Journal of Pharmaceutical Research Vol. 8, No. 1, January 2009 : 30-34.

ANTINOCICEPTIVE AND ANTIOXIDANT ACTIVITY OF VARIOUS PARTS OF *CLITORIA TERNATEA* (FABACEAE)

Bhalke RD* and Anarthe SJ
Sanjivani College of Pharmaceutical Education and Research, Department of Pharmacognosy, Post – Shingnapur, Kopragaon (M.S.)

Received on : 11.05.2008 Revised : 05.01.09 Accepted : 07.01.09

ABSTRACT

Present study reports analgesic activity and antioxidant activity of methanolic extracts of flowers, leaves, stems and seeds and roots of the plant *C. ternatea*. Hot plate and tail immersion methods were used for evaluation of central analgesic activity and acetic acid induced writhing model was used for evaluation of peripheral analgesic activity. *In-vitro* antioxidant activity was evaluated using DPPH, reducing power assay, lipid peroxidation and nitric oxide scavenging models.

Results indicate that methanolic extract of roots at 50 mg/kg, i.p. dose produced a significant increase in reaction time ($P<0.01$) in tail immersion method and increase in response latency period ($P<0.05$, $P<0.01$) in hot plate method, against standard drug pentazocin. Methanolic extract significantly ($P<0.01$) attenuated the number of writhing when compared to standard drug paracetamol in acetic acid induced writhing model.

Methanolic extract of roots of *C. ternatea* inhibited DPPH free radical, nitric oxide and lipid peroxidation scavenging activity at IC_{50} value 45.29, 52.83, 59.14 ìg/ml against corresponding standards ascorbic acid 44.91, 43.80, 52.28 ìg/ml. respectively.

Key words: *Clitoria ternatea*; analgesic activity; antioxidant activity.

INTRODUCTION

C. ternatea is medium size climber which blooms profusely. It is found in white flowered and blue flowered varieties. Commonly *C. ternatea* is known as the *aparajita.* This plant is used as laxative, diuretic, brain tonic, antiulcer, and in the treatment of headache and snakebite [1, 2].

Plant shows the significant activities like immunomodulary, antistress, antidepressant, anti-convulsant, anti-inflammatory, antipyretic effects[3, 4] and increase in memory[5]. Anthocynanins, flavonoids and flavanol glycosides have been isolated.[6- 8]

The present work evaluates the analgesic and antioxidant activity of various parts of the plant *C. ternatea.*

MATERIALS AND METHODS

Plant material

Flowers, leaves, stem seeds and roots of the plant *C. ternatea* was collected from the western rural area of the Shirdi, Ahmednagar District (M. S.) during June at the flowering stage of the plant. It was authenticated by the Botanical Survey of India, Pune. (Voucher specimen no. BRD-2)

Preparation of extracts

Flowers, leaves, stem seeds and roots of the plant *C. ternatea* were shade dried, reduced to coarse powder and subjected to cold maceration by using solvent methanol for 7 days. Extracts were vacuum dried.

Animals

Healthy wistar albino mice of either sex and of approximately the same age, weighing about 20-25 gm were used for study. They were housed in polypropylene cages maintained under standard condition (12hour light/12 hour dark cycle; 30±4°C, 36-60 humidity).

The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Chemicals

The following drugs were used: pentazocine lactate injection (Ranbaxy, Ahmedabad), paracetamol injection (Heilenlab, Goa), acetic acid (AR Grade, PCL, Pune), DPPH (Sigma chemicals), thiobarbituric acid (Loba Cchemie). Methanol, ferric chloride, sulphanilamide, sodium nitroprusside, O-phosphoric acid, ascorbic acid, napthyl ethylene diamine hydrochloride, trichloroacetic acid, potassium ferricyanide, potassium chloride butylated hydroxyl toluene, butylated hydroxyanisole, potassium dihydrogen phosphate were of AR Grade, PCL, Pune. Methanolic extract of various parts of *C. ternatea* were suspended in a minimum volume of DMF and then the volume was adjusted with water for injection, and administered intraperitoneally. All drug solutions were prepared fresh immediately before starting the experiment.

***Correspondence :** rasikabhalke@yahoo.co.in

Acute toxicity study

Swiss albino mice were divided into test groups comprising of six animals in each group. The test was performed using increasing i.p. dose of methanolic extract of flowers, leaves, stem, seeds and roots *C. ternatea* or pentazocine lactate from 10-500 mg/kg[9, 10]. Normal group received only vehicle (2% DMF). The mice were allowed for food and ad libitium, kept under regular observation for 24 hr for any mortality or behavioral changes. All extracts did not cause any significant behavioral changes and no mortality was observed.

