contributes to the development of neutric plaques, also known as senile, dendritic, or amyloid plaques. Nerve cells, alongside several different factors it consists of twisted protein fibers placed inside nerve cells. These fibers consist of a protein known AS tau, which generally takes place in neurons.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(4).1657-69

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(4).1657-69

When inappropriately processed, Tau molecules clump together to form Neurofibrillary tangles. This ailment may also be related to intelligence infection, plaque formation being one of the different immoderate in older persons or abnormal in some different way in individuals who eventually enhance Alzheimer's disorder [1]. Herbal remedy for AD has greater benefits when in contrast to presently current drug healing procedures with unavoidable aspect outcomes, it can additionally enhance the patients' exceptional of lifestyles as they can be bump off as Nutraceuticals and even any mild increase in dose may also no longer be trouble when consumed [2]. The single most productive supply of leads for drug improvement has been Ayurvedic medicinal plant life and over one hundred new products are already in scientific development. Indeed, numerous scientific research has described the use of a variety of Ayurvedic medicinal vegetation and their components for the treatment of Alzheimer's disease, phytochemical research of the specific components of the vegetation have proven the presence of many precious compounds, such as lignans, flavonoids, tannins, alkaloids and so on, that exhibit a broad spectrum of pharmacological activities. This includes anti-inflammatory, anti-amyloidogenic, anti-cholinesterase, antioxidant, and hypolipidemic effects. Herbal medication affords various alternatives to alter the growth and signs of AD [4]. This review will center attention on the most current research findings related to the possibility of a wide variety of medicinal plant life in the management of AD [3]. This neuro-degenerative disease process is characterized classically by two hallmark pathologies: β-amyloid plaque deposition and neurofibrillary tangles of hyperphosphorylated tau [2]. A component of healthy nerve cells, Amyloid precursor protein derivative β- Amyloid protein deteriorates and leads to the formation of Neutric plaques, they are also called as senile, dendritic or amyloid plaques. Nerve cells, along with various other components it consists of twisted protein fibers positioned within nerve cells. These fibers consist of a protein called tau, which normally occurs in neurons. When incorrectly processed, tau molecules clump together to form Neurofibrillary tangles [4]. The histological hallmarks of Alzheimer's disease include senile plaques (SPs) and neurofibrillary tangles (NFTs) [1]. In this review, we discuss the Scopolamine-induced experimental model of Alzheimer's type dementia. Scopolamine, an antimuscarinic agent, competitively antagonizes the effect of acetylcholine on the muscarinic receptors by occupying postsynaptic receptor sites with high affinity and increases AChE activity in the cortex and hippocampus. Due to cholinergic hypofunction, scopolamine reduces cerebral blood flow. Scopolamine also induces reactive oxygen species (ROS), resulting in free radical injury, an increase in brain MDA levels, and a worsening in antioxidant status in the scopolamine-treated group. Scopolamine causes neuroinflammation in the hippocampus through increasing oxidative stress and pro-inflammatory cytokines. Scopolamine is proved to increase levels of APP and Tau. Chronic scopolamine administration resulted in significant histological changes in the cerebral cortex, including neuronal degeneration. Scopolamine administration has been used both in healthy human volunteers and in animals model of dementia to determine the effectiveness of potential new therapeutic agents for Alzheimer's disease [5].

Current Medication of Alzheimer's disease and its Problems [6]:

TABLE 1: CURRENT MEDICATION OF ALZHEIMER DISEASE

Drug Name	Indication	Action	Adverse Effects
Donepezil Brand name: Aricept	Mild to severe AD	Prevents the breakdown of acetylcholine (ACh) by inhibiting the action of Acetylcholinesterase. Treats cognitive symptoms of AD	1. CNS: headache, seizures, insomnia, fatigue, aggression 2. CV: chest pain, hypertension, atrial fibrillation 3. GI: nausea, vomiting, GI bleeding 4. Metabolic: Weight loss, dehydration
Galantamine Brand name: Raza dyne	Mild to moderate AD	Prevents the breakdown of acetylcholine and stimulates receptors to release excess acetylcholine to treats cognitive symptoms of AD.	1. CNS: depression, dizziness, fatigue, insomnia 2. CV: bradycardia, AV -block 3. GI: diarrhea, nausea, anorexia, abdominal pain

			4. Hematologic: anemia
Rivastigmine Brand name: Exelon	Mild to moderate AD. Also used to treat dementia from parkinson's disease	Prevents the breakdown of acetylcholine by inhibiting the enzymes that degrade acetylcholine .treats cognitive symptoms of AD	1. CNS: headache, dizziness, confusion, nervousness, paranoia, malaise 2. CV: hypertension, chest pain, edema 3. Musculoskeletal: back pain, bone fractures
Memantine Brand name: Namenda	Moderate to severe AD.	Blocks glutamatergic (NMDA) receptors and controls glutamate activity. Treats cognitive symptoms of AD	1. CNS: stroke, aggressiveness, agitation, fatigue, confusion, pain, syncope 2. CV: heart failure, edema 3. GI: anorexia, constipation, nausea,

Plant Profile *Cissus quadrangularis* L: *Cissus quadrangularis* (Hadjod) is a perennial plant belonging to the Vitaceae family. It is native to India, Bangladesh and Sri Lanka. Africa and Southeast Asia are also home to this species. Brazil and the southern United States are importing it. *Cissus quadrangularis* grows to a height of 1.5 meters, with quadrangular-sectioned branches that are 8 to 10 cm long and 1.2 to 1.5 cm wide. A leathery edge runs along each angle. At the nodes, the toothed tri-lobe leaves, which are 2 to 5 cm broad, appear. Each node has a tendril that emerges from the opposite side. When the globular berries are mature, they turn red among racemes of little white, yellowish, or greenish blooms. It's a cactus-like, jointed climber found in more remote parts of India, as well as in nurseries. The absolute alcoholic concentration of the plant, on parenteral administration, kills the counter anabolic impact of the cortisone in the healing of bone breaks [7].

