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# ANTI- AMNESIC ACTIVITY OF ETHANOLIC FLOWER EXTRACT OF *IXORA COCCINEA*

B. N. B. Vaidehi *, E. Sai Priyanka, J. Ramya, G. Chaitanya Sai and G. Vinaykumar

Aditya College of Pharmacy, East Godavari District, Surampalem – 533437, Andhra Pradesh, India.

**Keywords:**

Alzheimer's, Anti-amnesic, Elevated plus maze, Stair-case method

**Correspondence to Author:**
**B. N. B. Vaidehi**

Aditya College of Pharmacy, East Godavari District, Surampalem – 533437, Andhra Pradesh, India.

**E-mail:** vaidehibondada@gmail.com

**ABSTRACT:** Usage of medicinal plants for the treatment of various diseases had been started from 1500 BC onwards. Loss of cognition is one of the age-related mental problems and a characteristic symptom of neurodegenerative disorders like Alzheimer's. Various natural drugs are used for treatment of Alzheimer's and Parkinsonism, but Available drugs provide symptomatic treatment with known side effects. So the present study is aimed to evaluate anti-amnesic activities of ethanolic flower extract of *Ixora coccinea* on mouse model as an attempt to search for new compounds against Alzheimer's disease-related memory impairment. *Ixora coccinea* is a common flowering shrub native to Southern India and Sri Lanka. Traditionally it is used as hepatoprotective, antimicrobial, anti-oxidant, anti-nociceptive, anti-mitotic and anti-inflammatory activities. Decoction of roots was used for nausea, hiccups, and anorexia. Numerous activities were reported on the entire plant and various parts of the plant like root, leaf, and flower .The anti-amnesic activity was screened by the stair case method and elevated plus maze method. The staircase method has been widely used in measuring perceptual learning. The Elevated plus-maze method was used in measuring memory. The results indicate that ethanolic flower extract of *Ixora coccinea* might be useful as anti-amnesic agent to delay the onset and reduce the severity of symptoms associated with dementia and Alzheimer's disease.

**INTRODUCTION:** *I coccinea* Linn. Is a small shrub cultivated throughout India. The common names are West Indian Jasmine, Rangan, Kheme, Ponna, Techi, Pan, Santan, Jarum-jarum, Jungle flame, Jungle geranium and many more. It consists of tropical evergreen trees and shrubs and holds around 500 species with its center of diversity in Tropical Asia.

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**Botanical Name:** *Ixora coccinea* L. The species name coccinea is a Latin derivative that means scarlet-colored [1].

**FIG. 1: *IXORA COCCINEA* FLOWERS**

**Botanical Name:** *Ixora coccinea L* [1]. The species name coccinea is a Latin derivative that means scarlet-colored.

**Synonym:** *Ixora grandiflora* Bot, *Ixora bandhuca* Roxbg Botanical study.

**Kingdom:** Plantae

**Order:** Gentianales

**Family:** Rubiaceae

**Subfamily:** Ixoroideae

**Tribe:** Ixoreae

**Genus:** Ixora

**Species:** coccinea

**Description:** The plant is a dense, multi-branched evergreen shrub, commonly 4-6 ft (1-2-2 m) in height, but capable of reaching up to 12 ft (3.6 m). Leaves are oblong are about 10 cm long with entire margins and are carried in opposite pairs or whorled on the stem.

They are sessile to short – petiolate, blades elliptic, oblong or obovate, usually leathery, base cordate to rounded, apex rounded, mueronate or shortly tapering; stipules basally sheathing, lobes Triangular and strongly acon – tipped. Flowers sessile; calyx lobes short, triangular, persistent, corolla tube usually 1-1.5 inches long, lobes lanceolate to ovate, less than 0.25 inches long, acute or sometimes fruit thinly fleshy and reddish black.

