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ASSESSMENT OF COGNITIVE EFFECT OF COMBINED EXTRACTS OF *ZINGIBER OFFICINALE* AND *OCIMUM SANCTUM* IN SCOPOLAMINE INDUCED AMNESIA

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ABSTRACT
In the current investigation the rhizome of Zingiber officinale and leaf of Ocimum sanctum were extracted using ethanol-water (70:30) and the anti-amnesic potential of the combined extracts was evaluated in experimental model. The extraction yield of the hydroalcoholic mixture was found to be 10.4 % for Zingiber officinale and 17.7 % for Ocimum sanctum. Preliminary phytochemical analysis suggested the presence of alkaloids, saponin glycosides, phenolics, terpenoids, and flavonoids in the leaf of the Zingiber officinale while alkaloids, sterols and glycosides were not present in Ocimum sanctum. The total phenolic content of the extracts of Z. officinale and O. sanctum were 73.61 ± 0.238 and 65.71 ± 0.265 GAE mg/g, respectively. The phenolic content was highest in the combined extract (CPE, 2:1) of all the three combinations with total phenolics 149.56 ± 1.247 GAE mg/g. The combination of these extracts (1:1, 1:2 & 2:1) were evaluated for anti-amnesic potential using Morris water maze and Elevated plus maze models. The number of movements in open arm of the elevated plus maze apparatus on administration of CPE, 2:1 was highest of all the extracts administered (5.14 ± 0.408). In the Morris method, the mice treated with CPE, 2:1 required only 14.22 ± 0.894 seconds on the 4th day to find the hidden platform as compared to 52.00 ± 3.577 for the scopolamine treated mice.

KEYWORDS: ***Zingiber officinale, Ocimum sanctum, extraction, Morris water maze, elevated plus maze***

INTRODUCTION
Dementia is a serious brain pathology that affects memory, reasoning, orientation, thoughtfulness, learning ability, and poignant reliability, among other functions of the superior cortical role. Memory loss precedes the gradual loss of other cognitive processes, including behavioural abnormalities, making it a neurodegenerative condition (Huang and Mucke, 2012). The cholinergic neurotransmission is essential for learning and memory (Auld et al., 2002).

The ginger rhizome, also known as *Zingiber officinale*, is frequently used in cooking and can be used fresh, dried, or powdered. Ginger essential oil can be extracted from the fresh rhizome. *Ocimum sanctum* L., also known as tulsi, is a 30–60 cm tall, upright, heavily branched subshrub with simple, opposite, fragrant green or purple leaves and hairy stems.

Experimental studies have shown several pharmacological activities of *Zingiber* species including nootropic (Joshi and Parle, 2006), antidepressant (Singh et al., 2012) and antinociceptive (Phukan and Adhikari, 2017) among others. The genus Ocimum has also been reported to possess a manifold of pharmacological actions like anti-inflammatory, analgesic and antipyretic (Godhwani et al., 1987), anticancer (Karthiresan, 1999), hypotensive (Singh et al., 2001), antimicrobial (Auil et al., 2005; Singh et al., 2005), anti-amnesic (Singh et al., 2016).

The objective of the present investigation was to combine the hydroalcoholic extracts of *Zingiber officinale* and *Ocimum sanctum* and evaluate their effects on scopolamine-induced amnesia in rats.

MATERIAL AND METHODS
Preparation of the Plant Material
The plant material after authentication, were washed with distilled water and dried under shade. The dried material were powdered using a blender at low speed. The powdered material were stored in closed container till use.

Extraction of Leaves (Sahira Banu and Cathrine, 2015)
100 g of powder was evenly packed in soxhlet apparatus and extracted with 300 ml of ethanol-water (70:30) by aid of heat. The extracts were filtered while hot and then concentrated by under vacuum. The concentrated extracts were transferred to 100 ml beakers and the solvents were evaporated on water bath. The oleo-resinous/semisolid extracts collected and the excessive moisture was removed by placing the extracts in desiccators.

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The dried extracts were stored in desiccators for further procedures of analysis.