MATERIALS AND METHODS:

Plant Material: The twigs (Leaves and stems) of *Cissus quadrangularis* L. were collected from the Medicinal garden of Oriental College of Pharmacy, Navi Mumbai in July 2020 and were authenticated by Mr. Mahesh Atale, M.Sc. Botany, Alarsin Pioneers in Ayurvedic Research, Andheri (E), Mumbai - 400093 and specimen were submitted to Pharmacological department, Oriental College of Pharmacy, Sanpada, Navi Mumbai - 400705. The plant stems were washed with tap water and shade dried at normal room temperature with the aid of circulating airflow using a fan. The stem was dried, and coarse powder was made of the stem in a mixture and it was stored in a container.

Preparation of Extract: The ethanolic extract of *Cissus quadrangularis* was obtained by the Soxhlet method. The fleshy stems of about 3 kg were washed, cut into small pieces, shade dried, and crushed into powder. Two hundred and fifty grams of the powdered stem was Soxhlet extracted with 95% ethanol. The extract was evaporated in vacuum under reduced pressure, to make asyrupy consistency, and the final extract was stored in a glass container at room temperature.

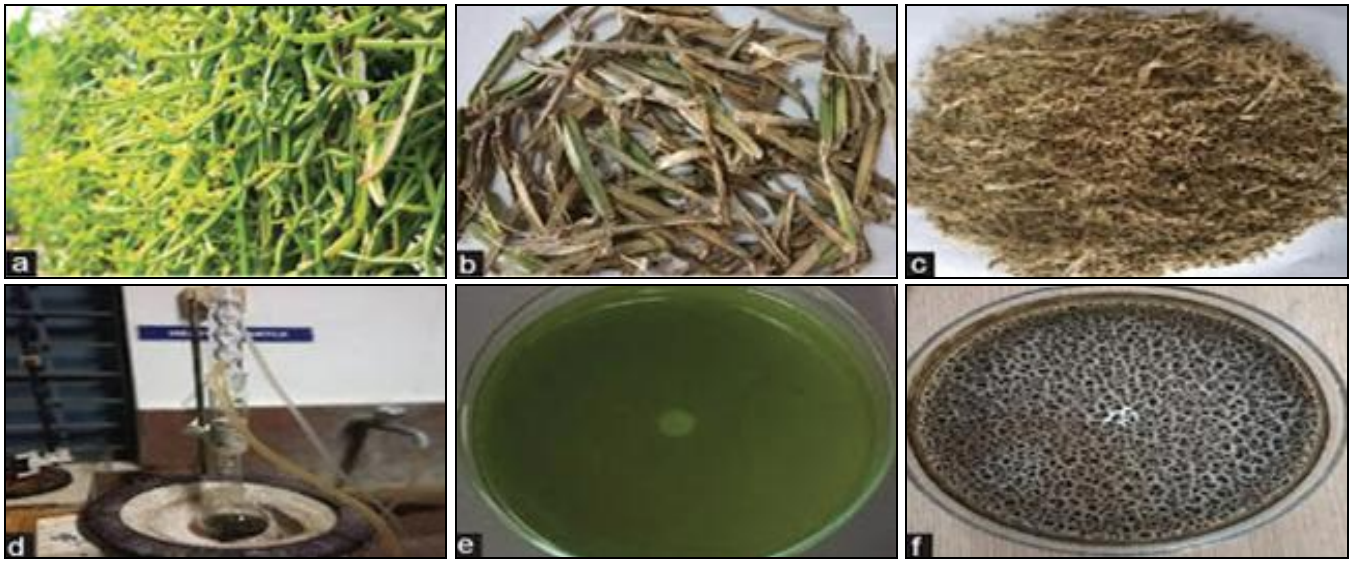

FIG. 1: PROCESS OF EXTRACT PREPARATION

Chemical Constituents: Several active chemical constituents, including phenolic compounds such as flavonoids, phytosterols, triterpenoids, stilbenes, and Vitamin C, Tannins and alkaloids, *etc*., serve as useful antioxidants are present in this plant. The *Cissus quadrangularis* stems were reported to have antioxidant properties. The stem of the *Cissus quadrangularis* contains carotene, mainly β-carotene, which is responsible for showing the anti-alzhiemer activity [8].

Medicinal Properties: Anti-osteoporotic activity, Antioxidant and Antimicrobial activity, Antibacterial activity, Anticonvulsant and sedative activity, CNS Activity, Anti-anxiolytic and Antiepileptic, Anthelmintic activity [9, 8, 10, 11, 12, 13].

Toxicity: The alcoholic extract's LD50 was estimated to be 3000 mg/kg. This plant has also known to be edible, making it safe and accordingly, $1/10^{th}$ and $1/20^{th}$ dose was selected for study, *i.e*., low dose 100 mg/kg, intermediate-dose 200 mg/kg, and high dose 300 mg/kg for the test groups with dose conversion [14].

Drug Treatment: The ethanolic extract was suspended in Normal saline in doses of 100, 200, 300 mg/kg p.o. for the pharmacological tests. The doses were selected based on the previous studies on ethanolic extract. *Cissus quadrangularis* stems extract (EECQ) was administered to individual rats in group [4, 5, 6, 7]. None of the rat was dead due to treatment till the end of the observation period. The *Cissus quadrangularis* stems extract (EECQ) caused no abnormality till the duration of treatment.

Qualitative Phytochemical Screening [15]:

TABLE 2: QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF POWDERED STEMS OF *CISSUS QUADRANGULARIS*

S. no.	Phytoconstituents	Test
1	Carbohydrates	Molisch's test Fehling's test
2	Proteins and free amino acid	Ninhydrin test
3	Alkaloids	Mayer's test
4	Cardiac Glycosides	Keller-Killiani test
5	Steroids	Liebermann-Burchard test
6	Terpenoids	Salkowaski test
7	Tannins	5% FeCl3 Solution
8	Flavonoids	Shinoda test
9	Phenols	Test for cartenoids
10	Saponins	Foam test

Equipments and Chemicals: Electronic balance, Morris water maze, Y-maze, novel object apparatus, syringes, and needles, ethanol, NS and scopolamine. Donepezil tablet, 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholine, thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Hydrogen peroxide (H_2O_2), Griess reagent.

