



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Original article doi: 10.1016/S2222-1808(15)60875-1

©2015 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Evaluation of antinociceptive, anti-inflammatory and anxiolytic activities of methanolic extract of *Terminalia citrina* leaves

Narhari Das^{1*}, Durajan Goshwami¹, Md. Sharif Hasan¹, Zobaer Al Mahmud¹, Sheikh Zahir Raihan¹, Md. Zakir Sultan²¹Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh²Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka-1000, Bangladesh

ARTICLE INFO

Article history:

Received 9 Apr 2015

Received in revised form 31 Apr, 2nd

revised form 4 May 2015

Accepted 20 May 2015

Available online 1 Jun 2015

Keywords:

Terminalia citrina

Antinociceptive activity

Tail flick

Elevated plus maze

Anti-inflammatory activity

ABSTRACT

Objective: To investigate the antinociceptive, anti-inflammatory and anxiolytic effects of methanolic extracts of *Terminalia citrina* (*T. citrina*) leaves (Family: Combretaceae).**Methods:** The antinociceptive activity was evaluated by acetic acid induced writhing method and radiant heat tail flick method while anti-inflammatory activity was evaluated by human red blood cell membrane stabilization method and anxiolytic activity by elevated plus maze model.**Results:** The methanolic extract of *T. citrina* leaves showed significant antinociceptive, anti-inflammatory and anxiolytic effects in dose dependent manner compared to their respective standard drugs.**Conclusions:** The present study demonstrated that *T. citrina* possesses antinociceptive, anti-inflammatory and anxiolytic effects.

1. Introduction

As nature gives us problems, so it gives the solution. Before civilization, when there were no hospitals and no man-made industries to cause pollution, there were diseases, and those diseases were cured by nature itself through the medicinal plants. Over the centuries, man has learnt to synthesize medicine, but some plants possess so wide range of pharmacological activities, that it is too difficult to synthesize the constituents in laboratory. Right screening of plants for finding a proper lead is required, focusing on bioactivities[1-6].

Terminalia citrina (*T. citrina*) (Bengali name: Haritaki, Family: Combretaceae) is a deciduous tree wide spread throughout the forest of Gazipur, Tangail, Sylhet, Chittagong, Rangamati and Chittagong hill tracts of Bangladesh. It is an important medicinal plant having various ethnopharmacological uses. Different parts of the plant

are used for various ailments. The fruit is used in long-term fever, loss of appetite and as sexual stimulant in Bangladesh[7], diarrhea, helminthes and other digestive disorders in Iran[8]. Its bark is diuretic and cardio tonic[9]. Seed is used in stomach aches and intestinal diseases[10]. The plant is also used in asthma, diarrhea, boils, burns, constipation, migraine, dental disease, haemoptysis, dizziness, bleeding hemorrhoids, eye disease, gastric hyperacidity, anaemia, arthritis, hoarse voice, dysentery, pyrexia, infections, traumatic cuts, cardiac diseases, cough, hepatomegaly, urolithiasis and for life longevity in Myanmar[11]. A detailed literature survey revealed that seed of plant was reported to possess antioxidant property[12] and five tannins identified as corilagin (1) (3), punicalagin (2) (4), 1,3,6-tri-O-galloyl-β-D-glucopyranose (3) (5), chebulagic acid (4) (6), and 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (5) (7) were isolated from methanol extract of fruit[13].

However, no detailed pharmacological study has been reported in the literature. Therefore, in the present investigation, we screened the leaf extracts of *T. citrina* for antinociceptive, *in vitro* anti-

*Corresponding author: Narhari Das, Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
Tel: +88- 01675490063
E-mail: narhari10@gmail.com

inflammatory and anxiolytic activities to provide a scientific basis for its use in traditional medicine.

2. Materials and methods

2.1. Plant material

The leaves of *T. citrina* were collected from Rangamati district, Bangladesh during the month of January 2013. The plants were mounted on paper and the sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB accession number-38094).