Preliminary Phytochemical Screening

Phytochemical analysis of the extracts was done to check the presence or absence of common plant secondary metabolites. The evaluation was done for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. Precipitate formation or color intensity was used as analytical response to these tests.

Preparation the combined extracts

The hydroalcoholic extracts obtained from *Zingiber officinale* and *Ocimum sanctum* were mixed in various ratios (1:1, 1:2 & 2:1) respectively and used for determination of the total phenolic content and anti-amnestic action using the reported methods reported in the succeeding sections. The anti-amnestic activity of the combined extracts was compared to that of the individual extracts and studied statistically for significance.

Total Phenolic Content (Tiwari et al., 2017)

The total phenolic content in the hydroalcoholic extracts of both the plants was determined by Folin-Ciocalteu method. For total phenolic content determination, 200 µL of each extract (1 mg/ml) was mixed with 3 ml purified water and 0.5 ml of Folin-Ciocalteu reagent. After 3 min, 4 ml of 75 g/L sodium carbonate aqueous solution was added, the mixture was vortexed for 15 sec and allowed to stand for 1 h in dark for color development and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Calibration curve was prepared by similar treatment of gallic acid instead of the extract. The obtained results were expressed as milligrams of gallic acid equivalent (GAE) per g of the dry sample, calculated according to the following formula:

T = C x V/M; where T is total phenolic content, C- concentration of gallic acid in extract, V- volume of extract solution, M is the weight of the extract in g.

EVALUATION OF ANTI-AMNESIC POTENTIAL

Animals

Healthy Wistar rats of either sex, weighing about 180-250g were used. The animals were housed in cages maintained at 12 day and night cycle and a temperature maintained at 17-26°C, fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Grouping of animal for treatment

The animals were divided in 12 groups with 5 animals in each group. The grouping and treatment per group (Table 1) is presented below.

Table 1 Grouping of animal for anti-amnestic study

Group No.	Treatment	Dose
1	Control	Normal Saline
2	Scopolamine (SCOP)	2mg/Kg, i.v for 21 days
3 & 4	ZOE	100 mg/Kg, oral +SCOP 2mg/Kg, i.v for 21 days
5 & 6	OSE	100 mg/Kg, oral +SCOP 2mg/Kg, i.v for 21 days
7 & 10	CPE (1:1)	100 mg/Kg, oral +SCOP 2mg/Kg, i.v for 21 days
8 & 11	CPE (1:2)	100 mg/Kg, oral +SCOP 2mg/Kg, i.v for 21 days
9 & 12	CPE (2:1)	100 mg/Kg, oral +SCOP 2mg/Kg, i.v for 21 days

Morris water maze test (Morris, 1984; Vorhees and Williams, 2006)

The testing system consisted of a circular pool (150 cm wide and 30 cm deep) filled with water and surrounded by visual cues. The pool was divided in four quadrants. A black circular hidden platform was placed in the northwest (NW) quadrant 2 cm under the water surface so that rat could escape swimming and drowning. Rat were screened for their swimming ability by recording the latency to reach the visible platform post training to exit the water tank onto the platform by using the visual cues. Each rat was placed inside the water tank facing the tank wall, at one of the four randomly selected entry points. The test was performed on four consecutive days (8 trials per day). The starting position was changed randomly for each trial and the animal was allowed to search for 60 s to find the hidden platform. At the end of the trials, the rat was allowed to remain on the platform for 30 s.

Elevated plus maze test (Scheider et al., 2011; Itoh et al., 1990)

The Elevated plus-maze comprised of two open (50cm × 10 cm) and two enclosed (50cm × 10 cm×40 cm) arms radiating from the central platform (10cm × 10 cm) to form a plus sign. The plus maze was elevated to a height of 50 cm above from the floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (9:00 – 14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. During the 5 min experiment, behavior of mice was recorded as (i) preference of the mice for its first entry into the open and closed arms, (ii) the numbers of entries into the open or closed arms, and (iii) time spent by the mice in each of the arms. The mice were considered to have entered an arm when and four paws were on the arm.

Statistical Analysis

All analysis was performed using graph pad prism 5 for Windows. All statistical analysis was expressed as mean ± standard deviation (SD). Data were analyzed by two-way ANOVA.