Animals: The CPCSEA acknowledged Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy; Sanpada approved the animals needed for the research work with protocol no. OCP/ IAEC/2020-21/07 entitled "Evaluation of anti-Alzheimer's activity of ethanolic extract of *Cissus quadrangularis* stems in Wistar albino rats". Animals procured were female Albino Wistar rats (100 to 120 g) were obtained from National Institute of Biosciences, Pune, Maharashtra 411051. The animals were kept in a well ventilated, air-conditioned animal house at a constant temperature of 24 ± 2 °C, with 12:12 hours dark: light cycle and a relative humidity of 55-60%. The animals were placed on bedding material in spacious polypropylene cages with a paddy husk. The animals were held on a normal diet with pellets and filtered water.

Acute Oral Toxicity Study [14, 16]: Acute Oral Toxicity Study has been performed previously on this plant as per OECD 423 guideline.

Experimental Design:

Grouping of Animals: Animals were divided into seven groups, each of six animals.

Group I: Control group oral administered by Distilled water (20 ml/kg).

Group II: Intraperitoneal injection by scopolamine hydrochloride (0.5 mg/kg) (Disease control).

Group III: Animal oral administered by donepezil tablet standard (2.5 mg/kg) and Alzheimer's induced with scopolamine (Standard).

Group IV: Animals oral administered by extract which is dissolved in NS (100 mg/kg) and Alzheimer's induced with scopolamine (Low dose).

Group V: Animals oral administered by extract which is dissolved in NS (200 mg/kg) and Alzheimer's induced with scopolamine (Intermediate dose).

Group VI: Animals oral administered by extract which is dissolved in NS (300 mg/kg) and Alzheimer's induced with scopolamine (High dose).

Group VII: Animals oral administered by extract which is dissolved in NS (300 mg/kg) (Extract only).

Anti-alzheimer's Study:

Morris Water Maze Test: The apparatus consisted of a circular pool (45 cm in height and 100 cm in diameter) with a featureless inner surface. The pool was filled with opaque water (maintained at 22 ± 2 °C) to a height of 30 cm and was divided into four quadrants of equal area which were marked as I, II, III and IV. There was a platform (29 cm × 6 cm) placed one centimeter below the level of water at the center of one of the four quadrants (the target quadrant).

The position of the platform was unaltered throughout the duration of the experiment. The test was commenced on day 10th of the treatment period when the rats were allowed to swim for 120 s without the platform in order to acclimatize.

During the next four consecutive days, each animal received four learning trials of 120 s with an intertrial interval of 60 s. For each learning trial the rat was placed in the water facing the pool wall diagonally opposite to the quadrant in which the platform was kept.

The time taken by the animal to locate the submerged platform was recorded as the escape latency time for each trial. If any of the animal were unable to locate the platform within 120 s, they were directed to the platform and allowed to rest there for 60s and in this case the escape latency time was recorded as 120 s. These sessions were recorded as the hidden platform trials or acquisition test. On day 15 (24 h after the final learning trial), the platform was removed from the pool and they were subjected to a probe trial session to assess memory retention.

Each rat was placed into the water diagonally opposite the target quadrant, and for 60 s it was allowed to swim and find the quadrant in which the platform was previously placed. The time spent by the animal in the target quadrant was recorded [17].

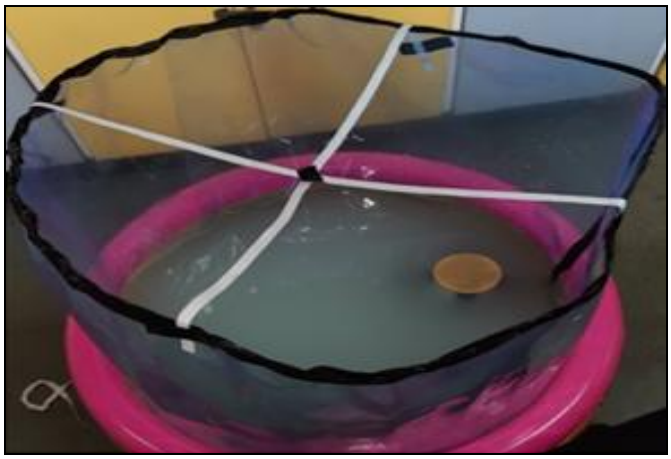

FIG. 2: MORRIS WATER MAZE

Novel Object Recognition Test: Object recognition was performed in a simple box, with or without a transparent wall. The procedure consists of three different phases: a habituation phase, an acquisition phase and a retention phase.

On the first day (habituation Phase), rats were individually subjected to a single familiarization session of 10 min. They were introduced to the empty area to become familiar with the apparatus.

On the 2nd day (acquisition phase), animals were subjected to a single 10-min session, during which floor- fixed two objects (A and B) were placed in a symmetric position in the central line of the area,10cm from each and 8 cm from the nearest wall (each object occupies approximately 5 cm space by its size).

The two objects, made of the same material with a similar colour and smell, were different in shape but identical in size. Rats were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of rats.

On the 3rd day (retention phase), rats were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object C in different shapes but in similar colour and size (A and C).

A recognition index (for retention session), calculated for each mouse, were expressed as the ratio Recognition index (RI) = Time exploring novel object/ (Time exploring novel object + Time exploring familiar object) × 100% [18].

