

# Evaluation of Neuroprotective Efficacy of Indian Shankpushpi Varieties in Alzheimer's disease – North Vs South

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## ABSTRACT

**Background:** Shankpushpi is a well-known Ayurvedic memory enhancing medicine associated with controversy. Among the various varieties, *Convolvulus pleuricaulis* and *Clitoria ternatea* are widely used in commercial memory enhancing formulations under the name of Shankpushpi. *Convolvulus pleuricaulis* is recognized as true shankpushpi in North side of India, however in southern part of India, *Clitoria ternatea* is accepted as Shankpushpi. **Objectives:** The present study aims to compare neuroprotective efficacy of *C. pleuricaulis* and *C. ternatea* by *in vitro*, *in vivo* methods and establish scientifically validated data to choose appropriate shankpushpi variety for commercial use. **Materials and Methods:** Both herbs were extracted in the Soxhlet apparatus with 70% ethanol for 5 - 6 hours at 60 - 65°C. The presence of neuroprotective principles - taraxerol and scopoletin in extracts was confirmed by the Thin Layer Chromatography. The preliminary screening for neuroprotective efficacy of extracts was done by *in vitro* free radical scavenging, Acetyl cholinesterase enzyme inhibition and LOX enzyme level estimation. *In vivo* study of extracts included behavioral assessment of adult rats by Y maze, Morris water maze using scopolamine induced Alzheimer's disease like model. **Result:** Extracts of both *C. pleuricaulis* and *C. ternatea* significantly scavenged free radicals, inhibited acetyl cholinesterase and LOX enzyme *in vitro*. But in *in vivo* study, significant retention of spatial and working memory was observed in rats administered with *C. pleuricaulis* as compared to *C. ternatea*. **Conclusion:** *C. pleuricaulis* more significantly shields against memory loss and dementia by reducing oxidative stress, inflammation, and memory impairment. Hence should be used in commercial neuroprotective formulation as chief source of Shankpushpi instead of *C. ternatea*.

**Key words:** Alzheimer's disease, Scopolamine, Dementia, Taraxerol, Scopoletin.

## INTRODUCTION

The need for herbal memory boosters has significantly grown over the last few years. The global market for brain health supplements is anticipated to reach USD 13.38 billion by 2028, growing at a CAGR of 8.0% from 2021 to 2028, according to the "Global Brain Health Supplements Market report 2021." The COVID-19 pandemic's substantial increase in brain health issues and the ageing population's increased risk of neurodegenerative disorders like Alzheimer's disease fueled widespread use of herbal supplements.<sup>2,3</sup> Shankpushpi is one of the "Medhya Rasayanas" described in Ayurveda.<sup>4</sup> As per the literature, *Canscora decussata* Schult, *Evolvulus alsonoides* Linn, *Convolvulus pleuricaulis* Choisy, and *Clitoria ternatea* Linn are the four herbs commonly associated with the word "Shankpushpi".<sup>5,6</sup> *Convolvulus pleuricaulis* belongs to the family Convolvulaceae.<sup>6</sup> Scopoletin, shankpushpine, convolamine, convoline,  $\beta$  - sitosterol, kaempferol, and phytosterols are the main active constituents of *C. pleuricaulis* responsible for the neuroprotective activity.<sup>6</sup> *Clitoria ternatea* plant belongs to the family Papilionaceae and taraxerol, taraxerone,  $\beta$  - sitosterol, kaempferol, anthocyanins, and flavonoids are major bioactive principles having neuroprotective potential.<sup>7-9</sup> Both herbs are different in morphology, phytochemistry but similar in neuroprotective activity. *C. pleuricaulis* profoundly grows in dry climate specifically in Bihar and Madhyapradesh region of India. Hence in North region recognized as true Shankpushpi or Northern Shankpushpi.<sup>10</sup> However, in South

region, Ayurvedic medicinal practitioners consider *C. ternatea* as chief Shankpushpi source based on the classic references given in Ayurvedic literature like Priya Nighantu (Piplayadi Varga, 53) Hence, it is also referred as Southern Shankpushpi.<sup>10-12</sup> Due to these controversial aspects, both herbs are preferred as shankpushpi. In the commercial market, both herbs are extensively used to prepare memory-enhancing formulations & supplements despite of the knowledge about which one is superior in neuroprotective activity. Very few studies are available based on the systematic comparative neuropharmacological evaluation of Southern Shankpushpi and Northern Shankpushpi. Thus, the current study compares the anti-Alzheimer's activity of both North Indian and South Indian shankpushpi - *C. pleuricaulis* and *C. ternatea* via *in vitro* and *in vivo* methods. This study presents a systematic comparative evaluation of the anti-Alzheimer's activity of both herbs and highlights which one performs the best, adding to the existing research regarding Shankpushpi.