2.2. Preparation of methanolic extract

The collected plant leaves were sun dried for several days and then oven dried for 24 h at 40 °C to facilitate grinding. The powdered plant leaves (1000 g) of *T. citrina* was extracted with about (4 L) methanol for 15 days and then filtered through a cotton plug followed by Whatman filter paper No. 1. The extract was then concentrated by using a rotary evaporator at reduced temperature (40–45 °C) and pressure to afford crude methanolic extract (50 g).

2.3. Animals

For the experiment Swiss albino mice of either sex, 4–5 weeks of age, weighing between (20–25 g) were collected from the Animal Resources Branch of Jahangirnagar University, Dhaka, Bangladesh. The mice were maintained under standard environmental condition and had free access to standard pellets as basal diet and water. All experimental protocols were approved by Dhaka University, Faculty of Pharmacy Ethics Committee.

2.4. Chemicals and reagents

Diclofenac, morphine and diazepam were collected from ACI Pharmaceuticals, Popular Pharmaceuticals and Gonoshasthaya Pharmaceuticals Ltd. Dhaka, Bangladesh respectively. Acetic acid was obtained from Merck, Germany. All others chemicals were obtained commercially and were of analytical grade.

2.5. Phytochemical screening

The preliminary phytochemical group test was carried out by following standard procedure[14].

2.6. Antinociceptive study

2.6.1. Acetic acid-induced writhing method

Antinociceptive activity was evaluated by the acetic acid induced

writhing method in mice[15]. Twenty Swiss albino mice were divided into four groups consisting of five animals in each. Each group received particular treatment as shown in Table 1. Then the response of the extract and standard (diclofenac sodium) treated groups was compared with those of the animals in the control group. Percentage inhibition of writhing in comparison to control group was taken as an index of analgesia and was calculated using the following formula:

$$\text{Inhibition (\%)} = [(Wc - Wt) \times 100] / Wc$$

Where Wc is the average number of writhing reflex in the control group and Wt is the average number of writhing reflex in the test group.

Table 1

Effect of oral administration of methanolic extract of *T. citrina* on acetic acid induced writhing in mice.

Group	Number of writhing	Inhibition of writhing (%)
Control	27.2 ± 1.06	—
Standard 50 mg/kg	12.2 ± 0.86**	55.14
<i>T. citrina</i> 200 mg/kg	21.6 ± 1.28*	20.58
<i>T. citrina</i> 400 mg/kg	19.6 ± 0.92**	27.94

Each value represents Mean ± SEM. **: $P < 0.001$, *: $P < 0.01$, compared with control. (One way ANOVA followed by Dunnett's *t*-test)

2.6.2. Tail flick method

Antinociceptive activity was evaluated by radiant heat tail flick method[16]. Test samples and control was given orally by means of a feeding needle to the mice at zero hour. A 30-minute interval was given to ensure proper absorption of the administered substances. After 30 min, the tail flicking time was measured by Analgesiometer (Medicraft, India). Morphine (2 mg/kg, *s.c.*) was used as standard. The strength of the current passing through the naked nicrome wire was kept constant at 3 A. The tail skin was kept at a distance of 1.5 cm from the heat source. The radiant heat [(55 ± 2) °C] in the tail was applied and maintained at 2.5 cm measured from the root of the tail. In order to avoid the tissue damage, the cut of reaction time was kept at 16 seconds.

2.7. In vitro anti-inflammatory activity

2.7.1. Preparation of red blood cells

Fresh blood was collected from healthy human donor not consuming any non-steroidal anti-inflammatory drugs for past two weeks into an anticoagulant (containing 3.8% trisodium citrate) in a clean sterile bottle. The blood was centrifuged at room temperature and the supernatant was carefully decanted. The packed human red blood cell (HRBC) was resuspended in normal saline followed by centrifugation. The process of washing and centrifugation was repeated until the supernatants were clear. A 10% HRBC suspension was then prepared with normal saline and used immediately[17].