FIG. 3: NOVEL OBJECT RECOGNITION MAZE

Y-Maze Test: Y-maze task is used to measure working memory performance which was assessed by recording spontaneous alternation behavior in a single session in a Y-maze made up of black painted wood. Each arm was 40 cm long, 12 cm height, 3 cm wide at the bottom, and 10 cm wide at the top and converged in an equilateral triangular central area. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The ability to alternate requires that the mice know which arm they have already visited. The series of arm entries, including possible returns into the same arm, will be recorded visually. Immediate working memory performance was assessed by recording spontaneous alternation behavior. Entry will be considered to be completed when the hind paws of the mouse had completely entered the arm. Alternation was defined as successive entries into the three different arms (A, B, and C) on overlapping triplet sets. The percentage of trials in which all three arms were represented, *i.e.*, ABC, CAB, or BCA but not BAB, was recorded as an alternation' to estimate short-term memory. On the 19th day, 90 min after the treatment of the last dose arm entries were recorded and percentage alteration was calculated [19].

FIG. 4: Y MAZE

Biochemical Test:

Preparation of Brain Sample: After assessing the learning and memory paradigms in scopolamine-induced amnesia, rats from each group were euthanized by using a carbon dioxide chamber; brains were removed quickly and placed in ice-cold saline. The frontal cortex, hippocampus and septum (and any other regions of interest) were quickly dissected out on a petri dish chilled on crushed ice. The tissues were weighed and homogenized in 0.1M Phosphate buffer (pH 8). The samples of rat brain homogenates were collected in different test tubes to analyze Acetyl cholinesterase, Catalase, MDA, nitric oxide. The supernatant used for enzymatic assays [20].

Estimation Acetyl Cholinesterase Enzyme Levels in the Brain: 0.4 ml aliquot of the homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100 µl of DTNB. The cuvette contents were mixed thoroughly by bubbling air, and absorbance was measured at 412 nm in a spectrophotometer. When absorbance reached a stable value, it was recorded as the basal reading. 20 µl of the substrate, i.e., acetylthiocholine, was added, and a change in absorbance was recorded. Change in the absorbance per minute was thus determined [21].

Determination of Catalase (CAT) Principle: Catalase activity was measured by the method of Aebi H. 0.1 ml of supernatant were added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. The activity of catalase was expressed as units/mg protein. The reaction occurs immediately after the addition of H_2O_2. Solutions were mixed well and the first absorbance (A1) was read after 15 seconds (t1) and the second absorbance (A2) after 30 seconds (t2). The absorbance was read at wavelength 240 nm [22].

Determination of Malondialdehyde (MDA): MDA was measured according to the method of Ohkawa *et al.* 1 ml. of suspension medium were taken from the tissue homogenate in a tube. 0.5 ml. of Trichloroacetic acid (TCA) was added to it, followed by 0.5 ml. of 8% Thiobarbituric acid

(TBA) reagent. The tubes were covered with aluminum foil and kept in the water bath for 30 min. at 80 ºC. After 30 min. the tubes were taken out and placed in the cold water for 30 min. These tubes were centrifuged for 15 min at 3000 rpm. The absorbance of the supernatant was taken at 540 nm, at room temperature against appropriate blank solution (1 ml distilled water, 0.5 ml of 30% TCA and 0.5 ml of 0.8% TBA.). MDA value was expressed as n moles MDA/mg of protein [23].

Determination of Nitric Oxide (NO): The production of nitric oxide was estimated by the accumulation of nitrate in the supernatant, which was determined by a colorimetric assay with the Griess reagent (1:1 solution of 1% sulfanilamide in 5% H_3PO_4 and 1% napthaylamine diamine dihydrochloric acid in water). An equal volume of the supernatant and the Griess reagent were mixed, and this mixture was incubated for 10 min in the dark at room temperature. The absorbance was taken at 540 nm using a spectrophotometer. The concentration of nitrite in the supernatant was estimated from a sodium nitrite standard curve [23].

Statistical Analysis: The data were analyzed using BioStat Pro (version 5.9.8). For each category, the results are expressed as mean ± SEM. A one-way variance analysis (ANOVA) was used to analyze statistical differences, followed by the Tukey-Kramer test. Tests at $P \leq 0.05$ were considered statistically significant. *** indicated p<0.001, ** indicates p<0.01, * indicates p<0.1* is used to denote the comparison of standard and test groups with the disease control group. To prepare the graphs, experimental data was obtained from all the animals in different groups, recorded and tabulated on a broadsheet using MS Excel program.

RESULTS & DISCUSSIONS:

Qualitative Phytochemical Screening [15]:

TABLE 3: RESULT OF QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF POWDERED STEMS OF *CISSUS QUADRANGULARIS*

S. no	Phytoconstituents	Test	Ethanolic extract of the stems of *Cissus quadrangularis*
1	Carbohydrates	Molisch's test	+ve
		Fehling's test	+ve
2	Proteins and free amino acid	Ninhydrin test	+ve
3	Alkaloids	Mayer's test	+ve
4	Cardiac Glycosides	Keller-Killiani test	+ve
5	Steroids	Liebermann-Burchard test	+ve
6	Terpenoids	Salkowaski test	+ve
7	Tannins	5% FeCl3 Solution	+ve
8	Flavonoids	Shinoda test	+ve
9	Phenols	Test for carotenoids	+ve
10	Saponins	Foam test	+ve

+ ve = Present, -ve = Absent

Anti-Alzheimer's Study:

Morris Water Maze: During the acquisition sessions (days 11-14) scopolamine treatment resulted in significantly longer escape latencies when compared to the controls; this effect was mitigated by co-administration of the test formulation. Significant decreases in escape latencies were observed on days 2-4 (treatment days 12-14) when compared to day 1 (treatment day 11) of the acquisition sessions in the control group but not in the scopolamine-treated rats.

