Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E: ISSN 2278-4136
P: ISSN 2349-8234
JPP 2014; 3(3): 146-154
Received: 03-08-2014
Accepted: 29-08-2014

Foyet Harquin Simplice
Department of Biological Sciences, Faculty of Science, University of Maroua, Cameroon. P.O. Box: 814, Maroua, Cameroon.

Abdou, Bouba Armand
Department of Agriculture, Animal husbandry and Derived products, High Institute of the Sahel, University of Maroua, Cameroon. P.O. Box: 46, Maroua.

Ngatanko Abaissou Hervé Hervé
Department of Life and Earth Sciences, Higher Teachers' Training College, University of Maroua, Cameroon. P.O. Box: 55, Maroua.

Manyi Forka Lucy
Department of Life and Earth Sciences, Higher Teachers' Training College, University of Maroua, P.O. Box: 55, Maroua, Cameroon.

Manyo Nkongho Annabel
Department of Life and Earth Sciences, Higher Teachers' Training College, University of Maroua, Cameroon.

Shu Nyenti Patence Neh
Department of Life and Earth Sciences, Higher Teachers' Training College, University of Maroua, Cameroon.

Asongalem Emmanuel Acha
Department on Biomedical Sciences, Faculty of Health Sciences, University of Buea, Cameroon.

Correspondence:
Foyet Harquin Simplice
Department of Biological Sciences, Faculty of Science, University of Maroua, Cameroon. P.O. Box: 814, Maroua, Cameroon.

Neuroprotective and memory improvement effects of a standardized extract of *Emilia coccinea* (SIMS) G. on animal models of anxiety and depression

Foyet Harquin Simplice, Abdou, Bouba Armand, Ngatanko Abaissou Hervé Hervé, Manyi Forka Lucy, Manyo Nkongho Annabel, Shu Nyenti Patence Neh, Asongalem Emmanuel Acha

Abstract
The present study evaluated the putative effects of the methanolic extract of *Emilia coccinea* leaves (MEC) on the central nervous system, including anxiety, depression-like behavior, and memory, in Wistar rats. The behavioral assays included open-field, elevated plus maze, forced swimming and Y-maze. The antioxidant activity of the extract was also measured *in vitro*. MEC showed a significant antioxidant activity. It significantly increased the number of open arm entries and time spent in the open arms of the elevated plus maze test. The rearing time as well as the time spent at the centre of the open field was significantly increased. MEC also significantly decreased the number of lines crossed and the climbing time on this task. In the forced swimming test, the extract was as effective as Imipramine in inducing shortening of immobility time while after 3 days of treatment, it significantly improves spatial memory in the Y-maze task.

Keywords: *Emilia coccinea*, Antioxidant, flavonoids, Anxiety, Depression, spatial memory

1. Introduction
Emilia coccinea (SIMS) G. (Asteraccae) is an annual herb commonly found throughout the plain of the Central Africa and in dry areas up to 2000 m altitude in the eastern Africa. This species belongs to the genus *Emilia* represented by about 100 species, with 50 of them found in Africa [1]. In traditional medicine, this plant is used for the treatment of fever, convulsions and epilepsy in children [2]. The sap is also applied to ulcers body rashes and abscesses. The dry leaves are used for the treatment of wounds, sores and sinusitis ulcer, ringworm [3], but also to treat jaundice, abdominal pains, and gastritis. In some tribe in the western part of Cameroon, the infusion of the dry leaves of this plant is used as a potent sedative and restorative. Previous phytochemical studies on *E. coccinea* have reported the presence of alkaloids, tannin, saponin, steroid, terpenoid, flavonoid and cardiac glycoside [4-5]. Quantitative estimation of the percentage of crude chemical constituents in the Nigerian *E. coccinea* was 0.92±0.22% of alkaloids, 0.81±0.10% of phenols, 0.96±0.10% of flavonoid, 2.30±0.20 of saponin and 11.85±0.31 of tannin [4].
According to the WHO report, approximately 450 million people suffer from a mental or behavioural disorder. This accounted for 12.3% of the global burden of disease, and this percentage may rise up to 15% by 2020 from predictions. The brain is susceptible to free-radical damage due to its comparatively high levels of oxygen metabolism and also relatively deficient in both free-radical scavenging enzymes and antioxidant molecules as compared to other organs. Oxidative stress by the imbalance between free radicals and the antioxidant system is a prominent and early feature in the pathogenesis of neuronal damage [6].
Different therapeutic regimens are employed to treat anxiety and depressive disorders; but their clinical uses are limited by their side effects such as psychomotor impairment, potentiation of other central depressant drugs and dependence liability. In the search for new therapeutic products for the treatment of neurological disorders, research on medicinal plant has also contributed significantly by demonstrating pharmacological effectiveness of different herbs in various animal models.
Various activities of the entire herb, including antibacterial, antioxidant and anti-inflammatory

activities have been reported in various studies [7], but no scientific data are available for the central nervous systems actions of the leaves of *E. coccinea* although this plant is used for the treatment of some neurological disorders in the western part of the Cameroon. The presence of flavonoids and phenolic compounds in the leaves of *E. coccinea* suggests that this plant possesses antioxidant properties and can have neuroprotective propensity. Therefore, the aim of this study was to examine the antidepressant-like, anxiolytic-like and sedative actions of the methanol extract of the methanolic extract of *Emilia coccinea* using animal models. Putative anxiolytic-like and antidepressant-like properties of *E. coccinea* were studied in the elevated plus-maze, open field and forced swimming test, while the effect on short term memory was investigated in Y-maze.

2. Methods

2.1. Plant material and extraction

Fresh leaves of *E. coccinea* were harvested in September 2013 at Etoug Ebe in the Centre Region of Cameroon and authenticated at the National Herbarium-Yaoundé, where the voucher specimen was conserved under the reference number 6297/HNC. The leaves were then washed and dried at room temperature (24-26 °C during 10 days).

Methanolic extract was prepared as follows: after drying fresh leaves and powdering it, 500 g of the powder were mixed with 500 ml of the solvent at room temperature and agitated for eight hours in a flask shaker using a magnetic agitator. The mixture was then filtered thought a Whatman paper. This was followed by the elimination of the solvent by a rotavapor. The given powder yielded 8.80% of a brown extract. The same process was done for the fresh leaves.