Roots and flowers are used in dysentery, dysmenorrhea, leucorrhoea, hemoptysis, and catarrhal bronchitis. Leaves are used in diarrhoea. Roots are also used in hiccup, nausea, loss of appetite and externally for the treatment of sores, eczema, chronic ulcers. Roots contain aromatic oil, tannin, fatty acids.

Leaves yield flavonols, kaempferol, quercetin, proanthrocyanidines, phenolic acids, and ferulic acids. Flowers yield cyanidins, flaconboides, and cooling material related to quercitin. Roots ground into a pulp, mixed with water, and used as a tincture for diarrhoea and dysentery. Traditionally it is used as hepatoprotective, antimicrobial, anti-oxidant, anti-nociceptive, anti–mitotic, and anti-inflammatory activities [2, 9].

**Chemical Constituents:** Fifty-four components have been identified in the essential oil of *I. coccinea* flower, representing 99.97% of the total components detected. The oil is composed mainly of triterpenes 62.60%, monoterpenes 31.73%, sesquiterpenes 3.35%, and an ester 2.29%. The major constituents of triterpenes were ursolic acid (27.34%), oleanolic (20.16%), and lupeol (15.10%). *Ixora coccinea* flower is of ursolic acid chemotype. Geranyl Acetate (8.74%) is the major mono-terpenes, followed by Linalyl acetate (6.79%), Neryl acetate (6.49%), Terpineol acetate (4.91%), and Borneol acetate (4.77%); Ethyl cinnamate (2.29%) an ester while the sesquiterpenes are Cyperene (2.72%) and α–Copaene (0.63%) [10]. The present study was undertaken to evaluate the anti-amnesic activity of ethanolic extract of *I. coccinea* Linn flowers against experimentally induced amnesia.

## MATERIAL AND METHODS:

**Plant Material:** The red flowers of the plant *Ixora coccinea* were collected from the gardens of Aditya College of pharmacy, Surampalem in East Godavari District, Andhra Pradesh, India. The plant authentication was confirmed by Dr. T. Raghavendra, botanist, Maharani College, Peddapuram.

**Preparation of Extract:** The freshly collected red flowers of *Ixora coccinea* were collected and washed thoroughly four times with running tap water and once with distilled water. The plant materials were shade dried for a period of one week and then coarsely powdered in a mixer grinder. 20g of powdered red flowers of *Ixora coccinea* were extracted successively with 200 ml of ethanol at 70-80 ºC in the Soxhlet extractor until the extract was clear. The extract was evaporated to dryness. After completion of the process, the concentrated crude extract is kept in a china dish and stored in a vacuum desiccator.

**Animals:** The animals (Wistar rats) of either sex were used for these experiments. Four-week-old rats (16-20 g) were used for an anti amnesic study. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea Feed) and water ad *libitum*. Permission and approval for animal studies were obtained from the institutional animal ethical committee (1269/ PO/E/S/08/CPSCEA: 25-08-2018).

**Phytochemical Screening:**

- The Phytochemical investigation was carried out on ethanolic flower extract of *Ixora coccinea* for the detection of phytochemical constituents (CK Kokate *et. al*, 2009)
- Briefly, tannins and flavonoids were determined by boiling 0.5 g of an extract with 20 ml of distilled water for 5 min in a water bath. Several drops of 0.1% ferric chloride and hydroxide solutions were added to 2 ml of the filtrate, respectively. Brownish green or blue-black coloration indicated the presence of tannins, while yellow coloration showed the presence of flavonoids.
- Saponins were determined by mixing Test solutions with water and shaken and observed for the formation of froth, which is stable for 15 min for a positive result.
- Proteins were determined by performing biuret, millon & Xanthoprotein tests.
- Alkaloids were determined by boiling 1 g of an extract with distilled water and acidified with 5 ml of 1% HCl in a water bath. Several drops of Meyer's reagent were added to 2 ml of the filtrate. The formation of creamy white/turbid precipitate indicated the presence of alkaloids.