TABLE 4: EFFECT OF ALCOHOLIC EXTRACTS OF STEMS OF *CISSUS QUADRANGULARIS* ON ESCAPE LATENCY TIME IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS

Groups/	ESCAPE LATENCY TIME (SECONDS)			
Days	Day 11	Day 12	Day 13	Day 14
Control group	86.45 ± 4.04	77.20± 0.62	56.62±0.89	27.33±0.92
Disease control(0.5mg/kg)	99.50 ± 2.6$^{\#}$	85.37± 0.98$^{\#}$	65.37±0.82$^{\#}$	37.95±1.05$^{\#}$
Standard control(2.5mg/kg)	81.08 ± 3.23***	55.00± 0.75***	27.91±0.72***	15.20±1.23***
Low dose (100mg/kg)	93.58 ± 2.482**	95.975± 1.39***	55.52±1.66***	27.29±1.53***
Medium dose(200mg/kg)	85.87 ± 1.26**	56.594± 1.04***	45.04±0.30***	23.50±0.71***
High dose(300mg/kg)	77.62 ± 1.42***	55.375±1.24***	37.87±2.23***	20.04±1.14***
Extract only (300mg/kg)	80.54 ± 1.74***	32.45± 2.07***	37.33±1.15***	19.45±0.83***

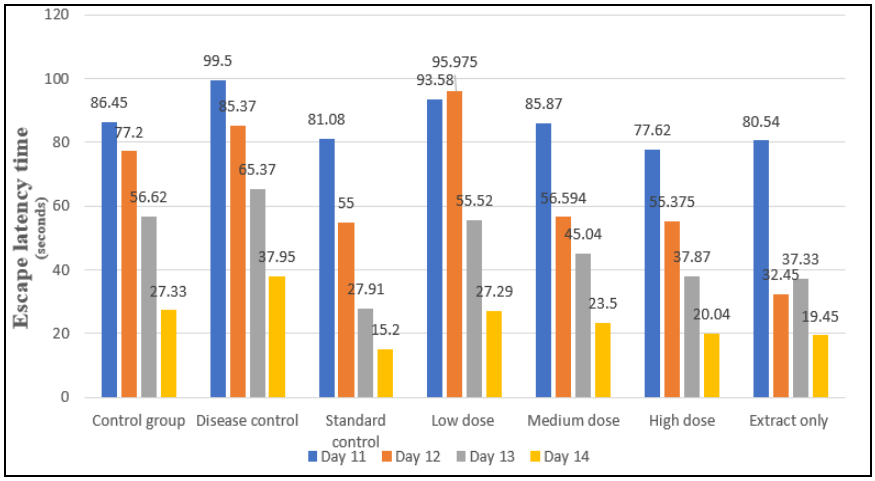

FIG. 5: EFFECT OF ALCOHOLIC EXTRACT OF STEMS OF *CISSUS QUADRANGULARIS* ON ESCAPE LATENCY TIME IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS

On day 15, scopolamine treated rats spent significantly less time swimming in the target quadrant when compared to the controls, whereas the time spent by the rats co-administered with the test formulation and scopolamine in the target quadrant showed a dose-dependent increase in time spend in each quadrant than the disease controls (Scopolamine).

TABLE 5: EFFECT OF ALCOHOLIC EXTRACT OF STEMS OF *CISSUS QUADRANGULARIS* ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS

Groups	Time spend in each quadrants (sec)
Control group	12.83 ± 0.600
Disease control(0.5mg/kg)	7.166 ± 0.749
Standard control(2.5mg/kg)	22.00 ± 1.211
Low dose (100mg/kg)	12.00 ± 0.577
Medium dose(200mg/kg)	11.83 ± 0.600
High dose(300mg/kg)	14.88 ± 0.683
Extract only (300mg/kg)	19.33 ± 1.256

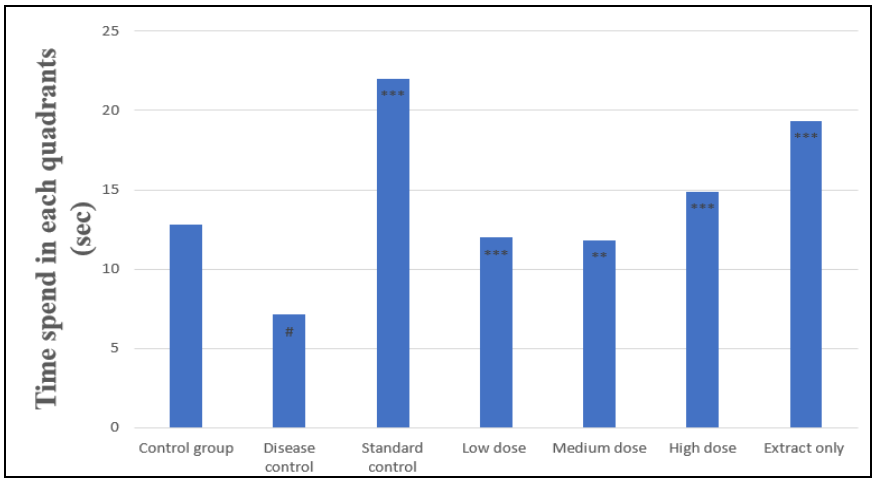

FIG. 6: EFFECT OF ALCOHOLIC EXTRACT OF STEMS OF *CISSUS QUADRANGULARIS* ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE INDUCED ALZHEIMER IN WISTAR ALBINO RATS. Note: Values are expressed as mean ± SEM (n=6). ***P <0.001 compared with disease control, **P<0.01 compared with disease control, and #P<0.05 compared with the control group. Data were analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

Novel Object Recognition Test: Dose-dependent increase in Recognition index when compared against disease control group (Scopolamine) was seen in all the four groups treated with ethanolic extract of dried stems of *Cissus quadrangularis*.