2.2. Experimental animals

Male Wistar albino rats (weighing 100-180 g) were obtained from the Laboratory of Biophysics and Biochemistry of the Department of Food Sciences and Nutrition, University of Ngaoundéré, Cameroon. The animals were housed in polyacrylic cages (6 animals / cage) and maintained at a temperature and light-controlled room (25 ± 2 °C, a 12-h cycle). The animals were acclimatized to laboratory conditions for 7 days before the start of experiment. Prior to and after treatment, the animals were fasted for 12 and 7 h, respectively. However, all animals were allowed to drink water *ad libitum*. Rats were treated in accordance with the guidelines of the Cameroonian Bioethics Committee (reg N°.FWA-IRB00001954) and in accordance with NIH- Care and Use of Laboratory Animals manual (8th Edition).

2.3. Chemicals

Diazepam hydrochloride and Imipramine were purchased from Novartis Turkey and used as reference drugs. All drugs and extracts were freshly prepared in saline on the day of the experiments and administered intraperitoneally (i.p.). Control animals received 10 ml/kg body of the vehicle in the same route of administration.

2.4. *In vitro* analysis

2.4.1. Determination of mineral composition

Micro and macro-elements were determined by dry ashing in muffle furnace 500 °C. 1 g of ground sample in a porcelain crucible was ashed in conventional resistance muffle furnace (CMF). The ash was diluted in 5ml of diluted mixture of HCl/HNO_3 acids, following by 20 mL of hot water and brought to 100 mL in deionised water. Ca, Mg, Na, K, Zn, Cu, Mn, and Fe were analyzed using Atomic Absorption Spectrometer. Phosphorous (P) was also determined as above but analyzed using Murphy Riley reagent and read colorimetrically [8].

2.4.2. Determination of total phenolic content

Total phenol content was determined spectrophotometrically in the extracts by using Folin–Ciocalteu method. 0.04 mL (0.0125 M) of the methanolic extract of *E. coccinea* was added to 1.36 mL distilled water and 0.2 mL of freshly prepared Folin–Ciocalteu reagent, followed by incubation in the darkness for 5 min. Then, 0.4 mL of 20% sodium carbonate solution was added. The test tubes were stirred with the help of a vortex and the samples were incubated at 40 °C in the darkness for 30 min. The UV–vis spectra of all the samples were recorded against the reference solution (zero gallic acid) and the absorbance was read at 760 nm. The measurements were done four times. For the gallic acid standard, a calibration curve (Pearson's correlation coefficient: $R^2 = 0.999$) was constructed and the total level of phenolics for each sample was determined in terms of gallic acid equivalents [9].

2.4.3. Determination of anti-oxidant activity

Two model systems: 2, 4-dinitrophenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and ferric Iron reduced activity assay were used to measure the antioxidant activities of the extract. In the two *in vitro* tests, ascorbic acid and quercetin were used as standard antioxidant compounds respectively.

2.4.3.1. Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of the methanolic extract of *E. coccinea* leaves were evaluated by determining its ability to reduce iron (Fe^{3+}) into Fe^{2+} using Oyaizu method [10]. The methanolic extract of *E. coccinea* leaves (0.1 mL) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium hexacyanoferrate solution ($K_3Fe(CN)_6$) at 1%. The mixture was incubated at 50 °C for 30 min. 2.5 mL of trichloroacetic acid (10%) was added and the mixture centrifuged for 10 min. 0.5 mL was pipetted into a test tube and mixed with 2.5 mL of distilled water and 0.5 ml of aqueous solution of $FeCl_3$ (0.1%). The absorbance was read at 700 nm in a spectrophotometer. Ascorbic acid was used as reference and the total reducing power (Ferric Iron reducing activity) was expressed as ascorbic acid equivalent.

2.4.3.2. Free radical scavenging activity (DPPH assay method)

The free radical scavenging activity of the methanolic extract of *E. coccinea* was evaluated as described by Zhang and Hamauzu [11]. Briefly, 2 mL of DPPH (0.1 mM prepared in methanol) was introduced in each test tube containing 0.25 µL of the fresh extract. The mixture was stirred for 5 min and incubated in darkness for 60 min at room temperature. For the control tube, methanol was used in the place of the extract while quercetin was used as reference at variable concentration. A curve was drawn from this reference and the absorbance read at 517 nm. Each assay was repeated four times and the results, recorded as mean of the fourth experiments.

The antioxidant activity of the extract was expressed as grams of quercetin equivalent/100g of the extract. The inhibition percentage was calculated from the following equation.

$PI\ (\%) = [(DO_{control} - DO_{essay})\ x100]/DO_{control}$

2.5. Behavioral evaluation

2.5.1. Open Field Activity test (OFT)

The open field apparatus was constructed of white polywood and measured 72 x 72 cm with 36 cm walls. Red lines were drawn on the floor with a marker and were clearly visible through the clear Plexiglas floor. Rats were treated (i.p) with single administration of the methanolic extract of *E. coccinea* leaves and the test were performed 30 min after the drug administration of the extract (200 and 400 mg/kg, i.p.) or saline (10 ml/kg). The standard drug diazepam (1 mg/kg, i.p.) was given once 30 min before the test. The rats were placed in the open field box for 5 min, and their behaviors were recorded. The behaviors scored included: time spent at the center square, number of the lines crossed on the floor of the maze, time spent at the border of the maze, grooming (duration of time the animal spent licking or scratching itself while stationary), and the climbing time [12].