2.7.2. Membrane stabilizing activity assay

The membrane stabilizing activity assay was carried out by

procedure that was described by Gadamsetty *et al.*[18]. The assay mixture consisted of hyposaline (0.25% w/v NaCl, 2 mL), 0.15 mol/L sodium phosphate buffer, pH 7.4 (1 mL), 1 mL of extract, erythrocyte suspension (2% v/v, 0.5 mL) and isosaline (0.85% (w/v) NaCl) to give total assay volume of 4.5 mL. The blood control (4.5 mL) was prepared with 1.0 mL of isotonic saline instead of extract, while drug control (4.5 mL) with 0.5 mL of isotonic saline instead of red blood cells. The reaction mixtures were incubated at 56 °C for 30 min. The tubes were cooled under running water followed by centrifugation at 3000 r/min for 20 min on visible spectrophotometer (Spectrumlab 23A). The supernatants were collected and the absorbance was read at 560 nm against reagent blank. The percentage membrane stability was estimated from the expression shown below. Naproxen was used as standard.

$$\% \text{ of Membrane stability} = 100 - \frac{(\text{Abs of drug sample} - \text{Abs of drug control}) \times 100}{\text{Abs of blood control}}$$

Blood control represented 100% lysis or zero percent stability.

2.8. Anxiolytic activity by elevated plus maze (EPM) test

The plus maze apparatus consisted of two open arms, measuring 16 cm × 5 cm, and two closed arms, measuring 16 cm × 5 cm × 12 cm, connected to a central platform (5 cm × 5 cm). The maze was elevated to a height of 25 cm above the floor. Prior to the test, mice were treated with respective drugs. After 45 min prior to experiment, each mouse was placed individually at the center of EPM with its head facing toward an open arm and observed for 5 min to record the number of entries into open arm, closed arm and time spent in each arm[19]. Diazepam (2 mg/kg, *p.o.*) was used as standard.

2.9. Statistical analysis

All values were expressed as the mean ± SEM and the results were analyzed statistically by One way ANOVA followed by Dunnett's *t*-test by using SPSS Ver.16. *P* < 0.05 compared to standard was considered to be statistically significant.

3. Results

3.1. Phytochemical screening

The phytochemical screening test showed the presence of

alkaloids, flavonoids, tannins, reducing sugar and carbohydrates in the leaves of *T. citrina*.

3.2. Acetic acid-induced writhing method

The effect of the ethanolic extract of leaves of *T. citrina* on acetic acid induced writhing in mice was shown in Table 1. Percentage of inhibition (writhing) of *T. citrina* extracts for 200 and 400 mg/kg body weight was 20.58% and 27.94% respectively and diclofenac sodium was 55.14%. Thus the extract showed significant antinociceptive activity compared with standard diclofenac sodium. The results were statistically significant (*P* < 0.001).

3.3. Tail flick method

The extract elongated the reaction time in a dose dependent manner as shown in Table 2. Percentage of elongation of *T. citrina* extract for 400 mg/kg body weight was 30.94% (*P* < 0.05) and 136.48% of standard morphine after 30 min administration of sample. The result was found to be statistically significant in comparison to control.

3.4. In vitro membrane stability activity

The results were shown in Table 3. The extract showed significant anti-inflammatory activity in a concentration dependent manner. The extract at a concentration of 2000 µg/mL showed 67.85% protection of HRBC in hypotonic solution compared with standard naproxen which showed 73.21% protection.

Table 3

Screening of *in vitro* evaluation of anti-inflammatory activity of methanolic extract of *T. citrina* leaves.

Test sample	Concentration (µg/mL)	Absorbance (560 nm)	Protection (%)
Control	—	0.112 ± 0.001	—
Standard (Naproxen)	100	0.093 ± 0.001 [*]	68.75
	1000	0.092 ± 0.001 [*]	69.64
	2000	0.088 ± 0.001 [*]	73.21
<i>T. citrina</i>	100	0.119 ± 0.001 [*]	45.53
	1000	0.100 ± 0.001 [*]	62.50
	2000	0.094 ± 0.002 [*]	67.85

Each value represents mean ± SEM; *: *P* < 0.001 compared with control; One way ANOVA followed by Dunnett's *t*-test.

3.5. EPM model

The results showed that the number of open arm entries and

Table 2

Assessment of central antinociceptive activity by tail flick method.