**Test for Coumarins:** 0.5 g of the moistened various extracts were taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. The test tube was placed for few minutes in boiling water, and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

**Evaluation of *In-vitro* Anti-Amnesic Activity of Ethanolic Flower Extract of *Ixora Coccinea*:** There is increasing evidence that severe stress affects cognitive functions and leads to the pathogenesis of various neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease, and aging. Both catecholamine and glucocorticoids are known to be linked to the pathogenesis of AD. Stress, particularly chronic adverse stress, is one of the environmental factors that have been suggested to contribute to the onset and progression of AD. The anti-stress and antioxidant activities will be correlated with the nootropic activity since the role of stress and free radicals have been implicated in the loss of memory, concentration, and also in AD.

**Experimental Design:**
**Group before Training/Learning:**

**Group 1:** Animal (n=3) were administered with vehicle.

**Group 2:** Animals (n=3) were administered with a lower dose (75 mg/kg body weight) of Ethanolic flower Extract of *ixora coccinea*.

**After Training:** After training, each group is divided into two subgroups. One subgroup subjected to stress other subgroup left normally without any stress. The administration of vehicle/ drug was normal as usual during this period.

**Group 1:** Animals (n=1) subjected to stress

**Sub Group 1:** Animal (n=1) normally left without stress

**Group 2:** subgroup 1: Animals (n=1) subjected to stress

**Sub Group 2:** Animals (n=1) left normally without stress

**Group 3:** Subgroup 1: Animals (n=1) subjected to stress

**Sub Group 3:** Animal (n=1) normally left without stress

**Stress Procedure:** The animals were subjected to chronic mild stress using the following protocol. The rats were forced to swim in a cylindrical vessel of height 60 cm and diameter 45 cm containing water at room temperature (28 °C). Water depth was maintained to 40 cm. The swim stress was conducted for about 15 min and 4 times a day for about a week; the animal is placed on a small platform (3 cm height & 3.5 cm diameter) fixed at the center of the chamber and surrounded by water 2 cm depth at 22 ºC for 24 h, deprived of food and

water during the night, following 3 h access to restricted food and 2 h access to an empty water bottle in alternative days about a week.

**Experimental Procedure:**

**Stair Case Test:** Rats are divided in to 3 Groups, Group 1 served as control, Group 2 and Group 3 were administered with ethanolic *ixora coccinea* flower extract orally 75 mg/kg and 150 mg/kg body weight respectively. Extract was administered 60 min prior to the experiment. The mouse was gently placed on the floor of the box with its back to the staircase [11].

**Training:** on the first day, during a 3 min period, the number of steps climbed and the number of rearings made were recorded. A step was considered climbed when all four paws were placed on the step. On the second day, after 60 min of drug administered, again a number of steps climbed, and the number of the rearings were recorded. After learning on 2nd day each particular group was divided in two groups as normal and stress groups. Then each subgroup of a particular group was subjected to stress from 2nd day to 7th for 4 days without training.

**Testing:** Then on the 7th day, again the rats in each group were placed on the floor of the staircase and the no. of steps climbed and no of rearings made was recorded.

**FIG. 2: MICE PERFORMING STRESS PROCEDURE AND STAIRCASE METHOD**

**Elevated Plus Maze Method:** The Elevated plus Maze consisting of two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm) was elevated to the height of 25 cm. On the first day, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the closed arm with all its four legs. TL was recorded on the first day. The mouse was allowed to explore the maze for 10 sec and then returned to its home cage. Memory retention was examined for 24 h (day 2) after the first day trial & on the day-9.

Mice received the vehicle, *Ixora coccinea* (75 mg/kg) & *Ixora coccinea* (150 mg/kg), 30 min before their placement on the elevated plus-maze as before and TL was noted [12, 13, 14].

The ‘‘inflexion ratio (IR)’’was calculated using the formula, IR = (L1 – L0)/L0 Where, L0 = transfer latency on day-2/day-9 in sec. L1 = initial transfer latency in sec.