2.5.2. Elevated plus-maze test (EPM)

Behavior in the elevated plus-maze (EPM) is used to assess exploratory, anxiolytic and motor activity. The possible anxiolytic effects of the methanolic extract of *E. coccinea leaves* were assessed, basically using the same method described by Casarrubea *et al*. [13]. The EPM consists of four arms, 49 cm long and 10 cm wide, arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 50 cm above the floor. Two arms were enclosed by walls 30 cm high and the other two arms were exposed. Rats were treated i.p. with single administration of the methanolic extract of *E. coccinea* leaves (200 and 400 mg/kg; i.p) or saline (10 ml/kg; i.p). The positive control diazepam (1 mg/kg, i.p) was given once 30 min before the test. Thirty minutes after the i.p. injection of the extract or saline, each animal was placed at the center of the maze facing one of the enclosed arms. During a 5 min test period, the number of open and enclosed arm entries, as well as the time spent in open and enclosed arms, were recorded as previously described [14]. Entry into an arm was defined as the point when the animal places all four paws into the arm. After the test, the maze was carefully cleaned with wet cotton (70% ethanol solution) and allowed to dry before the next animal.

2.5.3. Forced swimming test (FST)

The FST is the most widely used pharmacological models for assessing antidepressant activity [15]. The development of immobility when the rodents are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior [16]. The possible antidepressant effects of the methanolic extract of *E. coccinea* leaves were assessed, basically using the same method described by Kawaura *et al*. [17] with minor modifications. Rats were treated with single administration of the methanolic extract of *E. coccinea* leaves (200 and 400 mg/kg; i.p) or saline (10 ml/kg; i.p). The standard drug imipramine (10 mg/kg, i.p) was given once 30 min before the test. On the first day of the experiments (pretest session), rats were individually placed into transparent Plexiglas cylinder (50 cm high and 20 cm wide) filled to a 30 cm depth with water at 26 ± 1 ºC. The animals were left to swim for 15 min before being removed, dried and returned to their cages.

The procedure was repeated 24 h later, in a 6 min swim session (test session) 30 min after the last dose of the methanolic extract of *E. coccinea* leaves, imipramine or saline. During the test session, the following behavioral responses were recorded: immobility time (time spent floating with the minimal movements to keep the head above the water) and swimming time (time spent with active swimming movements). Increases in active responses, such as climbing or swimming and reduction in immobility, was reconsidered as behavioral profiles consistent with an antidepressant-like action [15].

3. Y-Maze test

Y-maze analysis has been shown to be a reliable, noninvasive test to determine cognitive changes in wistar rat through the measurement of the spontaneous alternation behavior in the Y-maze task. The maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. All animals were tested in a randomized order at the beginning and at the end of the experimental protocol. Rats were treated once daily with the methanolic extract of *E. coccinea* leaves (200 and 400 mg/kg; i.p), diazepam (2 mg/kg, i.p.), Diazepam (2 mg/kg, i.p.) plus *E. coccinea* (400 mg/kg; i.p) or saline (10 ml/kg; i.p) during 3 consecutive days. 30 min after the last administration of the methanolic extract of *E. coccinea* leaves, diazepam or saline solution, rats were placed at the end of one arm and allowed to move freely through the maze for 8 min. The time limit in Y-maze test was 8 min, and every session was stopped after 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as three consecutive entries in three different arms (i.e. A, B, C or B, C, A, etc). The percentage alternation score was calculated using the following formula: Total alternation number/ (Total number of entries − 2) x 100. Furthermore, total number of arm entries was used as a measure of general activity in the animals. The maze was wiped clean with 70% ethanol between each animal to minimize odour cues [18-19].

3.1 Statistical analysis

Data were presented as mean ± SEM values. One-way ANOVA followed by Tukey multicomparaison “t”-test was performed using Graph Pad Prism version 5.00 for Windows, Graph Pad Software, San Diego California USA, www.graphpad.com. A probability level of 0.05 or less was accepted as significant. Pearson’s correlation coefficient and regression analysis were used to evaluate the connection between the working memory errors and some parameters like locomotion, grooming and rearing in the Y-maze test.

4. Results

4.1. Total phenolic content and *in vitro* antioxidant activity of *Emilia coccinea*

The results of the phenolic content showed that the dry leaves of this plant contained 863.04±5.42 mg of GAE/100 g of dry material. This represents a very good content of total

phenolics compounds in the dry leaves and is four fold the total phenolic content of the fresh leaves. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. The preparations were able to reduce the stable free radical DPPH to the yellow-coloured 1,1-diphenyl-2-picrylhydrazyl, with 19.08±0.62 g of quercetin equivalent/100 g of dry material, indicating a weak activity against this radical. The total reducing power was about 4.71±0.04 g of Vit C equivalent/100 g of dry material (Table 1).

Mn and Zn were found to be very high (549.83±0.60 and 46.87±0.01 mg/100 g respectively) in our samples of *E. coccinea* than those reported on other plants [20] (Table 2)

Table 1: Total phenolic content and *in vitro* antioxidant activity of *Emilia coccinea*

Test	Unit	Dry *Emilia coccinea*	Fresh *Emilia coccinea*
Water content	g/100 g	4.41± 0.03[a]	86.05±0.53[b]
Total phenolic content	Mg of GAE/100 g of DM	863.04±5.42[b]	204.15±2.04[a]
Total reducing powder	g of Vit C equivalent/100 g of DM	4.71±0.04[b]	3.24±0.39[a]
Antiradical activity (DPPH)	g of quercetin equivalent/100 g of DM	19.08±0.62[b]	6.40±0.06[a]

Results present as the mean ± S.E.M. of 4 experiments. Values with different letters within a line are significantly different at 0.05 level.

Table 2: Mineral composition of *Emilia coccinea* leaves

Elements	Mg (g/100 g)	Mn (mg/100 g)	Ca (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	Cu (mg/100 g)
E.C	0.52±0.003	549.83±0.60	3.26±0.01	265.35±0.52	46.87±0.01	10.23± 0.003

Results present as the mean ± S.E.M. of 4 experiments. Samples expressed in mg/100g dry weight basis (N=4).

4.2. Effects of the methanolic extract of *E. coccinea* leaves in the open field test in rats

The results given in table 3 indicate that the diazepam (2.0 mg/kg) treated rats showed a significant decrease in number of lines crossed, climbing and rearing as well as the time spent at the border of the field. The time spent at the centre of the field was increased significantly at the same time. MEC treated rats (200 mg/kg) exhibited a significant increase in the time spent at the centre of the maze while the extract was a sedative in all of the applied doses with decreased in the number of line crossed. The animals were most immobile and inactive at the dose 400 mg/kg. The number of lines crossed by the negative control group was greater than that of the extract-treated groups, but, the number of lines crossed by extract-treated groups was not significantly different to that of the diazepam-treated group, as shown in table 4.