Group	30 min		60 min		90 min	
	Reaction time (second)	% Elongation	Reaction time (second)	% Elongation	Reaction time (second)	% Elongation
Control	6.14 ± 0.59		6.20 ± 0.40		6.42 ± 0.28	
Standard 2 mg/kg	14.52 ± 0.49 ^{**}	136.48	13.30 ± 0.44 ^{**}	114.51	11.40 ± 0.40 ^{**}	77.57
<i>T. citrina</i> 200 mg/kg	5.02 ± 0.32	18.24	5.06 ± 0.37	18.38	5.48 ± 0.41	15.10
<i>T. citrina</i> 400 mg/kg	8.04 ± 0.39 [*]	30.94	8.10 ± 0.44 [*]	30.64	8.38 ± 0.65 [*]	30.52

Each value represents mean ± SEM. **: *P* < 0.01; *: *P* < 0.05 compared with control; One way ANOVA followed by Dunnett's *t*-test.

time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard diazepam (Table 4).

Table 4

Effects of methanolic extract of *T. citrina* leaves on behavior of mice.

Group	Open arm entries	Close arm entries	Time spent in open arm (second)	Time spent in close arm (second)
Control	2.33 ± 0.21	2.83 ± 0.40	6.83 ± 0.47	293.16 ± 0.47
Diazepam 2 mg/kg	5.16 ± 0.54**	3.83 ± 0.47	18.50 ± 1.08**	281.50 ± 1.08**
<i>T. citrina</i> 200 mg/kg	2.66 ± 0.33	3.33 ± 0.42	7.33 ± 0.66	292.66 ± 0.66
<i>T. citrina</i> 400 mg/kg	3.83 ± 0.30*	3.16 ± 0.47	11.00 ± 1.31*	289.00 ± 1.31*

Each value represents mean ± SEM; **: $P < 0.01$; *: $P < 0.05$ compared with control; One way ANOVA followed by Dunnett's *t*-test.

4. Discussion

Natural products derived from plants have been used in folklore medicine to treat different diseases. So with the objective to contribute to the knowledge of the medicinal flora and considering the use of this plant, a pharmacological study of the methanol extract of the leaves of *T. citrina* was done. To our knowledge, this is the first time that the antinociceptive, *in vitro* membrane stabilizing property and anxiolytic activities in experimental models were reported.

The antinociceptive activity of the extract was demonstrated using two classical *in vivo* models. On the one hand, the extract showed a significant anti-inflammatory activity *in vitro* HRBC membrane stabilizing model. Similarly, *T. citrina* extract demonstrated to possess anxiolytic activity in EPM model. In acetic acid induced writhing method, it was observed that there was a dose dependent reduction in the number of writhes in the test animals. Acetic acid-induced abdominal writhing model represents pain sensation by releasing arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism[20]. Prostanoids such as prostaglandin E2 and prostaglandin F2 as well as lipoxigenase products have been found at a higher level in the peritoneal fluid after intraperitoneal injection of acetic acid. The antinociceptive effect occurs due to its action on visceral receptors that is sensitive to acetic acid by inhibiting the production of algogenic substances or inhibiting the transmission of painful messages at the central level[21]. The acetic acid induced writhing response is a sensitive method to evaluate peripherally acting antinociceptive activity. Thus, the reduction in the number of writhing indicates that extract might exert antinociceptive activity by inhibition of prostaglandin synthesis or action of prostaglandins. In the tail flick model, reaction time was increased significantly for the test samples and standard drug when compared to the predrug reaction time (control group) 30 min after drug administration. The tail flick test is considered to be selective to examine compounds acting through opioid receptor, the extract increased mean basal latency time which indicates that it may act via centrally mediated antinociceptive mechanism[22]. Narcotic antinociceptive

inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. The extract exhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic antinociceptive. It has been reported that tannins and alkaloids have ability to inhibit pain perception[15]. So these phyto-constituents might be responsible for the antinociceptive activity.