## MATERIALS AND METHODS

### Plant material

Both North Shankpushpi and South Shankpushpi i.e. *C. pleuricaulis* and *C. ternatea* plants were collected from MAPA - Ayurvedic Medicinal Garden, Government College of Pharmacy, Amravati, Maharashtra in December 2021. Plant authentication was done at Department of Pharmacognosy, Government College of Pharmacy, Amravati. Voucher specimens of two plants (No.

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GCOPA/CP/21-22/9/MS, No. GCOPA/CP/21-22/10/MS) have been deposited to the Department of Pharmacognosy, Government College of Pharmacy, Amravati.

## Reagents and chemicals

Acetylcholinesterase enzyme (*Electrophorus electricus*) from Sigma Aldrich (Bangalore, India), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) was from Sigma Aldrich (Bangalore, India), Acetylthiocholine iodide (ATCI) from Himedia (Mumbai, India), Scopolamine Hydrobromide injection from local market (Amravati), Di- sodium hydrogen phosphate from CDH (Central Drug House (P) Ltd, New Delhi, India), Sodium dihydrogen Phosphate from CDH (Central Drug House (P) Ltd, New Delhi, India), Sodium hydroxide from CDH (Central Drug House (P) Ltd, New Delhi, India), Scopoletin and Taraxerol (Yucca Enterprises, Mumbai), Tween 80 from CDH (Central Drug House (P) Ltd, New Delhi, India), DPPH (2,2 - diphenyl-1-picryl hydrazyl hydrate) from CDH (Central Drug House (P) Ltd, New Delhi, India), Lipoxygenase enzyme (Himedia, Mumbai) .

## Preparation of extracts

Whole plants of *C. pleuricaulis* and *C. ternatia* plants were shed dried and powdered. 100 g of each plant powder was extracted with 70 % ethanol in the Soxhlet apparatus at 60° - 65° C for 5 – 6 hours till complete extraction. After completion of extraction, the filtrate was concentrated and dried at a low temperature. Both extracts (CPE and CTE) were kept in the refrigerator at 4° C till further analysis.

## Qualitative analysis of extracts by Thin layer Chromatography

Thin-layer chromatography was performed to ensure the presence of neuroprotective principles in both plant extracts. Scopoletin and taraxerol were selected as neuroprotective biomarker principles. For scopoletin estimation in *C. pleuricaulis*, Toluene: Chloroform: Acetone (4:2.5:3.5) as mobile phase as per mentioned in Indian Pharmacopoeia 2010 and Anisaldehyde sulphuric acid as spraying agent was used. For taraxerol estimation in *C. ternatia*, hexane-ethyl acetate (80:20, v/v) was used as the mobile phase.<sup>13</sup> Developed spots on both plates were observed in the detection chamber at 254 and 365 nm.

## In-vitro antioxidant activity of plant extracts

This study was performed by DPPH free radical scavenging assay protocol with slight modifications as follows: 11.2 mg of DPPH (2, 2 - diphenyl-1-picryl hydrazyl hydrate) was dissolved in ethanol in 100 ml volumetric flask wrapped in aluminium foil. Stock solutions of CPE (*C. pleuricaulis* extract) and CTE (*C.ternatia* extract) were prepared by dissolving 10 mg extract in 10 ml ethanol having final concentration of 1 mg/ml. from this stock solution further serial dilutions of 10, 20, 30, 40, 50 µg/ml were made. Similarly, serial dilutions of Ascorbic acid 10, 20, 30, 40, 50 µg/ml were made in ethanol from 1 mg/ml stock solution of Ascorbic acid. In a test tube, added 5 ml of each plant extract to 1 ml of DPPH and kept in dark for 20 min for incubation. After 20 min, absorbance of mixture was taken at 520 nm. Similar process was followed for ascorbic acid diluted samples. For blank, 1 ml DPPH was added to 5 ml ethanol. % inhibition was calculated by following formula:<sup>14</sup>

$$\% \text{RSA} = \frac{\text{Abs. of Blank} - \text{Abs. of sample}}{\text{Abs. of blank}} \times 100$$

## In-vitro evaluation of acetylcholinesterase inhibition activity

*In vitro* Anti - Alzheimer's activity evaluation of both South and North Shankhpushpi extracts was done by Ellman's colorimetric method.<sup>15</sup> For this study, ATCI (Acetylthiocholine iodide) as a substrate (0.075 M) and AchE (Acetylcholinesterase) (0.5 u/ml) were derived from