4.3. Effects of the extract in the EPM

In the EPM, the MEC (200, 400 mg/kg, i.p.) was found to significantly ($P < 0.05$) decrease the number of entries and the time spent by the rats in the closed arms compared to the control animals (Fig. 1 and 2). With the diazepam (2 mg/kg), the standard drug used in this test, the number of closed arm entries as well as the time spent in the closed arms was significantly decreased ($P < 0.05$). By considering the total number of entries in both the arms (enclosed and open arms), as an index of locomotory activity of the animals, the difference between the total number of lines crossed by the saline treated animals (33±0.96) was not significantly different from those of MEC treated animals at the doses of 200 and 400 mg/kg (25±1.30 and 28±0.84 respectively), ($F(2.86) = 0.70$, $P \geq 0.05$). This difference was also nonsignificant when compared to the diazepam -treated animals (24±1.20) (Data not shown).

Table 3: Effects of the MEC and diazepam in the open field test in rats.

Groups	Dose (mg/kg)	Number of line crossed	Climbing time (s)	Rearing Time (s)	Time spent at the center (s)	Time spent at the border (s)
Control	-	33±3.2	19.4 ±3.12	78.8±16.56	3.80±0.96	268.00±23.60
MEC	200	8.2±2.24***	5.80±2.56***	47.20±11.76	21.20±6.76*	267.00±23.20
MEC	400	5.8±1.76***	3.00±1.20***	24.00±9.60**	12.80±3.04	241.00±16.00
Diazepam	2	5.4±4.32***	2.00±1.20***	1.20±0.32***	114.00±12.00**#£	166.0±44.00****#£

Animals were treated with single dose of the extract (200 or 400 mg/kg, i.p.) or distilled water. In the positive control, diazepam was given only once (2 mg/kg, i.p.) 30 min prior to the test. Results present as the mean ± S.E.M. of 6 animals. Data analysis was performed using One way ANOVA followed by Tukey multicomparaison "t" –test. *** $P < 0.0001$ vs. saline-treated animals; £ $P < 0.0001$ vs. 200 mg/kg; # $P < 0.0001$ vs. group 400 mg/kg.

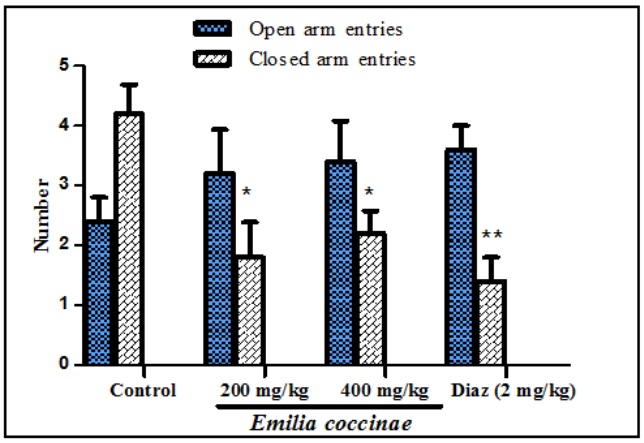

Fig 1: Effect of the methanolic extract of *E. coccinea* leaves on the number of entries in open and closed arm in elevated maze test. Experiments were performed 30 min after the administration of the extract or diazepam (Diaz). Each column represents mean ± S.E.M. of 6 animals. Data analysis was performed using One way ANOVA followed by Tukey multicomparaison "t" –test. * $P < 0.05$ vs. saline-treated animals; ** $P < 0.01$ vs. saline-treated animals.

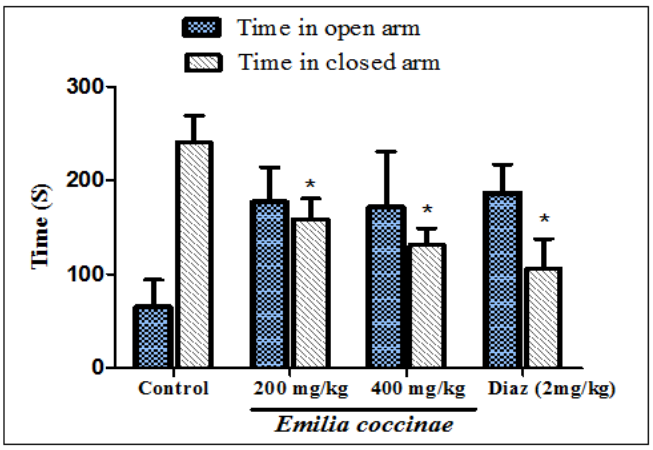

Fig 2: Effect of the methanolic extract of *E. coccinea* leaves on the time spent in open arm and closed arm in elevated maze test. Experiments were performed 30 min after the administration of the extract of *E. coccinea* or diazepam (Diaz). Each column represents mean ± S.E.M. of 6 animals. Data analysis was performed using One way ANOVA followed by Tukey multicomparaison "t" –test. * $P < 0.05$ vs. saline-treated animals.

4.4. Effects of the extract in the FST

The figure 3 shows the effect of MEC for the duration of immobility time in the FST model. One-way ANOVA revealed that there were no significant differences between *E. coccinea* -treatment groups ($F (39.41) = 3.09$, $P > 0.05$).

Post-hoc analysis showed that the MEC (200 and 400 mg/kg) and imipramine treated groups were significantly different ($P < 0.0001$) from the vehicle treated group. MEC significantly increased in the dose dependent manner the duration of swimming time, indicating the antidepressant effect of the extract. This antidepressant effect of the MEC at the dose of 100 mg/kg was comparable to that of imipramine (2 mg/kg).