It is evident that the methanolic extract protected the human erythrocyte membrane against lysis induced by hypotonic solution. The erythrocyte membrane may be considered a model of the lysosomal membrane which plays an important role in inflammation[23]. The compounds which prevent the lysis of membrane caused by the release of hydrolytic enzymes contained within the lysosomes may relieve some symptoms of inflammation[24]. When the HRBC is subjected to hypotonic stress, the hemoglobin release from HRBC will be prevented by anti-inflammatory drugs because of the membrane stabilization. It has been demonstrated that certain herbal preparations were capable of stabilizing the red blood cell membrane and this may be indicative of their ability to exert anti-inflammatory activity[23]. The mode of action of the extracts or drugs, may bind to the erythrocyte membranes with subsequent alteration of the surface charges of the cells. It has been reported that certain saponins and flavonoids exerted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro*, while tannins and saponins possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules[25]. The presence of flavonoids and tannins in the extract may be attributed to the observed anti-inflammatory activity.

The EPM is an animal model of anxiety useful to predict the anxiolytic effects of benzodiazepines (BZD)[26]. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform[27]. In EPM, animals will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Anxiolytic drugs increase the number of entries and time spent in open arms in comparison to closed arms. They also increase the ratio of open arm to total arm entries. The administration of methanolic extract of *T. citrina* leave increased the number of entries in the open arms and prolonged the time spent into open arms. It has been found that flavonoids bind with high affinity at BZD site of the GABAA receptor[26]. The mechanism of anxiolytic action of extract could be due to the binding of this phytochemical to the GABA-BZD complex. The presence of flavanoid in the extract as revealed by phytochemical screening may be responsible for the observed anxiolytic activity.

The present study was observed that leaves of *T. citrina* showed a significant antinociceptive, anti-inflammatory and anxiolytic activity which deserves further investigation to isolate the active

constituents responsible for these activities and to establish the mechanisms of action.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors are grateful to the Pharmacology Laboratory, Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Bangladesh for providing necessary chemical, instruments and laboratory facility.