TABLE 6: EFFECT OF ALCOHOLIC EXTRACT OF STEMS OF *CISSUS QUADRANGULARIS* ON RECOGNITION INDEX IN SCOPOLAMINE INDUCED ALZHEIMER IN WISTAR ALBINO RATS

Groups	Recognition index
Control group	55.68 ± 1.062
Disease control(0.5mg/kg)	42.16 ± 1.306
Standard control(2.5mg/kg)	60.74 ± 1.272
Low dose (100mg/kg)	55.30 ± 0.899
Medium dose(200mg/kg)	56.11 ± 0.534
High dose(300mg/kg)	57.94 ± 1.079
Extract only (300mg/kg)	59.12 ± 1.010

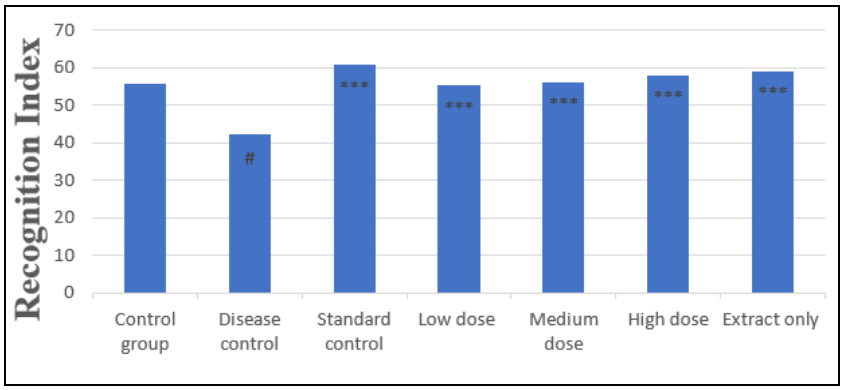

FIG. 7: EFFECT OF ALCOHOLIC EXTRACTS OF STEMS OF *CISSUS QUADRANGULARIS* ON RECOGNITION INDEX IN SCOPOLAMINE INDUCED ALZHEIMER IN WISTAR ALBINO RATS. Note: Values are expressed as mean ± SEM (n=6). ***P <0.001 compared with disease control, **P<0.01 compared with toxicant control and #P<0.05 compared with the control group. Data were analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

Y Maze Test: The scopolamine treatment was associated with a significant decrease in short-term memory performance, as indicated by a decreased spontaneous alternation percentage, compared with the control group.

All four doses of alcoholic extract of dried stems of *Cissus quadrangularis* pretreatment significantly showed a dose-dependent spontaneous increase in the alternation percentage, which was decreased by scopolamine.

TABLE 7: EFFECT OF ALCOHOLIC EXTRACTS OF STEMS OF *CISSUS QUADRANGULARIS* ON SPONTANEOUS ALTERNATION PERCENTAGE IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS

Groups	% Alterations
Control group	54.29 ± 0.803
Disease control(0.5mg/kg)	32.28 ± 0.609
Standard control(2.5mg/kg)	57.21 ± 0.542
Low dose (100mg/kg)	46.98 ± 0.417
Medium dose(200mg/kg)	49.36 ± 0.595
High dose(300mg/kg)	53.72 ± 1.545
Extract only (300mg/kg)	53.70 ± 0.454

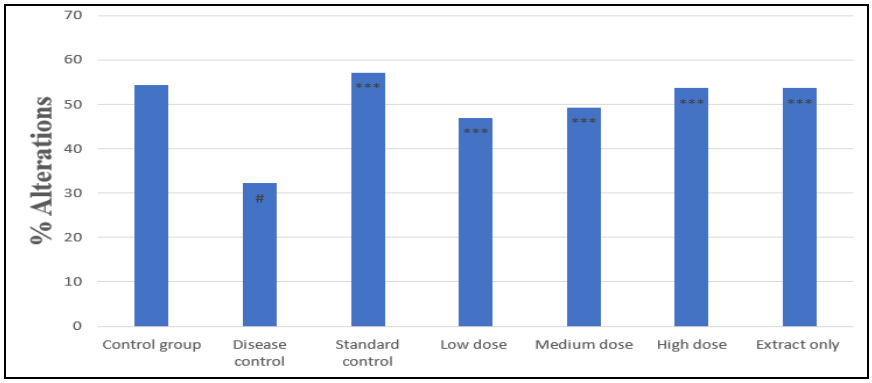

FIG. 8: EFFECT OF ALCOHOLIC EXTRACTS OF STEMS OF *CISSUS QUADRANGULARIS* ON % ALTERATION IN SCOPOLAMINE INDUCED ALZHEIMER IN WISTAR ALBINO RATS. Note: Values are expressed as mean ± SEM (n=6). ***P<0.001 compared with disease control, **P<0.01 compared with toxicant control, and #P<0.05 compared with the control group. Data were analyzed using one-way ANOVA followed by the Tukey-Kramer test. ANOVA: Analysis of variance, SEM: Standard Error of Mean.

Biochemical Studies:

Estimation of Acetyl Cholinesterase Enzyme Levels in the Brain: All the four test doses of the ethanolic extract of dried stems of *Cissus quadrangularis* showed dose-dependent decrease in acetylcholinesterase level when it was compared against the disease control group (Scopolamine).

TABLE 8: EFFECT OF ALCOHOLIC EXTRACT OF DRIED STEMS OF *CISSUS QUADRANGULARIS* ON ACETYLCHOLINE ESTERASE LEVEL IN SCOPOLAMINE-INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Enzyme Levels
Control group	0.081 ± 0.00036
Disease control(0.5mg/kg)	0.092 ± 0.00036
Standard control(2.5mg/kg)	0.071 ± 0.00047
Low dose (100mg/kg)	0.088 ± 0.00213
Medium dose(200mg/kg)	0.080 ± 0.00297
High dose(300mg/kg)	0.076 ± 0.00176
Extract only (300mg/kg)	0.080 ±0.002136