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RESULTS AND DISCUSSION

The extraction yield in ethanol-water (70:30) was found to be 10.4 % for *Zingiber officinale* and 17.7 % for *Ocimum sanctum*.

Phytochemical Screening

The phytochemical screening of extract was done for detecting the presence alkaloids, glycosides, tannins, saponins, flavonoids, steroids and terpenoids. All the extracts were tested for the presence of various categories of phytochemicals and the results are presented in Table 2.

The findings suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, and flavonoids in the plants.

Table 2 Phytochemical analysis of the extracts

Phytochemical Tested	Observation	*Z. officinale* extract	*O. sanctum* extract
Alkaloids	Orange color precipitate/ solution	+	-
Saponins	Continual frothing	+	+
Cardiac glycosides	Brown ring at junction	+	-
Tannins	Green colored precipitate	+	+
Flavonoids	Yellow colored precipitate	+	+
Steroids	Formation of Green Color	+	-
Terpenes/terpenoids	Appearance of Grey color	+	+

Total Phenolic content

The quantification of total phenolic content in the extract of *Zingiber officinale* and *Ocimum sanctum* was done by Folin-Ciocalteu method. The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 3. The total phenolic content of found in the extract of *Zingiber officinale* and *Ocimum sanctum* were 73.61 ± 0.238 and 65.71 ± 0.265 GAE mg/g, respectively.

Table 2 Total phenolic content of extracts

Plant	Total phenolic content (GAE mg/g)
Zingiber officinale	73.61 ± 0.238
Ocimum sanctum	65.71 ± 0.265
CPE, 1:1	84.37 ± 1.633
CPE, 1:2	116.9 ± 3.155
CPE, 2:1	149.56 ± 1.247

Data expressed as gallic acid equivalent (GAE) mg per g of the extract, Values are mean ± SD of triplicate determinations; CE – Combined extract of *Zingiber officinale* and *Ocimum sanctum* extract.

Determination of Anti-amnesic Potential

The extracts were individually and in combination (1:1, 1:2 & 2:1) subjected to *in vivo* determination of anti-amnesic potential using morris water maze and elevated plus maze models. As evident from table 4, scopolamine treated group increased the entries of mice in the closed arm entries along with the total time spent in the closed arm. All the extracts were able to improve the open arm activity exhibited by the animals. The time spent in open arm by the mice treated with vehicle was not significant while CPE, 2:1 exhibited a significant result compared to SCOP ($p<0.0001$) in two way ANOVA. The number of movements in open arm on administration of CPE, 2:1 was highest of all the extracts administered and was found to be 5.14 ± 0.408 while it was just 1.5 ± 0.547 in Scopolamine treated mice.

Table 4 Memory assessment in elevated plus maze paradigm

Treatment	Dose (mg/kg)	Number of open arm entries	Number of close arm entries	Total arm Entries
Vehicle	0.5 ml/kg, i.p.	2.16 ± 0.408^{ns}	4.66 ± 0.516***	7.0 ± 0.752
SCOP	2 mg/kg, i.p.	1.5 ± 0.547	6.16 ± 0.752	7.66 ± 0.816
ZOE	100 mg/kg, p.o.	2.36 ± 0.066*	3.11 ± 0.408***	5.47 ± 0.474
OSE	100 mg/kg, p.o.	2.48 ± 0.301***	1.87 ± 0.516***	4.35 ± 0.817
CPE, 1:1	100 mg/kg, p.o.	3.33 ± 0.547***	1.75 ± 0.752***	5.08 ± 1.299
CPE, 1:2	100 mg/kg, p.o.	4.26 ± 0.408***	1.62 ± 0.516***	5.88 ± 0.924
CPE, 2:1	100 mg/kg, p.o.	5.14 ± 0.408***	1.48 ± 0.816***	6.62 ± 1.224

Values represent mean ± SD (n = 5). ^{ns}- not significant; * ($p<0.05$); ***($p<0.001$)

The elevated plus-maze is a validated predictive test for anxiety-like behavior of rodents in which the animal prefers to stay in the closed arms rather than open arm. The CE were able to significantly improved the on scopolamine- induced learning

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and memory impairment in mice as exhibited by the positive response in elevated plus-maze.