References

- [1] Benites J, Gutierrez E, Lopez J, Rojas M, Rojo L, Costa Mdo C, et al. Evaluation of analgesic activities of tremetone derivatives isolated from the Chilean *altiplano* medicine *Parastrephia lepidophylla*. *Nat Prod Commun* 2012; **7**: 611-4.
- [2] Burnett J, Newman B, Sun D. Targeting cancer stem cells with natural products. *Curr Drug Targets* 2012; **13**: 1054-64.
- [3] Christen P, Cuendet M. Plants as a source of therapeutic and health products. *Chimia (Aarau)* 2012; **66**: 320-3.
- [4] Hung HY, Qian K, Morris-Natschke SL, Hsu CS, Lee KH. Recent discovery of plant-derived anti-diabetic natural products. *Nat Prod Rep* 2012; **29**: 580-606.
- [5] Lovkova Mla, Buzuk GN, Sokolova SM, Kliment'eva NI. [Chemistry of medicinal plants (a review)]. *Prikl Biokhim Mikrobiol* 2001; **37**: 261-73. Russian.
- [6] Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 2012; **75**: 311-35.
- [7] Hossain MS, Hanif A, Khan M, Bari S, Jahan R, Rahmatullah M. Ethnobotanical survey of the Tripura tribe of Bangladesh. *Am Euras J Sustain Agric* 2009; **3**(2): 253-61.
- [8] Amiri MS, Joharchi MR. Ethnobotanical investigation of traditional medicinal plants commercialized in the markets of Mashhad, Iran. *Avicenna J Phytomed* 2013; **3**: 254-71.
- [9] Khare CP. *Indian medicinal plants, an illustrated dictionary*. Berlin: Springer-Versa; 2007, p. 654.
- [10] Lev E, Amar Z. Ethnopharmacological survey of traditional drugs sold in Israel at the end of the 20th century. *J Ethnopharmacol* 2000; **72**(1-2): 191-205.
- [11] Soe K, Ngwe TM. *Medicinal plants of Myanmar, Combretaceae*. Myanmar: Forest Resource Environment Development and Conservation Association (FREDA); 2004.
- [12] Palasuwan A, Soogarun S, Lertlum T, Pradnawat P, Wiwanitkit V. Inhibition of Heinz body induction in an *in vitro* model and total antioxidant activity of medicinal Thai plants. *Asian Pacific J Cancer Prev* 2005; **6**: 458-63.
- [13] Burapadaja S, Bunchoo A. Antimicrobial activity of tannins from *Terminalia citrina*. *Planta Med* 1995; **61**: 365-6.
- [14] Ganguly A, Mahmud ZA, Uddin MMN, Rahman SA. *In-vivo* anti-inflammatory and anti-pyretic activities of *Manilkara zapota* leaves in albino Wistar rats. *Asian Pac J Trop Dis* 2013; **3**(4): 301-7.
- [15] Hossain H, Jahan IA, Islam HS, Kanti DS, Arpona H, Arif A. Phytochemical screening and anti-nociceptive properties of the ethanolic leaf extract of *Trema cannabina* Lour. *Adv Pharm Bull* 2013; **3**(1): 103-8.
- [16] Goshwami D, Rahman MM, Muhit MA, Islam MS. Antinociceptive activity of leaves of *Lasia spinosa*. *Arch Appl Sci Res* 2012; **4**(6): 2431-4.
- [17] Amujoyegbe OO, Agbedahunsi JM, Akinpelu BA, Oyedapo OO. *In vitro* evaluation of membrane stabilizing activities of leaf and root extracts of *Calliandra portoricensis* (JACQ) benth on sickle and normal human erythrocytes. *Int Res J Pharma Pharma* 2012; **2**(8): 198-203.
- [18] Gadamssety G, Maru S, Tyagi A, Chakravarthula SN. Anti-inflammatory, cytotoxic and antioxidant effects of methanolic extracts of *Drypetes sepiaria* (Euphorbiaceae). *Afr J Tradit Complement Altern Med* 2013; **10**(5): 274-82.
- [19] Lakshmi BVS, Sudhakar M, Ramya RLV. Anti-anxiety activity of *Moringa oleifera* assessed using different experimental anxiety models in mice. *J Pharm Res* 2014; **8**(3): 343-8.
- [20] Adedapo AA, Sofidiya MO, Afolayan AJ. Anti-inflammatory and analgesic activities of the aqueous extracts of *Margaritaria discoidea* (Euphorbiaceae) stem bark in experimental animal models. *Rev Biol Trop* 2009; **57**: 1193-200.
- [21] Sekhar NC, Jayasree T, Ubedulla S, Dixit R, Manohar VS, Shankar J. Evaluation of antinociceptive activity of aqueous extract of bark of *Psidium guajava* in albino rats and albino mice. *J Clin Diagn Res* 2014; **8**(9): HF01-4.
- [22] Vogel HG. Analgesic, anti-inflammatory, and anti-pyretic activity. In: Vogel H, editor. *Drug discovery and evaluation: pharmacological assays*. 3rd ed. Berlin: Springer-Verlag; 2008, p. 983-1113.
- [23] Kumbhare MR, Sivakumar T, Morankar PG, Kalantri M. Membrane stabilizing potential and antimicrobial activity of the pods of *Caesalpinia pulcherrima* (Caesalpinaceae) against selected microbes. *Indo Am J Pharm Res* 2013; **3**(4): 3472-80.
- [24] Gadamssety G, Maru S, Sarada NC. Antioxidant and anti-inflammatory activities of the methanolic leaf extract of traditionally used medicinal plant *Mimusops elengi* L. *J Pharm Sci Res* 2013; **5**(6): 125-30.
- [25] Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. *Int J Plant Physiol Biochem* 2010; **2**(4): 46-51.
- [26] Tiwari P, Verma R, Ahirwar D, Chandy A, Dwivedi S. Evaluation of anxiolytic effect of *Syzygium aromaticum*: a traditional herb of India. *Asian Pac J Trop Dis* 2014; **4**(Suppl 1): S77-80.
- [27] Clement Y, Chapouthier G. Biological bases of anxiety. *Neurosci Biobehav Rev* 1998; **22**(5): 623-33.