**FIG. 3: MICE PERFORMING ELEVATED PLUS MAZE METHOD**

**Statistics:** The observations are given as means ± S.E.M. The data was analyzed by one- way ANOVA followed by Tukey’s test using Graph Pad Prism [9]. P <0 .05 was considered as significant.

**Results of Phytochemical Analysis:** The present Phytochemical analysis of Ethanolic flower extract of *Ixora coccinea* showed positive result for alkaloids, glycosides, carbohydrates, saponins, coumarin glycosides, flavanoids and steroids.

## RESULTS & DISCUSSION:

**TABLE 1: PHYTOCHEMICAL STUDY OF ETHANOLIC FLOWER EXTRACT OF *IXORA COCCINEA***

| S. no. | Compound | Chemical Test | Ethanolic extract |
|---|---|---|---|
| | | Mayers | +Ve |
| 1 | Alkaloids | Hagers | +Ve |
| | | Murexide | -Ve |
| 2 | | Legal Test | +Ve |
| | Glycosides | Bontrager's Test | +Ve |
| | | Molisch Test | +Ve |
| | | Fehlings | +Ve |
| | | Disaccharides | +Ve |
| | | Salwinoffs | +Ve |
| 3 | Carbohydrates | Iodine | -Ve |
| | | Biurtte | -Ve |
| 4 | Proteins | Millons | -Ve |
| 5 | Saponins | Foam Forming | +Ve |
| 6 | Coumaringlycosides | | +Ve |
| | | Schinoda | +Ve |
| | | Alkaline Sol | +Ve |
| | | $Fecl_3$ | -Ve |
| | | NaOH | +Ve |
| 7 | Flavanoids | Lead Acetate | –Ve |
| 8 | Tannins | $Fecl_3$ | -Ve |
| 9 | Steroids | Salkowski | +Ve |

+ Ve Indicates Present -Ve Indicates Absent

**Staircase Method:**

**No of Steps Climbing and Rearings Using Staircase Method:** In normal control group (which were not subjected to stress) there was significant decrease in no of steps climbed (22.25 ± 0.95) and no. of rearings (24.5 ± 0.57) on day 7 compared to day 1 and 2.

In normal group 2 (75 mg/kg extract-treated) there is a significant decrease in no. of steps climbed (18.25 ± 0.95) and no. of rearings (12.25 ± 1.25) on day 7 compared to control. In normal group 3 (150 mg/kg extract-treated) there is a significant decrease in no. of steps climbed (10 ± 0.81) and no. of rearings (7.5 ± 0.5) on day 7 compared to low dose. In stress-induced control group, on 7th day there was a decrease in no. of steps climbed and no. of rearings which clearly indicates stress-induced amnesia and depression. In stress-induced subgroups, the extract (75 and 150 mg/kg) treated groups exhibited a significant decrease in no. of steps climbed and no. of rearing on day 7 compared to the controlled, treated stress subgroup. Results were given in **Tables 2** & **3.**

**TABLE 2: EFFECT OF ETHANOLIC FLOWER EXTRACT OF *IXORA COCCINEA* ON NO. OF STEPS CLIMBED AND REARINGS IN NORMAL RATS USING STAIRCASE**

| Treatment | Day1 | | Day2 | | Day7 | |
|---|---|---|---|---|---|---|
| | No. of Steps climbed | No. of rearings | No. of steps climbed | No. of rearings | No. of steps climbed | No. of rearings |
| Control | 38.25±0.95 | 35.25±0.5 | 30.5±0.57 | 27.25$^{@}$±1.5 | 22.25*±0.95 | 24.5**±0.57 |
| *Ixora* | | | | | | |
| Coccinea (75 mg/kg) | 25±1.63 | 23.25±0.5 | 20.25±0.95 | 19.25$^{@}$±0.95 | 18.25*±0.95 | 12.25**±1.25 |
| *Ixora* | | | | | | |
| Coccinea (150 mg/kg) | 15.5±1 | 13.75±0.95 | 13±0.5 | 9.25$^{@}$±0.5 | 10*±0.81 | 7.5**±0.5 |