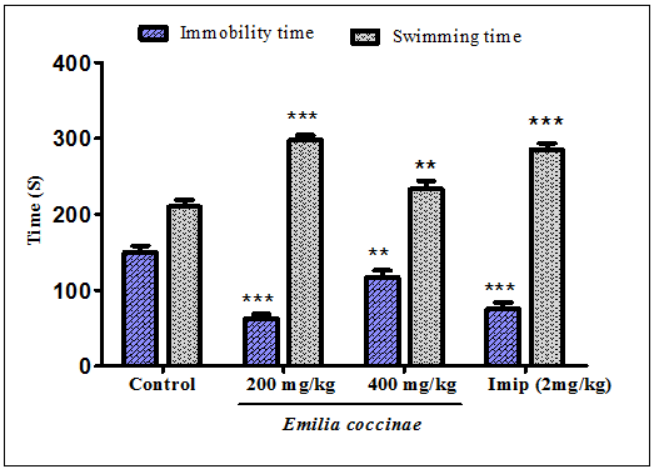

Fig 3: Effect of the methanolic extract of *E. coccinea* leaves on the immobility and swimming time in forced swimming test. Experiments were performed 30 min after the administration of the extract or imipramine (Imip). Each column represents mean ± S.E.M. of 6 animals. Data analysis was performed using One way ANOVA followed by Tukey multicomparaison "t" –test. ** $P < 0.001$; *** $P < 0.0001$ vs. saline-treated animals.

4.5. Effects of the extract in the Y-Maze task

In Y-maze task, we observed after three days administration a significant increase of spatial memory in animal treated with high-dose (400 mg/kg) of the methanolic extract of *E. coccinea* leaves ($F (3.68) = 6.47$, $P < 0.01$) (Fig. 4a), indicated by an increase of spontaneous alternation percentage compared to control group, suggesting effects on short term-memory. At the same time, the plant extract (200, 400 mg/kg) and diazepam (2 mg/kg), significantly, and at a dose -dependent manner, decreased the total number of arm entries of the animals when compared to control group ($F (3.09) = 8.69$, $p < 0.0007$) (Figure 4b). More importantly, when linear regression was determined, no significant positive correlation between spontaneous alternation vs. number of entries in the maze ($n=7$, $r^2=0.237$, $p=0.2678$) (Figure 5) was noted. It can also be clearly realized that Diazepam, $GABA_A$ agonist slightly impaired short term memory of rats, although this was not significant compared to control animals.

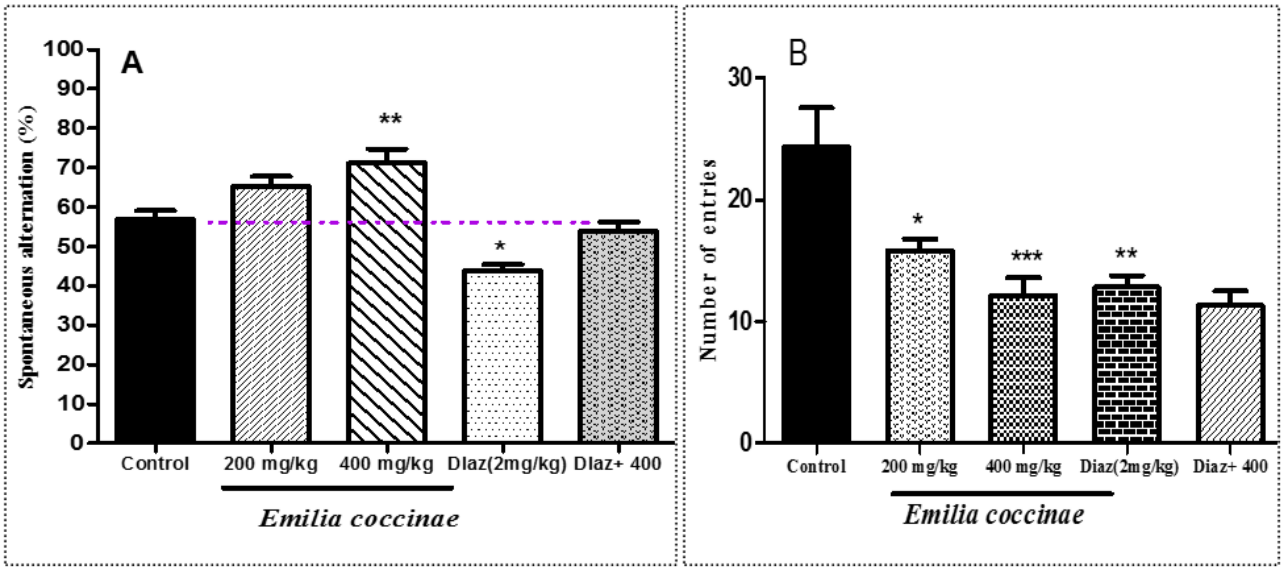

Fig 4: Effect of the methanolic extract of *E. coccinea* leaves and diazepam (Diaz) on the spontaneous alternation percentage (A) and number of entries (B) in Y-maze task. Experiments were performed 30 min after 3 days administration of the extract. Each column represents mean ± S.E.M. of 7 animals. Data analysis was performed using One way ANOVA followed by Tukey multicomparaison "t" –test. * $P < 0.05$; ** $P < 0.001$; ** $P < 0.0001$ vs. control animals; $^{£}$ $P < 0.001$ vs. 400 mg/kg treated animals.