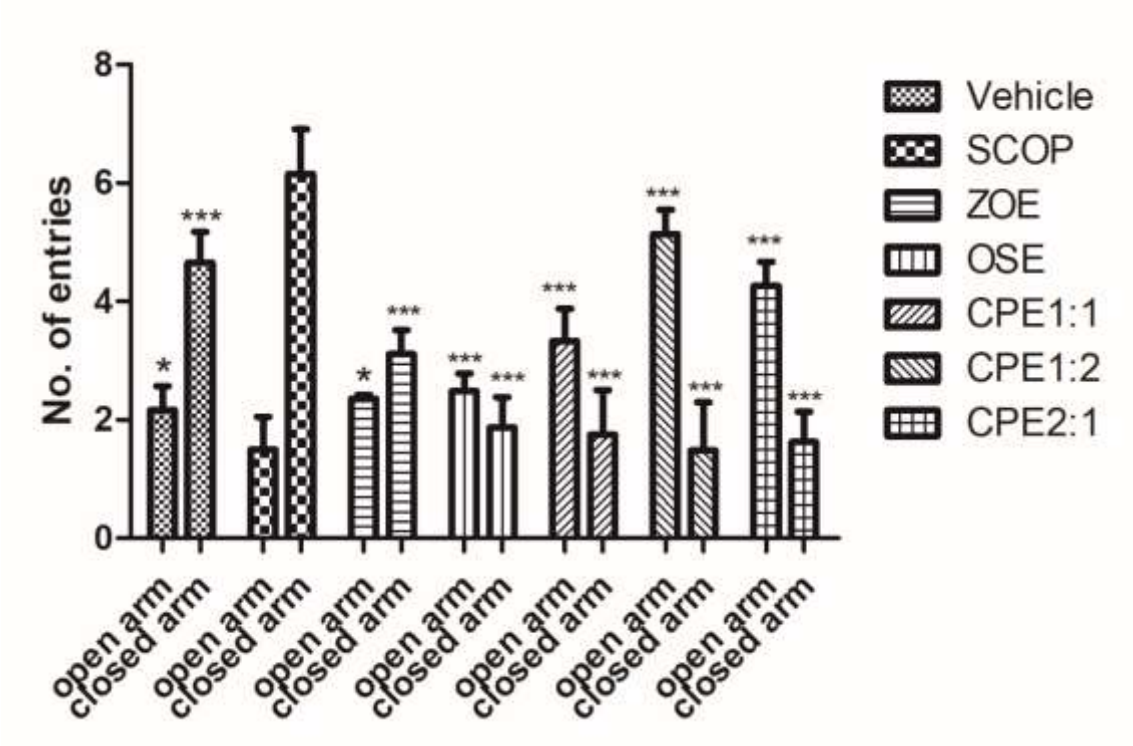

Figure 1 Effect of extracts on mice in EPM test

The average latency to find the hidden platform is exhibited by MWM (Morris water maze) test. Our observations reveal that all experimental groups exhibiting learning tendency by finding the hidden platform by the fourth experimental day. Results of MWM test in experimental animals confirmed a significant effect of the extracts, in comparison to control group on the latency to climb the hidden platform. The two-way ANOVA confirmed a significant interaction between treatment (control vs. SCOP treated) × trial days ($p<0.001$) and SCOP + CE treated × trial days ($p<0.001$) (Figure 2).