All Values are shown as: mean ± SEM, n=3 in each group Statistical analysis was carried out by one way ANOVA followed by TUKEY's test. *P < 0.05 No. of steps climbed compared to IC (75mg/Kg), @ P < 0.05 No. of rearings on Day: 2, compared to control **P < 0.05 No. of rearings on Day: 7 compared to control

**TABLE 3: EFFECT OF ETHANOLIC FLOWER EXTRACT OF *IXORA COCCINEA* ON NO. OF STEPS CLIMBED AND REARINGS ON 2$^{ND}$ AND 9$^{TH}$ DAY IN STRESS-INDUCED RATS USING STAIRCASE**

| Treatment | Day1 | | Day2 | | Day7 | |
|---|---|---|---|---|---|---|
| | No of steps climbed | No. of rearings | No. of steps climbed | No. of rearings | No. of steps climbed | No of rearings |
| Control | 51.25±1.70 | 45.75±0.95 | 44.5**±1.29 | 37*±0.81 | 38**±0.81 | 27.25*±1.25 |
| *Ixora coccinea* (75 mg/kg) | 32±1.41 | 25±1.41 | 25**±0.81 | 18*±1.41 | 17**±0.81 | 13.25*±1.5 |
| *Ixora* | | | | | | |
| coccinea (150 mg/kg) | 19±1.81 | 16.25±1.25 | 16.25**±0.95 | 13.25*±0.5 | 16.25**±0.5 | 8.75*±1.7 |

All Values are shown as: mean ± SEM, n=3 in each group Statistical analysis was carried out by one –way ANOVA followed by TUKEY's test. **P < 0.05 No. of steps climbed compared to control *P < 0.05 No. of Rearings compared to control

**TABLE 4: EFFECT OF ETHANOLIC FLOWER EXTRACT OF *IXORA COCCINEA* ON TRANSFER LATENCY (IN SEC) ON 2ND AND 9TH DAY IN NORMAL RATS USING ELEVATED PLUS MAZE**

| Treatment | Transfer latency (in sec) | | | Inflection ratio | |
|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 9 | Day 2 | Day 9 |
| vehicle | 72.5±0.19 | 36.75*±0.96 | 27.11*±1.05 | 0.98 | 1.67 |
| *Ixora coccinea* (75 mg/kg) | 66.15±0.96 | 30*±0.73 | 20.13*±0.75 | 0.99 | 2.28 |
| *Ixora* Coccine*a* (150mg/kg) | 40±0.90 | 33.75*±0.75 | 22.23*±0.19 | 1.28 | 2.46 |

All Values are shown as: mean ± SEM, n=3 in each group; Statistical analysis was carried out by one–way ANOVA followed By Tukey's test, *P < 0.05 when compared with control

**TABLE 5: EFFECT OF ETHANOLIC FLOWER EXTRACT OF *IXORA COCCINEA* ON TRANSFER LATENCY (IN SEC) ON 2ND AND 9TH DAY IN STRESS-INDUCED RATS USING ELEVATED PLUS MAZE**

| Treatment | Transfer latency (in sec) | | | Inflection ratio | |
|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 9 | Day 2 | Day 9 |
| Vehicle + stress | 72.5±0.19 | 36.75*±0.96 | 45.5*±1.19 | 0.97 | 0.59 |
| *Ixora* Coccinea (75 mg/kg) *Ixora* | 66.15±0.96 | 31.25*±0.7 | 39.15*±1.2 | 1.11 | 0.68 |
| Coccinea (150 mg/kg) | 51.15±1.05 | 32.13*±04 | 31.09*±0.9 | 1.25 | 0.92 |

All Values are shown as: mean ± SEM, n=3 in each group; Statistical analysis was carried out by one–way ANOVA followed by TUKEY's test.*P < 0.05 when compared with control

**Transfer Latency using Elevated Plus Maze:** In elevated plus maze, the transfer latency *i.e.,* the time taken for the entry in to One of the closed arms from the end of open arm was recorded. The transfer latency was significantly reduced and increased in inflection ratio in 2nd day in extract administered groups than control groups. Normal subgroups which left normal without any stress exhibits a significant decrease in transfer latency and increase in inflection ratio on day 9 also.