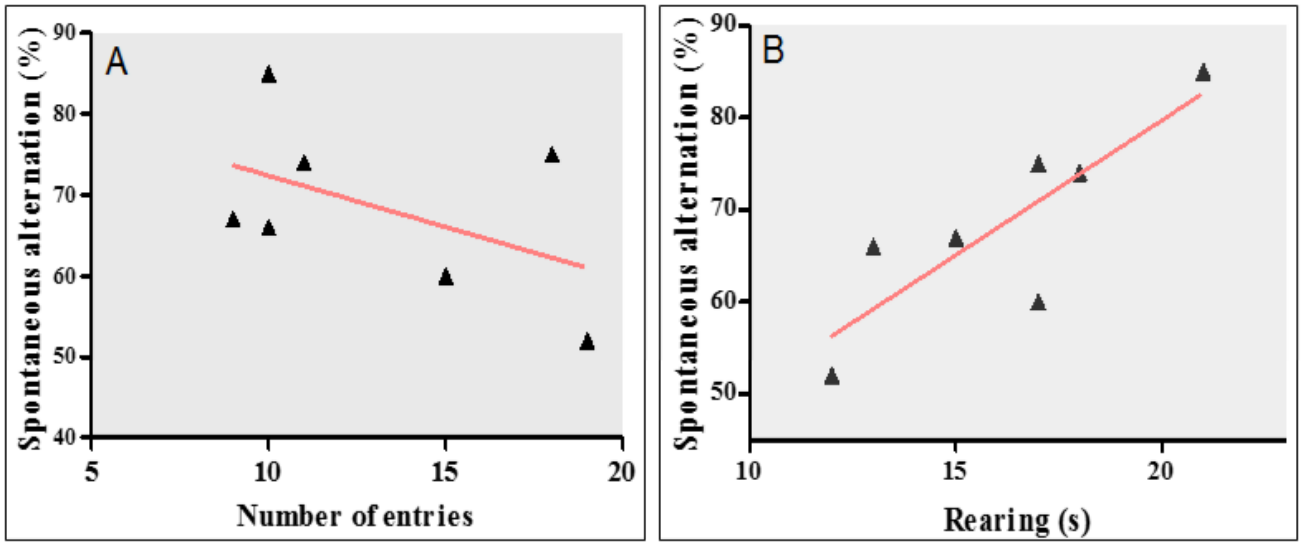

Fig 5: Correlation between working memory errors vs. locomotor activities and rearing behavior of rats treated with the MEC at the dose of 400 mg/kg.

5. Discussion

The present study provides behavioral evidence for the anxiolytic and antidepressant-like activities of *E. coccinea*. The EPM and OF test are regularly used to study anxiolytic effects of plant extract while the forced swimming tests are widely accepted behavioral models for the assessment of antidepressant activity. *E. coccinea* has been used to treat some neurological-related diseases in traditional medicine such as convulsion and epilepsy, but its specific neuropharmacological activities have not yet been demonstrated. The findings of the current investigation show for the first time that MEC, standardized in its content of flavonoids with doses of quercetin (19.08 g /100 g of DM), Vit C (4.71 08 g /100 g of DM), Gallic acid (863.04 mg /100 g of DM), Mn and Zn (549.83±0.60 and 46.87±0.01 mg/100 g of DM respectively) possesses a significant anxiolytic and antidepressant properties.

The open field test is a paradigm used for evaluating the effect of drugs on gross general behavior and is used to measure the level of nervous excitability [21-22]. When removed from their acclimatized home cages and placed in a novel environment, animals express their anxiety and fear by showing decreased ambulation and exploration, immobilization or freezing, and modification in normal rearing and grooming behavior. Increased micturation and defecation due to augmented autonomic activity is also observed. These paradigms are attenuated by classical anxiolytics and potentiated by anxiogenic agents [23]. In the open field behavioral task, the MEC was seen to increase time spent at the center of the maze and decrease peripheral square movements; the observed decrease in central square movements could be due to the impairment of locomotory

activity. The decrease in locomotory (number of lines crossed and rearing) activity in the open field test of rats treated with the extract produces more evidence for its central nervous depressant activity. The decrease in the rearing activity (vertical movement), as well as grooming, an emotional activity parameter, was also significantly affected by treatment with the MEC. Diazepam used as a positive control drug, also significantly reduced anxiety state in the open-field test with some depressive side effects.

A behavioral assay extensively used for the studies of acute behavioral stress reactivity is the elevated plus maze (EPM). When animals were taken from their home cage and given access to either an open maze alley or a closed maze alley, they spent more time exploring the closed arms as a characteristic of an approach-avoidance conflict [24]. The EPM test has several characteristics that make it particularly useful. It reliably detects anxiolytic and anxiogenic activity of a variety of therapeutic and experimental drugs of different classes. Unlike models that require extensive conditioning, it relies on an innate conflict between competing "drives", the balance of which is affected by the level of anxiety. Thus, it requires no training, deprivation, pain or aversive stimuli. The response involves the redirection of an ongoing activity (i.e., exploration) rather than the suppression of behavior, which could be confounded by sedation or ataxia. Several plants increase the exploration of open arms in the elevated plus-maze test and are used to diminish anxiety in folk medicine. Among them are *Trichilia catigua* and *Plumeria rubra* [25-26].

Conventional anxiety indices in the elevated plus-maze test comprise percent open arm entries and percent time spent in these areas in the maze, with anxiolytics generally increasing and anxiogenics decreasing these measures. In this regard, in the elevated plus-maze test, the MEC (200 and 400 mg/kg, i.p.) increased the exploration and the time spent in the open arms in a non-dose-related way. The number of entries and the time spent in the enclosed arms were also significantly reduced when compared to the control group: indicating that the MEC has an anxiolytic-like effect. As expected, diazepam reduced the animal's natural aversion to the open arms and promoted maze exploration. Literature reports describe the action of benzodiazepines, such as diazepam, as anxiolytics when used at the lowest doses, but these effects are associated with the sedation and myorelaxant effects at higher doses. Our results clearly indicated that the dose of diazepam used in this study also act as sedative.

In the forced swimming test, the animals are forced to swim in a very restricted space from which there is no way to escape. They rapidly develop a state of despair behavior characterized by a low motivation for escaping as shown by the increased periods of immobility. In this experiment, the immobility displayed by rodents when subjected to unavoidable stress such as forced swimming is thought to reflect a state of despair or lowered mood, which is thought to reflect depressive disorders in humans. This behavioral test is sensitive to serotoninergic compounds, such as the selective serotonin reuptake inhibitor fluoxetine [12]. The immobility time has also been shown to be reduced by treatment with tricyclic antidepressant drugs like imipramine, which typically increase the swimming efforts of the animal seeking a solution to the problem and, therefore, they decrease the duration of immobility in the forced swimming test [27]. In this study, the single administration of the MEC provoked significant reduction of the immobility time of rats subjected to forced swimming when compare to the control group. This result shows that the extract possesses antidepressant activity on the central nervous system. It is noteworthy that in the FST test, false positive results can be obtained from agents that stimulate locomotory activity [28]. In the open field test, we clearly showed that the MEC significantly reduced the locomotory activity of the animals (number of lines crossed and rearing), this confirms the assumption that the antidepressant-like effect of the extract in the FST is specific [29].