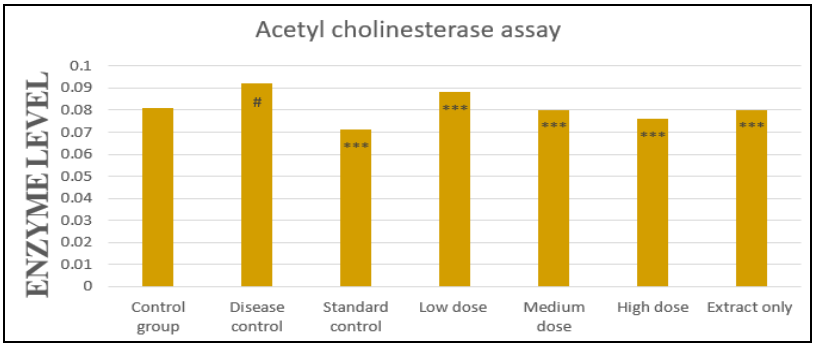

FIG. 9: EFFECT OF ALCOHOLIC EXTRACT OF DRIED STEMS OF *CISSUS QUADRANGULARIS* ON ACETYLCHOLINE ESTERASE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS. Note: Values are expressed as mean ± SEM (n=6). ***P <0.001 compared with disease control,**P<0.01 compared with toxicant control, and #P<0.05 compared with the control group. Data were analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

Determination of Catalase (Cat) Principle: All the four test doses of the ethanolic extract of dried stems of *Cissus quadrangularis* showed a dose-dependent increase in catalase activity when it was compared against the disease control group (Scopolamine).

TABLE 9: EFFECT OF ALCOHOLIC EXTRACT OF DRIED STEMS OF *CISSUS QUADRANGULARIS* ON CATALASE LEVEL IN SCOPOLAMINE-INDUCED ALZHEIMER IN ALBINO WISTAR RATS

GROUPS	CATALASE LEVELS
Control group	34.67 ± 0.120
Disease control(0.5mg/kg)	27.38 ± 0.116
Standard control(2.5mg/kg)	39.39 ± 0.154
Low dose (100mg/kg)	33.79 ± 0.080
Medium dose(200mg/kg)	36.14 ± 0.328
High dose(300mg/kg)	37.62 ± 0.164
Extract only (300mg/kg)	38.51 ± 0.229

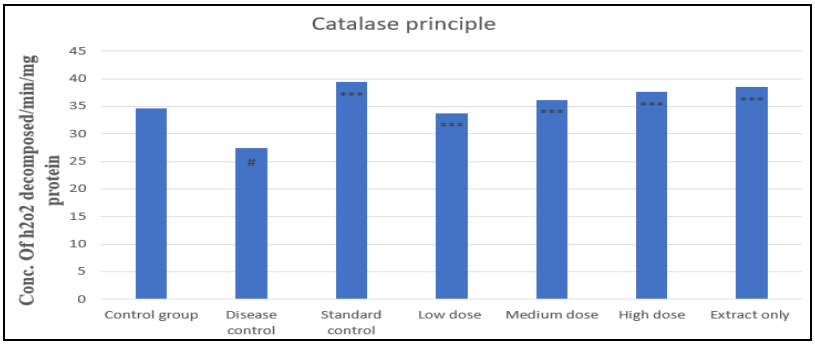

FIG. 10: EFFECT OF ALCOHOLIC EXTRACT OF DRIED STEMS OF *CISSUS QUADRANGULARIS* ON CATALASE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS. Note: Values are expressed as mean ± SEM (n=6). ***P<0.001 compared with disease control,**P<0.01 compared with toxicant control and #P<0.05 compared with control group. Data was analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test.

Determination of Malondialdehyde (MDA): All the four test doses of the ethanolic extract of dried stems of *Cissus quadrangularis* showed dose-dependent decrease in Malonyldialdehyde level when it was compared against disease control group (Scopolamine).

TABLE 10: EFFECT OF ETHANOLIC EXTRACT OF DRIED STEMS OF *CISSUS QUADRANGULARIS* ON MALONDIALDEHYDE (MDA) LEVEL IN SCOPOLAMINE-INDUCED ALZHEIMER IN ALBINO WISTAR RATS

GROUPS	MDA LEVELS
Control group	0.025 ± 0.00101
Disease control(0.5mg/kg)	0.030 ± 0.00031
Standard control(2.5mg/kg)	0.010 ± 0.00045
Low dose (100mg/kg)	0.024 ± 0.00057
Medium dose(200mg/kg)	0.023 ± 0.00076
High dose(300mg/kg)	0.017 ± 0.00073
Extract only (300mg/kg)	0.020 ± 0.00135

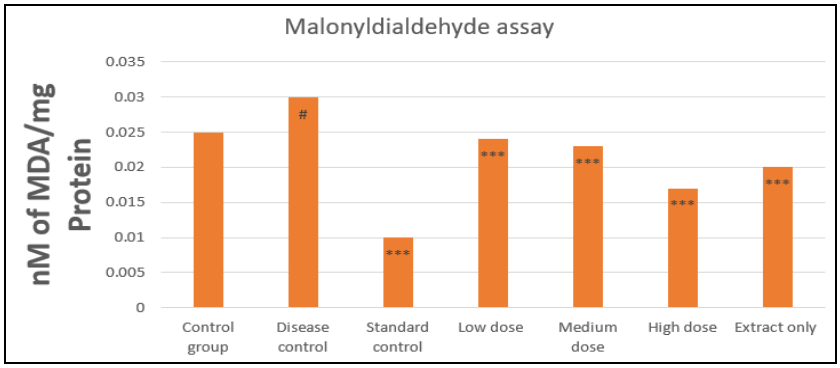

FIG. 11: EFFECT OF ETHANOLIC EXTRACT OF DRIED STEMS OF *CISSUS QUADRANGULARIS* ON MALONYLDIALDEHYDE (MDA) LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS. Note: Values are expressed as mean ± SEM (n=6). ***P<0.001 compared with disease control,**P<0.01 compared with toxicant control and #P<0.05 compared with the control group. Data were analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

Determination of Nitric Oxide (NO): All the four test doses of the ethanolic extract of dried stems of *Cissus quadrangularis* showed a dose-dependent decrease in Nitrite level when it was compared against the disease control group (Scopolamine).