*Electrophorus electricus* Eel used as an enzyme. DTNB 5, 5-dithio-bis-(2-nitrobenzoic acid) (0.01 M) was used as a chromophore. The protocol was as follows:

In a cuvette, 3 ml mixture of Phosphate buffer (PH 8), 100 µl AchE enzyme, and 100 µl plant extract or std. Donepezil hydrochloride was incubated at 37° C for 5 min to ensure binding of the enzyme with phytoconstituents in extracts. After 5 min added 100 µl of DTNB and allowed to stand for a few minutes. After a few minutes, added 20 µl of ATCI to initiate the reaction. Readings were taken after 2 minutes of addition at 412 on a UV Spectrophotometer. For control, replaced the test compound solution with water (100 µl). Measure the absorbance at 412 nm and calculate the % inhibition by the following formula:<sup>15</sup>

$$\% \text{ Acetylcholinesterase inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of plant extract}}{\text{Abs. of Control}}$$

## In vitro LOX enzyme assay

The effect extracts (CPE and CTE) on LOX enzyme activity was measured by following method:<sup>16</sup> The activity was determined spectrophotometrically by monitoring the appearance of the conjugated diene Hydroperoxide at 234 nm. The substrate for LOX assay was prepared according to the method described by Axelrod *et al.* (1981). Linoleic acid (28 mg) was weighed into a 10 mL glass measuring cylinder and an equal weight of Tween-20 plus 2 mL of distilled water were added. Sufficient amount (50 µL) of 2N NaOH was added to obtain a clear solution. The volume of the solution was made up to 10 mL with distilled water. Each time the substrate was prepared fresh and used for the enzyme assay. The reaction mixture contained 2.7 mL of Sodium phosphate buffer (0.2 M, pH 6.5) and 0.3 mL of substrate. The reaction was initiated by adding the enzyme, extract, and the change in absorbance at 234 nm was recorded for three minutes using UV spectrophotometer.<sup>17</sup>

The enzyme activity was expressed as a change in the absorbance ( $\Delta A_{234}$ ) /mg protein/min. Formula used for calculations:

$$V_0 = \Delta A_{234 \text{ nm}} (\epsilon l \Delta t)^{-1}$$

$V_0$ : enzymatic activity,  $\Delta A_{234 \text{ nm}}$ : absorbance variation at 234 nm,  $\epsilon$ : molar extinction coefficient of linoleic acid hydroperoxides at 234 nm,  $l$ : optical path,  $\Delta t$ : time (180 s)

## In vivo analysis of Anti - Alzheimer's activity of plant extracts

### Animals

Adult male Sprague–Dawley rats (wt. 250 – 300 g, age 2–3 months) were used for this study. Animals were kept in polypropylene cages (n = 6) at Animal House facility, Government College of Pharmacy, Amravati under standard Laboratory conditions of relative humidity (50 ± 5%), light (08:00–20:00 h) and temperature (25 ± 2 °C). One-week acclimatization period was followed. Animals had free access standard rat pellet chow (Nutrivet Feed Pvt Ltd, Pune) and water. 7 days acclimatization period was followed before initiation of the study. All the experiments were conducted between 9 am to 6 pm. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Government College of Pharmacy, Amravati (Reg. No 1370/ CPCSEA)

## Preparation of dose and drug treatment

North Indian (CPE) and South Indian Shankhpushpi (CTE) extract was assessed for Anti - Alzheimer's or neuroprotective efficiency at three oral dosage levels of 50, 200, 500 mg/kg. All doses were prepared by suspending each plant extract (CPE and TCE) in a 2 % v/v aqueous solution of tween 80. Donepezil Hydrochloride tablets were used as the

reference standard. Scopolamine Hydrobromide (Buscon-Boehringer Ingelheim, Germany) was used as a standard anticholinergic drug to induce Alzheimer's disease-like symptoms in rats. In each group, there were six animals (n = 6). The Control group received 2% aqueous tween 80 solutions as a treatment whereas test groups received plant extracts suspended in 2% aqueous tween 80 (CPE and CTE) at a dose of 50, 200, 500 mg/kg orally followed by standard anticholinergic drug scopolamine hydrobromide injection (0.5 mg/kg i.p) after 30 min of plant extract administration for 14 days. At the same time, the reference standard group received Donepezil hydrochloride suspended in 2% aqueous tween 80 at a dose of 20 and 50 mg/kg orally and scopolamine hydrobromide injection (0.5 mg/kg i.p) at 30 min intervals for 14 days. The negative control group received scopolamine hydrobromide injection (0.5 mg/kg i.p) for 14 days. This dosing protocol was used to assess the neuroprotective potential of CPE and CTE in rats. Animals were trained for behavioral study from 12<sup>th</sup> to 14<sup>th</sup> day of the study 45 min after giving scopolamine. An actual behavioral test was conducted on the 14<sup>th</sup> day before the sacrifice of animals.