The extract treated groups were showed better results on 9th day than control group, and the results were dose-dependent. In stress-induced subgroups, the extract (75 and 150 mg/kg) treated groups exhibited a significant decrease in transfer latency and increased in inflection compared to vehicle-treated stress groups. The results were dose-dependent and indicated a protective effect against stress. These results indicate anti amnesic activity of extract. Results were given in **Tables 4** and **5.**

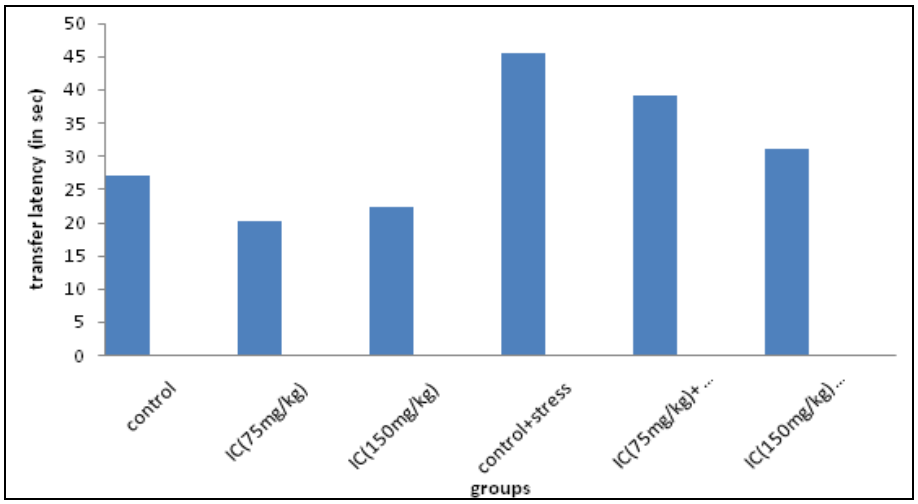


**FIG. 4: GRAPHICAL REPRESENTATION OF ELEVATED PLUS-MAZE METHOD**

Ethanolic flower extract of *Ixora coccinea* was evaluated for anti amnesic activity by Staircase Method and Elevated Plus Maze method. From the results obtained after testing by both Methods, it was concluded that ethanolic flower extract of *Ixora coccinea* possesses potent Anti- amnesic activity at conc. of 150 mg/kg when compared with the control group of rats.

**CONCLUSION:** Ethanolic flower extract of *Ixora coccinea* was done and subjected to Phytochemical Screening. The phytochemical screening results revealed the presence of alkaloids, glycosides, steroids, Carbohydrates, saponins, flavonoids, and coumarin glycosides in the ethanolic flower extract of *Ixora coccinea*. Ethanolic flower extract of *Ixora coccinea* was evaluated for anti-amnesic activity by Staircase method and the Elevated plus Maze method. The ethanolic flower extract of *Ixora coccinea* at various conc. of 75 mg/kg, 150 mg/kg were screened for anti amnesic activity and showed statistically significant results indicating that a high dose of the extract (150 mg/kg) possessed potent antiamnesic activity compared with a stress-induced stress-free control group of rats.

The anti-amnesic activity of ethanolic flower extract of *Ixora coccinea* was due to the presence of various physicochemical constituents like Alkaloids, Glycosides, coumarin glycosides, carbohydrates, flavonoids, and saponins.

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