TABLE 11: EFFECT OF ETHANOLIC EXTRACT OF DRIED STEMS OF CISSUSQUADRANGULARISON NITRITE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Nitrite Levels
Control group	07.06 ± 0.021
Disease control(0.5mg/kg)	20.04 ± 0.243
Standard control(2.5mg/kg)	09.14 ± 0.104
Low dose (100mg/kg)	15.69 ± 0.170
Medium dose(200mg/kg)	14.41 ± 0.294
High dose(300mg/kg)	12.31 ± 0.295
Extract only (300mg/kg)	12.13 ± 0.207

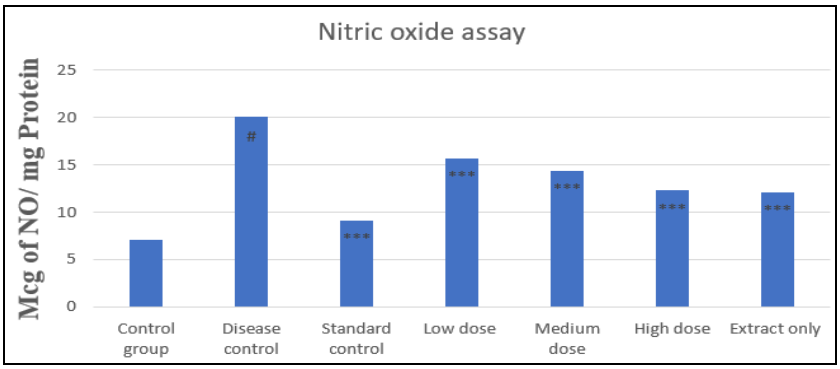

FIG. 12: EFFECT OF ETHANOLIC EXTRACT OF DRIED STEMS OF *CISSUS QUADRANGULARIS* ON NITRITE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS Note: Values are expressed as mean ± SEM (n=6). ***P<0.001 compared with disease control, **P<0.01 compared with toxicant control and #P<0.05 compared with the control group. Data was analyzed using one-way ANOVA followed by the Tukey-Kramer test. ANOVA: Analysis of variance, SEM: Standard Error of Mean.

DISCUSSION: Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities. Patients also frequently have non-cognitive symptoms, such as depression, apathy, and psychosis that impair daily living, Alzheimer's disease can occur at any age, even as young as 40 years. It is mainly caused by beta-amyloid plaques, which are the dense deposit of protein and cellular material that accumulate outside and around nerve cells. Neurofibrillary tangles, which are twisted fiber that builds up inside the nerve cell.

A depletion of short-term memory, failure to learn new knowledge, mood changes, difficulty recognizing terms, forgetting identities, and losing things are demonstrated by earlier illness. There is still considerable research into medications capable of preventing or at least effectively altering the trajectory of AD, referred to as 'disease-modifying' medicines [24]. Dried stems of *Cissus quadrangularis* tested for anti-Alzheimer model in novel object recognition experiment which is employed in animal models of neurological disorders to assess memory, particularly memory recognition. The decision to explore the unfamiliar object represents the utilization of memory for learning and identification. *Cissus quadrangularis* tested for antialzheimer model in novel object recognition test showed dose-dependent increase in recognition index.

The Morris water maze task has been widely used to test spatial learning and memory in rodents. It showed a decrease in escape latency time and increased time spent in the target quadrant in a dose-dependent manner when treated with ethanolic extract of dried stems of *Cissus quadrangularis* L [25]. Y maze is widely used to assess behavioural tasks in preclinical research for studying spatial learning and memory. Y maze test for AD rats pretreated with ethanolic extracts of dried stems of *Cissus quadrangularis* showed a dose-dependent increase in % alternation. In addition to behavioral tests, ethanolic extract of *Cissus quadrangularis* showed a dose-dependent reduction in AchE level as the cognitive impairment is due to the loss of ACh, which results from the hydrolytic action of AChE. As catalase is an antioxidant enzyme continuously produced by numerous metabolic reactions, a dose-dependent increase in catalase activity has been observed in AD rats pretreated with *Cissus quadrangularis* stems extract [26]. AS elevated levels of MDA are indicative of increased oxidative stress a decrease in the levels of the lipid peroxidation product Malonyldialdehyde (MDA) have been observed in AD rats that were pretreated with *Cissus quadrangularis* stems fruit extract. Reactive oxygen species are responsible for the age-related degradation of cognitive performance, which ultimately may cause Alzheimer's disease in elderly persons, the AD rats pretreated with *Cissus quadrangularis* stem extract showed a significant decrease in the levels of nitrite when compared against disease control group [27].

CONCLUSION: Preliminary phytochemical analysis of an ethanolic extract of *Cissus quadrangularis* showed the presence of carbohydrates, flavonoids, carotenoids, glycosides, tannins, alkaloids, phenolic compounds. It can be concluded from the study that the ethanolic extract of *Cissus quadrangularis* stem possesses significant anti-Alzheimer activity, which is probably due to carotene, mainly β-carotene, which is responsible for showing the antialzhiemer activity, which plays an active role in providing free radical scavenging effect. Thus *Cissus quadrangularis* plant can be used for the treatment of neurological disorders and may be recommended as a supplement for the anti-Alzheimer activity

ACKNOWLEDGEMENTS: The author thanks her guide Dr. Mrs. Vanita G. Kanase, and the department of pharmacology for constantly supporting and providing facilities during the work.

CONFLICTS OF INTEREST: The authors have no conflicts of interest regarding this investigation.

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How to cite this article:
Sarvagod SS and Kanase VG: E valuation of anti-alzheimer's activity of alcoholic extract of *Cissus quadrangularis* stems in wistar albino rats. Int J Pharm Sci & Res 2022; 13(4): 1657-69. doi: 10.13040/IJPSR.0975-8232.13(4).1657-69.