### Water maze test

The protocol was followed as per the procedure described by Uabundit N et al., 2010. Water maze apparatus was used (180 cm in diameter x 58 cm tall) made of acrylic and painted with black color to assess the spatial working memory of positive control, negative control, and treatment receiving rats. The tank was filled with tap water. The temperature of the water was maintained at 25°C. The maze was divided into four quadrants (NE, NW, SE, and SW) by imaginary lines crossing the center of the pool. A movable escape platform was kept in the 1<sup>st</sup> quadrant at a center position below the water level. At the time of training, the water was covered with non-toxic milk powder to hide the platform. The location of the platform and tank position was the same place for each animal throughout the training and experiment to help the rats to memorize the various environmental cues related to platform throughout the experiment. Each rat was placed gently in the water facing the pool wall from one of the four starting points (N, E, S, or W) along the perimeter of the pool, and the animal was allowed to swim until it found and climbed on to the platform. During the training session, the rat was gently placed on the platform if it failed to reach the platform in 60 s. In either case, the subject was left on the platform for 15 s and removed from the pool. The time for animals to climb on the hidden platform was recorded as escape latency. Time spent in the region that previously contained the platform was recorded as the retention time. In each trial, the animal was quickly dried with a towel before being returned to the cage. All tests were carried out within 45 min after the administration of vehicle or plant extract or standard drug, a cholinesterase inhibitor, which served as the positive control.<sup>18,19</sup>

### Y - Maze test

Y - Maze study is widely used to assess the short-term working spatial memory.<sup>20</sup> For this study, 3 arms Y - Maze apparatus was used made of acrylic material 50 cm x 10 cm (Wall Height 20 cm). Before the experiment during training, each rat from the control, positive, negative, and test groups were allowed to explore the maze for 60 s. at the time of the experiment, each rat was allowed to explore the maze for 2 min. after completion of the test, rats were returned to their respective cages. The spontaneous alterations of the rats were recorded. % alteration of each rat was calculated by the following formula:<sup>20</sup>

$$\% \text{ alteration} = \frac{\text{Correct alterations}}{\text{Total number of Alterations}} \times 100$$

### Statistics

Results were expressed as mean ± SD. The inter-group variation was measured by Single-factor ANOVA. Statistical significance was considered at p < 0.05. The statistical analysis was done using MS Excel

## RESULTS

### Qualitative analysis of extracts by Thin layer Chromatography

The percentage yield of 70% ethanolic extracts North Indian Shankpushpi (CPE) and South Indian Shankpushpi (CTE) was 9 ± 0.13 % and 14 ± 0.87 % respectively. The results for the thin layer chromatography in Table 1 and Figure 1, confirm the presence of neuroprotective principles scopoletin and taraxerol in extracts of *C. pleuricaulis* (CPE) and *C. ternatia* (CTE). The Rf values of Taraxerol and scopoletin are near similar 0.52 and 0.58 respectively.

### In vitro evaluation of anti-oxidant activity

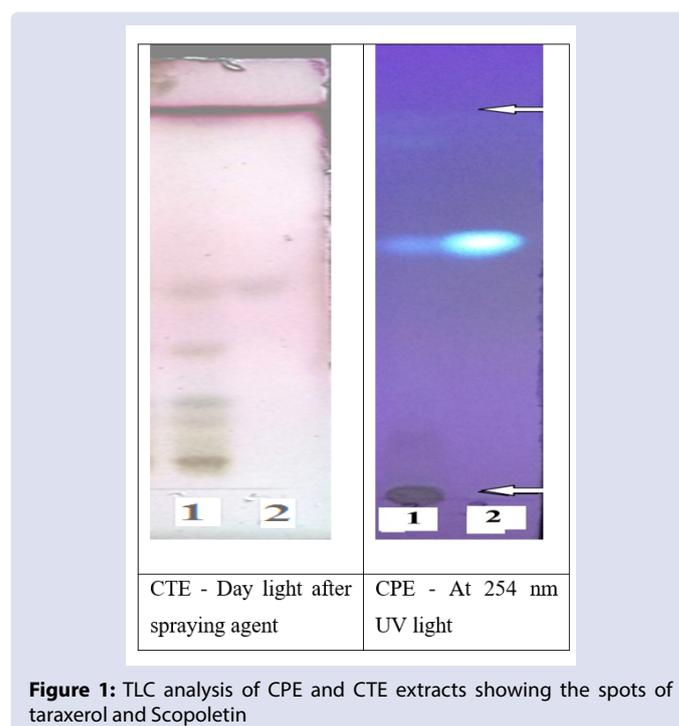
In free radical scavenging assay, both North Indian Shankpushpi i.e *C. pleuricaulis* (CPE) and South Indian Shankpushpi i.e *C. ternatia* extract (CTE) displayed moderate inhibition of the free radicals generated by DPPH. The antioxidant potential of *C. pleuricaulis* extract (CPE) (22.10 ± 1.45 %) found to be slightly greater than *C. ternatia* extract (CTE) (19.23 ± 0.24 %) but lower than that of standard drug Ascorbic acid (As shown in table 2 and Figure 2). Data found statistically significant when analyzed by ANOVA single factor.