Working memory allows animals to remember information that is useful for a single session of an experiment but not for subsequent sessions and spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory. The Y-maze task is a specific and sensitive test of spatial recognition memory in rodents. The test relies on an innate tendency of rats to explore a novel environment [30]. The Y-maze used in this study involves no aversive stimuli and was considered suitable for evaluating memory and the specific part of the brain involved in performance of this task include the hippocampus [31-33].

As shown in our results, the MEC at the dose of 100 mg/kg did not significantly increase the number of spontaneous alternation. However rats treated with high dose of the methanolic extract of *E. coccinea* (400 mg/kg) showed a significant improvement in spatial learning with an increase number of spontaneous alternations and reduction of a percentage of bias, when compared to control. This result suggests that the plant extract (400 mg/kg) displays improvement effect on acquisition of the short term-memory of the rats within Y-maze task. This effect is however linked to a marked significant decrease in exploratory behavior, probably due to the myorelaxant effect of the extract. At this level of our study it is not possible to suggest any possible mechanism of action of the extract since the process for the acquisition of short-term memory is a very complex biological process. At the same time the implication of the $GABA_A$ agonist in the impairment of the learning and memory in the spontaneous alternation paradigm is clearly evident. However, the results obtained from the linear regression results suggest that the improvement of the acquisition of the short term memory could not be related to the locomotory activities of the animals treated with the MEC. In our experiment, the rats receiving both the MEC and diazepam did no show any sign of memory impairment. This result suggested that the extract may counteract the effects of diazepam, as a GABA antagonist and in that case its anxiolytic effect will be through serotoninergic pathway. This result may also indicate that the MEC does not act through the GABA receptors, during the short term memory process but through other receptors types like glutaminergic, cholinergic or dopaminergic receptors. The implication of these receptors in the process of learning and memory is known well established [34].

Extracts of many plant species that contain a number of polyphenolic compounds have been shown to present antioxidant properties. The antioxidant activity of polyphenolics has been attributed to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors [35]. In the present study, a higher antioxidant activity was observed with the MEC. A very high content of total phenolics has been determined in the *E. coccinea* leaves

(863.04±5.42 mg of GAE/100 g of dry material, 19.08±0.62 g of quercetin equivalent/100 g of dry material), we cannot exclude that the scavenging activity could result from their presence, namely, on the basis of a synergistic effect with other metabolites [36].

The presence of Cu, Zn and Mn ions in our sample, which are metallic co-factor of anti-oxidant enzyme, give credence on the anti-oxidant properties of *E. coccinea*. In this way, antioxidant properties have been related to some of pharmacological effects of secondary metabolites of the plant. Weinreb *et al*. [37] showed that the neuroprotective activities of the green tea are based on the antioxidant activities of epicatechins. Thus, it is possible that both functional and antioxidant activities of the MEC observed in the present work are related.

In conclusion, the present study clearly demonstrated that the methanolic extract of *E. coccinea* leaves treatment could significantly prevent anxiety and depression state. The positive effect of the treatment on memory suggests the therapeutic potential of this extract in aging and age-related neurodegenerative disorders where cognitive impairment is involved. However, for other behavioral effects of MEC and underling mechanism(s) of action, further preclinical investigations are necessary.

6. Competing interest

The authors declare that they have no competing interests.

7. Authors' contributions

FHS, MFL, MNA and SNPN carried out the study; ABA, and FHS, designed the experiments. FHS and NAHH wrote the manuscript; FHS and ABA supervised the work. All authors read and approved the final manuscript.

8. Acknowledgments

Foyet Harquin Simplice was supported by TWAS grant N°: 12-132 RG/BIO/AF/AC_G.

9. References

1. Bosch CH. *Emilia coccinea* (Sims) G.Don In: Grubben, G.J.H. & Denton, O.A. (Editors). PROTA 2, Vegetables/Légumes. PROTA, Wageningen, Netherlands, 2004.
2. Agoha RC. Medicinal plants of Nigeria. Offsetdikker Jifaculteit waskunden, Natnurwenten schopp; pen, Netherlands, 1981; 22-158.
3. Burkill HM. The useful plants of West Tropical Africa Vol 3, Families J-L. Royal Botanical Garden kew, 1984, 522.
4. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some nigerian medicinal plants. African Journal of Biotechnology 2005; 4(7):685-688.
5. Teke GN, Kuiate JR, Ngouateu OB, Gatsing D. Antidiarrhoeal and antimicrobial activities of *Emilia coccinea*(Sims) G. Don extracts. Journal of Ethnopharmacology 2007; 112:278-283
6. Uttara B, Singh VA, Zamboni P, Mahajan RT. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. Current Neuropharmacology 2009; 7(1):65-74.
7. Okiei W, Ogunlesi M, Ademoye MA. An assessment of the antimicrobial properties of extracts of various polarities from *Chasmanthera dependens, Emilia coccinea* and *Cuscuta australis*, herbal medications for eye diseases. Journal of Applied Sciences 2009; 9:4076-4080.
8. Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 1962; 27:31-36.
9. Gao X, Bjo kL, Trajkovski V, Uggla M. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. Journal of Agricultural and Food Chemistry 2000; 80:2021-2027.
10. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 1986; 44:307-315.
11. Zhang D, Hamauzu Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry 2004; 88:503-509.
12. Foyet HS, Tsala ED, Bouba AA, Hritcu L. Anxiolytic and antidepressant-like effects of the aqueous extract of *Alafia multiflora* stem barks in rodents. Hindawi Publishing Corporation. Advances in Pharmacological Sciences, 2012.
13. Casarrubea M, Roy V, Sorbera F, Magnussonb MS, Santangelo A, Arabo A *et al*. Significant divergences between the temporal structure of the behavior in Wistar and in the spontaneously more anxious DA/Han strain of rats tested in elevated plus maze. Behavioral brain research 2013; 250:166-173.
14. Adeyemi OO, Akindele AJ, Yemitan OK, Aigbe FR, Fagbo FI. Anticonvulsant, anxiolytic and sedative activities of the aqueous root extract of *Securidaca longepedunculata* Fresen. Journal of Ethnopharmacology 2010; 130(2):191-195.
15. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future need. Trends in Pharmacological Sciences 2002; 23(5):238-245.
16. Ulak G, Mutlu O, Yıldız Akar F, Komsuoğlu FI, Tanyeri P, Erden BF *et al*. Neuronal NOS inhibitor 1-(2-trifluoromethylphenyl)-imidazole augment the effects of antidepressants acting via serotonergic system in the forced swimming test in rats. Pharmacology Biochemistry and Behavior 2008; 90(4):563-8.
17. Kawaura K, Miki R, Urashima Y, Kawahara R, Soeda F, Shirasaki T, Takahama K *et al*. Pharmacological mechanisms of antidepressant-like effect of tipepidine in the forced swimming test. Behavioral Brain Research 2012; 226:381-385.
18. Siedlak SL, Casadesus G, Webber KM, Pappolla MA, Atwood CS, Smith MA *et al*. Chronic antioxidant therapy reduces oxidative stress in a mouse model of Alzheimer's disease. Free Radical Research 2009; 43(2):156-164.
19. Hernández-Chan NG, Góngora-Alfaro JL, Álvarez-Cervera FJ, Solís-Rodríguez FA, Heredia-López FJ, Arankowsky-Sandoval G *et al*. Quinolinic acid lesions of the pedunculopontine nucleus impair sleep architecture, but not locomotion, exploration, emotionality or working memory in the rat. Behavioral Brain Research 2011; 225:482- 490.
20. Bouba AA, Njintang YN, Foyet HS, Scher J, Mbufong CMF. Proximate composition, minerals and vitamins