Table 5 Time to reach hidden platform in Morris water maze test

Treatment	Dose (mg/kg)	Time to reach platform (sec)			
		Day 1	Day 2	Day 3	Day 4
Vehicle	0.5 ml/kg, i.p.	57.66 ± 4.32*	53.83 ± 3.544*	52.24 ± 2.422^{ns}	48.67 ± 2.786^{ns}
SCOP	2 mg/kg, i.p.	63.16 ± 4.792	59.33 ± 4.456	56.00 ± 4.816	52.00 ± 3.577
ZOE	100 mg/kg, p.o.	49.43 ± 2.857**	46.24 ± 2.529**	41.88 ± 1.940**	38.22 ± 2.483**
OSE	100 mg/kg, p.o.	48.21 ± 3.970**	44.86 ± 3.449**	39.17 ± 2.483**	35.24 ± 0.894**
CPE, 1:1	100 mg/kg, p.o.	42.37 ± 2.483**	39.53 ± 1.940**	36.11 ± 2.522**	33.64 ± 1.449**
CPE, 1:2	100 mg/kg, p.o.	39.61 ± 0.924**	33.92 ± 2.857**	28.53 ± 4.483***	28.11 ± 1.887**
CPE, 2:1	100 mg/kg, p.o.	36.18 ± 3.970***	21.18 ± 3.449***	17.24 ± 2.483***	14.22 ± 0.894

Values represent means ± SD (n = 5), ^{ns}- not significant; **($p<0.05$); ***($p<0.001$)

The mice treated with CPE, 2:1 required only 14.22 ± 0.894 seconds on the 4th day to find the hidden platform as compared to 52.00 ± 3.577 for the scopolamine treated mice. It was also suggestive from the results that in all the treatment groups, the animal were able to find the hidden platform much quickly from the 3rd day. The mice treated with CPE, 1:2 did not show much significant improvement on the 4th day of experiment in a searching the hidden platform.

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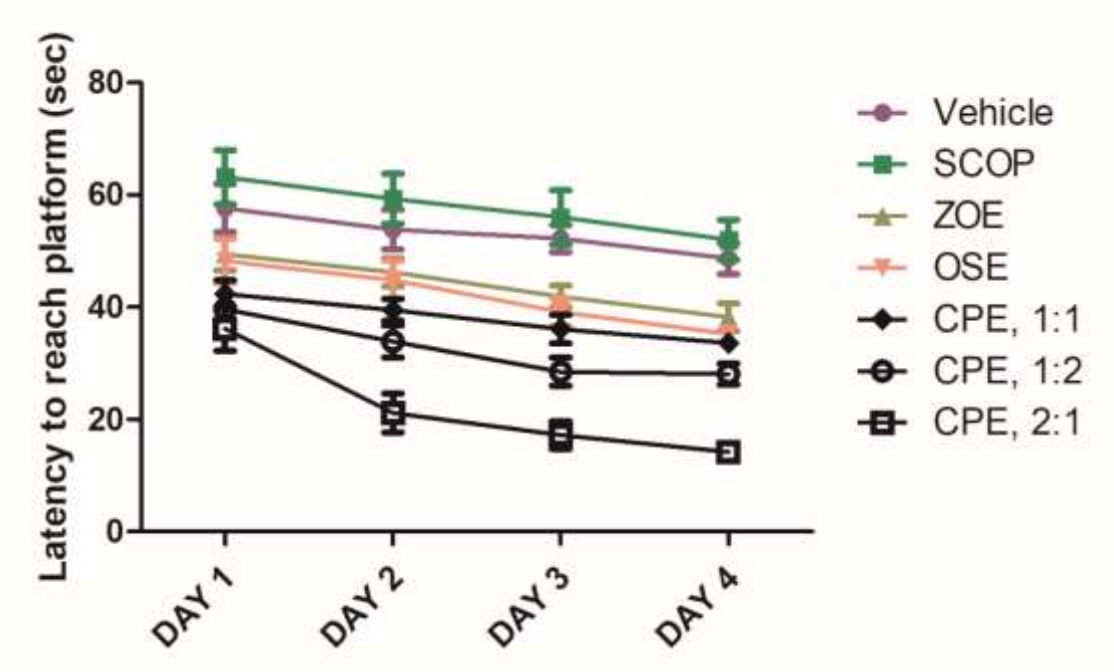

Figure 2 Latency to reach platform

CONCLUSION

The objective of the present study was to assess the anti-amnesic potential of combined extracts of *Zingiber officinale* and *Ocimum sanctum* using the elevated plus maze and Morris water maze models. The hydro-alcoholic fraction of both the plants displayed anti-amnesic action. The results led to the conclusion that by mixing extracts of different species of plants one can obtain synergistic or additive biological action thereby opening newer therapies for dementia and amnesia.

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