**Table 1: Thin Layer chromatographic analysis of scopoletin and taraxerol in extracts of *C. pleuricaulis* (CPE) and *C. ternatia* (CTE).**

Sr. no	Name of Extract	Spot	Rf Value
1	CTE	Spot No. 1 - CTE	0.52
		Spot No. 2 - taraxerol std	0.52
2	CPE	Spot No. 1 - CPE	0.58
		Spot No. 2 - Scopoletin std	0.58

**Table 2: In vitro Anti-oxidant activity of CPE, CTE and std. drug Ascorbic acid.**

Sr no.	Name of sample	% Inhibition at 5 µg/ml
	Ascorbic acid	59.79 ± 4.02 %
	CPE	22.10 ± 1.45 %
	CTE	19.23 ± 0.24 %



**Figure 1: TLC analysis of CPE and CTE extracts showing the spots of taraxerol and Scopoletin**

### In-vitro evaluation of acetylcholinesterase inhibition by extracts

Both Hydroalcoholic extracts of North Indian Shankpushpi (CPE) and South shankpushpi (CTE) inhibited anticholinesterase enzyme *in vitro* to certain extent. When compared activity of two extracts, it is observed that, CPE inhibited Anticholinesterase enzyme ( $52.14 \pm 1.19$  %) significantly than that of South Indian Shankpushpi *C. ternatia* (CTE) ( $46.14 \pm 2.31$  %).

### In-vitro evaluation of effect of extract on LOX enzyme activity

Neuroinflammation is considered as one of the leading causes of Alzheimer's disease initiation. Effect of extracts on LOX enzyme, an enzyme responsible for stimulation of the inflammatory biomarkers was assessed as per the protocol of Borthakur *et al.* 1987 with slight modifications at UV spectrophotometer. In this study, salicylic acid was used as standard drug for comparison. Both hydroalcoholic extracts (CPE and CTE) reduced the LOX level *in vitro*. But when compared with each other, CPE reduced the LOX level ( $15.20 \pm 2.06$   $\delta$ 234/mg protein/min) with slightly higher rate than that of CTE ( $11.03 \pm 5.10$   $\delta$ 234/mg protein/min) but lesser than that of salicylic acid ( $42.08 \pm 3.12$   $\delta$ 234/mg protein/min)

### In - vivo analysis of Anti - Alzheimer's activity of plant extracts

#### Assesment of spatial working memory by Morris water maze test

In the Morris water Maze test, the escape latency period was found significantly higher in scopolamine treated rats which indicates successful induction of dementia in rats. Whereas in extract and standard drug-treated rats, the escape latency period was found to be decreased significantly. Data was analyzed by Single factor ANOVA and found significant with P-value ( $p = 0.032 < 0.05$ ). The order of escape latency period was found to be Donepezil hydrochloride ( $13.5 \pm 10.70$

**Table 3:** % inhibition acetylcholinesterase enzyme by Std. Donepezil Hydrochloride, *C. pleuricaulis* (CPE) and *C. ternatia* (CTE) extracts.

Sr. no	Name of std/Extract	% AchE inhibition
1	CPE	$52.14 \pm 1.19$
2	CTE	$46.14 \pm 2.31$
3	Donepezil Hydrochloride	$97.24 \pm 1.13$

**Table 4:** Effect of Std. salicylic acid, *C. pleuricaulis* (CPE) and *C. ternatea* (CTE) extracts on LOX enzyme level *in vitro*.