contents of some wild plants used as spaces in Cameroun. Food Nutrition Sciences 2012; 3:423-432.
21. File SE, Fernandes C. Dizocilpine prevents the development of tolerance to the sedative effects of diazepam in rats. Pharmacology Biochemistry and Behavior 1994; 47:823-6.
22. Hines TJ, Minton BR. Effects of environmental enrichment on rat behavior in the open field test. Proceedings of the National Conference on undergraduate research (NCUR) 2012 Weber State University, Ogden, 2012, 29-31.
23. Varsha J, Bharatkumar GP. Effect of hydroalcoholic extract of *Sphaeranthus indicus* against experimentally induced anxiety, depression and convulsions in rodents. International Journal of Ayurveda Research 2010; 1(2):87-92.
24. Lapiz-Bluhm MD, Bondi CO, Doyen J, Rodriguez GA, Bédard-Arana T, Orilak DS *et al*. Behavioral assays to model cognitive and affective dimensions of depression and anxiety in rats. Journal of Neuroendocrinology 2008; 20:1115-1137.
25. Chassot JM, Longhini R, Gazarini L, Mello JCP, Weffort-de Oliveira RM. Preclinical evaluation of *Trichilia catigua* extracts on the central nervous system of mice. Journal of Ethnopharmacology 2011; 137:1143 -1148.
26. Chatterjee M, Verma R, Lakshmi V, Sengupta S, Verma AK, Mahdi AA *et al*. Anxiolytic effects of *Plumeria rubra* var. *acutifolia* (Poiret) L. flower extracts in the elevated plus-maze model of anxiety in mice. Asian Journal of Psychiatry 2013; 6(2):13-118.
27. Herrera-Ruiz M, Zamilpa A, Gonzalez-Cortazar M, Reyes-Chilpa R, Leon E, Garcia MP, Tortoriello J, Huerta-Reyes M. Antidepressant effect and pharmacological evaluation of standardized extract of flavonoids from *Byrsonima crassifolia*. Phytomedicine 2011, 18:1255-1261.
28. Bourin M, Fiocco AJ, Clenet F. How valuable are animal models in defining antidepressant activity? Hum. Psychopharmacology 2001; 16:9-21
29. Sanchez-Mateo CC, Bonkanka CX, Prado B, Rabanal RM. Antidepressant properties of some *Hypericum canariense* L and *Hypericum glandulosum* Ait extracts in the forced swimming test in mice. Journal of Ethnopharmacology 2005; 97:541-547.
30. Yusuf S, Adelaiye BA, Agunu A. Effect of *Ziziphus mauritiania* (L.) seed extracts on spatial recognition memory of rats as measured by the Y-maze test. Journal of Natural Products 2009; 2:31-39.
31. Borght KVD, Havekes R, Bos T, Eggen BJL, Zee EAVD. Exercise improves memory acquisition and retrieval in theY-Maze Task: Relationship with hippocampal neurogenesis. Behavioral Neurosciences 2007; 121(2):324-334.
32. Bagheri M, Joghataei MT, Mohseni S, Roghani M. Genistein ameliorates learning and memory deficits in amyloid β(1–40) rat model of Alzheimer' s disease. Neurobiology Learning and Memory 2011; 95:270-276.
33. Liu DS, Zhou YH, Liang ES, Li W, Lin WW, Chen FF *et al*. Neuroprotective effects of the Chinese Yi-Qi-Bu-Shen recipe extract on injury of rat hippocampal neurons induced by hypoxia/reoxygenation. Journal of Ethnopharmacology 2013; 145:168-174.
34. Myhrer T. Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. Brain Research Reviews 2003; 41:268-287.
35. Zhang G, Hu M, He L, Fu P, Wang L, Zhou J *et al*. Optimization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities in vitro. Food and Bioproducts Processing 2013; 91(2):158-168.
36. Saito M, Sakagami H, Fujisawa S. Cytotoxicity and apoptosis induction by butylated hydroxyl anisole (BHA) and buty lated hydroxytoluene (BHT). Anticancer Research 2003; 23:4693-4701.
37. Weinreb O, Mandel S, Bar-Am O, Yogev-Falach M, Avramovich-Tirosh Y, Mit T *et al*. Multifunctional neuroprotective derivatives of rasagiline as anti-alzheimer's disease drugs. Neurotherapy 2009; 6:163-174.