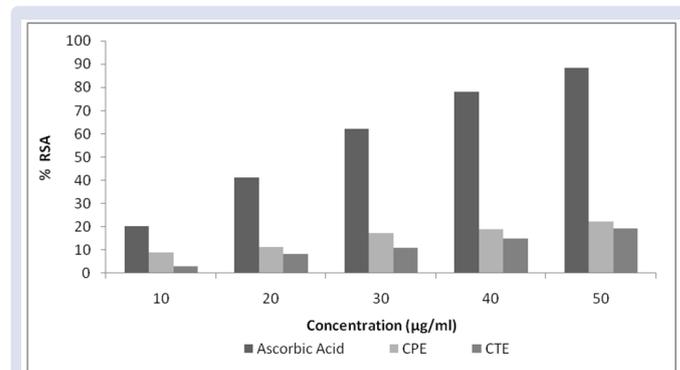
Sr. No	Name of std/Extract	change in the absorbance ( $\delta$ 234) /mg protein/min
1	CPE	$15.20 \pm 2.06$
2	CTE	$11.03 \pm 5.10$
3	Salicylic acid	$42.08 \pm 3.12$

**Table 5:** Assessment of Anti-Alzheimer's effect of hydroalcoholic extract of *C. pleuricaulis* and *C. ternatia* on scopolamine induced dementia by Morris water maze.

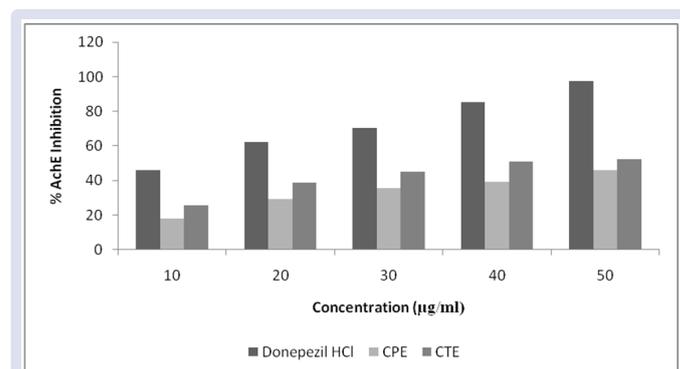
	Escape latency 60 s	Retention Time within 60 s
Control	$24.66 \pm 6.74$	$50 \pm 7.59$
Scopolamine Hydrobromide	$45.16 \pm 12.41$	$8 \pm 6.16$
Donepezil Hydrochloride 100 mg/kg	$13.5 \pm 10.70$	$46.83 \pm 12.04$
CPE 500 mg/kg	$20.66 \pm 13.39$	$36.33 \pm 13.66$
CTE 500 mg/kg	$33.5 \pm 14.73$	$31.16 \pm 9.23$

**Table 6:** Assessment of Anti-Alzheimer's effect of hydroalcoholic extract of *C. pleuricaulis* and *C. ternatia* on scopolamine induced dementia by Y maze.

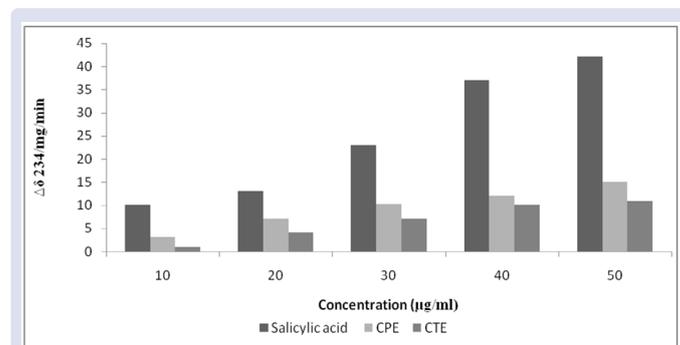
Treatment	% Alteration
Control	$49.33 \pm 14.51$
Scopolamine Hydrobromide (0.5 mg/ml)	$13.33 \pm 12.11$
Donepezil Hydrochloride 50 mg/kg	$70 \pm 17.88$
CPE 500 mg/kg	$66.66 \pm 8.16$
CTE 500 mg/kg	$50 \pm 12.64$



**Figure 2:** Antioxidant Activity of CPE, CTE and std. drug Ascorbic acid. All values are mean  $\pm$  SD. Analyzed by one-way ANOVA, data found to be statistically significant with  $p < 0.05$

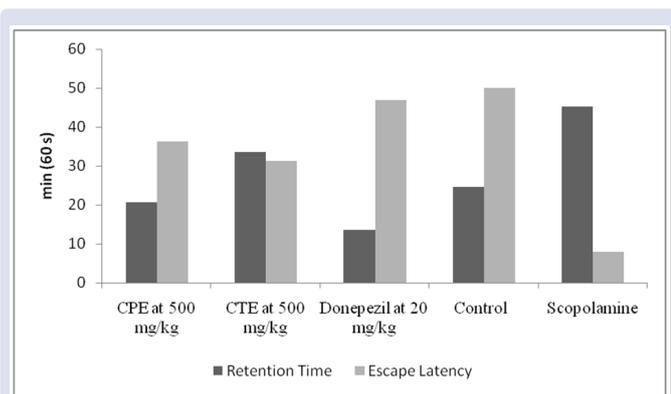


**Figure 3:** % AchE inhibition of CPE, CTE, Donepezil Hydrochloride. All values are mean  $\pm$  SD. Analyzed by one-way ANOVA, data found to be statistically significant with  $p < 0.05$

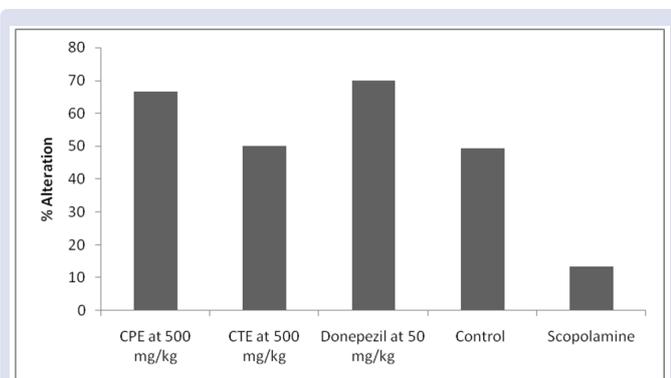


**Figure 4:** *In vitro* LOX enzyme assay of CPE, CTE and Salicylic acid. All values are expressed as mean  $\pm$  SD. When analyzed by one-way ANOVA, data found to be statistically significant with  $p < 0.05$

Sec) > North Indian Shankpushpi (CPE) ( $20.66 \pm 13.39$  Sec) > South Indian Shankpushpi (CTE) ( $33.5 \pm 14.73$  Sec) as compared to the standard drug. Similarly, the retention time was found to be significantly



**Figure 5:** Retention time and Escape latency of treatment, control, scopolamine and std. drug groups of rats in Morris water Maze. All values expressed as mean  $\pm$  SD. Data analyzed by ANOVA found statistically significant with  $p < 0.05$



**Figure 6:** % Alteration of treatment, control, scopolamine and std. drug groups of rats in Y – Maze. All values expressed as mean  $\pm$  SD. Data analyzed by ANOVA found to be statistically significant with  $p$  value  $< 0.05$

increased in CPE-treated rats ( $36.33 \pm 13.66$  sec) as compared to the CTE-treated rats ( $31.16 \pm 9.23$  sec). The P-value of retention time data was found to be significant ( $p$ -value =  $0.015 < 0.05$ ) (as shown in table 5 and figure 5).

### Assessment of short term spatial working memory by Y maze test

In the Y maze test, Scopolamine hydrobromide administered to rats showed significantly decreased percentage of correct alteration indicating the induction of dementia and decreased spatial working memory. In extract-treated rats, the percent of alteration or correct alterations was found to be increased significantly despite daily administration of scopolamine injection. North Indian Shankpushpi (CPE) extract-treated rats performed better in the Y maze study with ( $66.66 \pm 8.16$  %) correct alterations than that South Indian Shankpushpi (CTE) administered rats ( $50 \pm 12.64$  %). However, both extracts showed a lower rate of correct alterations as compared to the standard drug Donepezil hydrochloride ( $70 \pm 17.88$  %). The Control group showed a percentage of alteration of ( $49.33 \pm 14.51$  %). This also indicates significant neuroprotective activity of both extracts.

## DISCUSSION

Among the four varieties of shankpushpi, *Convolvulus pleuricaulis* Choicy and *Clitoria ternatea* Linn are commercially important and are widely used in memory-enhancing formulations under the common name Shankpushpi. In *C. ternatia*, triterpenoid taraxerol is the chief active principle present in roots responsible for the

neuroprotective activity while Scopoletin is the chief neuroprotective active principle of *C. pleuricaulis*.<sup>21</sup> Although *Convolvulus pleuricaulis* Choicy (North Shankpushpi) is officially accepted as true shankpushpi, however, in the southern part of India, *Clitoria ternatea* (South Shankpushpi) is widely used as Shankpushpi in memory-enhancing formulations instead of *C. pleuricaulis* based upon its reference as shankpushpi in ancient ayurvedic literature. Hence, the use of these two varieties as an alternative to each other in commercial products is quite controversial and may lead to the use of less therapeutically potent varieties in the formulation. Very few studies have reported the evidence-based difference in the neuropharmacological effect of these two varieties. According to a study conducted by Sethiya N. K et al., 2019, Methanolic extract of *Convolvulus pleuricaulis* Choicy found a superior neuropharmacological effect than that of Methanolic extract of *Clitoria ternatea* based on the *in vitro* beta amyloid inhibition in a cell line, antioxidant, anti-inflammatory activity, and *in vivo* pole climbing and Morris Water Maze behavioral analysis.<sup>5</sup> In extract characterization, they found the presence of stigmaterol, scopoletin, rutin, and Ursolic acid in the methanolic extract of *C. ternatia* while Scopoletin and Stigmaterol in the methanolic extract of *C. pleuricaulis*.<sup>5</sup>

In the present research work, we analyzed neuropharmacological effects of hydroalcoholic extracts of *C. ternatia* and *C. pleuricaulis* by *in-vitro* anti-oxidant, anticholinesterase, anti-inflammatory assays, and *in vivo* by Morris Water Maze, Y –maze methods of spatial working memory assessment. For this purpose, we extracted the whole plant of *C. ternatia* and *C. pleuricaulis* with 70% ethanol to avoid traces of toxicity due to the solvent in the final extract. The percentage yield of 70% ethanolic extracts North Indian Shankpushpi (CPE) and South Indian Shankpushpi (CTE) was found to be  $9 \pm 0.13$  % and  $14 \pm 0.87$  % respectively. Thus, these outcomes show that the pharmacognostic yield of *C. pleuricaulis* whole plant is comparatively lower than that of the *C. ternatea*. Taraxerol and scopoletin are the major phytoconstituents of *C. ternatea* and *C. pleuricaulis* having previously reported neuroprotective activity by inhibiting AchE, oxidative stress as well as inflammatory responses.<sup>21,22</sup> Thus, selected these phytochemicals as biomarkers to confirm the presence of neuroprotective principles in extracts by TLC analysis. Both taraxerol and scopoletin were found to be present in respective extracts with Rf values of 0.52 and 0.58 respectively. In *in-vitro* neuropharmacological screening, both extracts exerted moderate free radical scavenging in DPPH assay, *in vitro* inhibition of acetylcholinesterase enzyme and Lipoxygenase enzyme. The order of *in vitro* inhibition was found to be CPE > CTE which is supportive of the previous findings.

Behavioral studies of animals provide a good platform to visualize the pharmacological effects of drugs without sacrificing animals. For this purpose, Alzheimer's disease-like symptoms were induced in rats by Scopolamine administration (0.5 mg/kg) for 14 days period. Hence, to confirm the *in vitro* Anti- Alzheimer's activity findings of plant extracts, the Water Maze Test and Y maze Test were conducted using scopolamine and extract-treated rats. The water maze study provided a clear idea regarding the effect of drugs on rats' spatial working memory.<sup>23</sup> Several studies are reporting that scopolamine binds to the muscarinic receptors and disrupts the acetylcholine function causing a significant decline in memory and cognitive activities.<sup>24-26</sup> As a result of our study, the escape latency frequency was found to be increased in only scopolamine-administered rats (As shown in Table 5, Figure 5) and retention time decreased (Table 5, Figure 5). They found themselves confused while seeking the correct path toward the place of the hidden platform in a specific quadrant despite several pieces of training. Extract-treated rats specifically CPE showed significantly improved spatial working memory despite continuous administration of scopolamine with a decrease in escape latency period (Table 5, Figure 5) while retention time was found to be increased as compared to CTE. In the Y maze study also, CPE extract-treated rats showed an increased number of correct alterations as compared to the CTE and scopolamine-treated rats (Table 6, Figure 6). These outcomes confirmed the neuroprotective

efficacy of both herbs and also the superiority of *C. pleuricaulis* as a potent neuroprotective in neurodegenerative diseases like Alzheimer's disease. These *in vivo* study outcomes also found a similarity to the outcomes of the *in vitro* acetyl cholinesterase inhibitory activity of both plant extracts.

## CONCLUSION

The above *in vitro* and *in vivo* study findings validate the claim that northern shankpushpi *i.e.* *C. pleuricaulis* is superior to provide neuroprotection in neurodegenerative diseases like Alzheimer's disease than southern Shankpushpi *i.e.* *C. ternatea*.

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## CONFLICTS OF INTEREST

None

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