Evaluation of antinociceptive activity

Hot plate method

Central analgesic activity of methanolic extract of flowers, leaves, stem, seeds and roots of the plant *C. ternatea* was evaluated using hot plate method[11]. The mice of either sex were divided into seven groups of six animals each. The first group served as control and received only vehicle (2% DMF), second group was administered standard drug pentazocine lactate (50mg/kg, i.p.) [12]dissolved in 2% DMF in water for injection. The animals of third to seventh group were treated with methanolic extract of flowers, leaves, stem, seeds and roots of the plant *C. ternatea* (50 mg/kg, i.p.) suspended in 2%DMF in saline water respectively. Mice were placed individually on the hot plate maintained at 55□1^{0}C. The basal reaction time was noted before and at 30, 60, 90, 120, 150, 180 min after the administration of treatment[13]. The experiment was terminated 20 sec after their placement on the hot plate to avoid damage to the paws[14]. Zero minute reading was the pre -drug reaction time.

Tail immersion method [15, 16]

Central analgesic activity of methanolic extract of flowers, leaves, stem, seeds and roots of the plant *C. ternatea* was evaluated using tail immersion method. The mice of either sex were divided into seven groups of six animals each. The first group served as control and received only vehicle (2% DMF), second group was administered standard drug pentazocine lactate (50mg/kg, i.p.) dissolved in 2% DMF in water for injection. The animals of third to seventh group were treated with methanolic extract of flowers, leaves, stem, seeds and roots of the plant *C. ternatea* (50 mg/kg, i.p.) suspended in 2%DMF in saline water respectively.

The lower 5 cm portion of the tail was immersed in water bath maintained at 55^{0}C .The time in seconds for mice to withdrawal the tail from the water was recorded as the reaction time, with a cut of period of immersion set as **20 seconds [17].** The basal reaction time was noted before and at 30, 60, 90, 120, 150, 180 min after the administration of treatment.

Acetic acid-induced writhing test

Peripheral analgesic activity was evaluated using acetic acid-induced writhing test[18, 19]. Mice of either sex were prescreened 48 hrs before the actual experiment and those sensitive to acetic acid-induced writhing were divided into seven groups, of six animals each. . The first group served as control and received only vehicle (2% DMF), second group received the standard drug paracetamol (50mg/kg i.p.)[12] dissolved in 2% DMF in water for injection and animals of third to seventh group were treated with methanolic extract of flowers, leaves, stem, seeds and roots of the plant *C. ternatea* (50 mg/kg, i.p.) suspended in 2%DMF in saline water respectively 30 minutes before intraperitoneal injection of 0.1 ml of 0.6% solution of acetic acid. Mice were placed individually in glass beakers after administration of acetic acid and five minutes were allowed to elapse. The mice were then observed for a further period of 30 minutes and the number of writhes recorded for each animal.

Evaluation of Antioxidant activity

Determination of free radical scavenging activity [20, 21]

The free radical scavenging activity of methanolic extract of flowers, leaves, stem, seeds and roots of the plant *C. ternatea* was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to the method of (Blios., 1958; Chindambra Murthy, 2002). 1ml of different concentrations of extract solution and standard were taken in different vials. To this 5ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37°C for 20min. Absorbance was measured against methanol as blank at 517nm and % scavenging was calculated to the corresponding blank reading. The antioxidant activity of the extracts was expressed as the IC_{50} value which was defined as concentration in µg/ml of extracts required to scavenge 50 % DPPH free radical. Absorbance of the DPPH was taken as the control. Percent antiradical activity was calculated by using the following formula

$$\% \text{ Antiradical activity} = \frac{\text{Control Abs- sample Abs}}{\text{Control Abs.}} \times 100$$

Reducing power assay [22, 23]

The reduction capability of methanolic extract of flowers, leaves, stem, seeds and roots of the plant *C. ternatea* was determined by the method of Oyaizu (1986) and ; Mau A (2002). In this assay, the yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each antioxidant samples. All the extracts in 1ml of deionised water were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and 2.5ml 1% potassium ferricyanide. The mixture was incubated at 50 ºC for 20min. 2.5ml of trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. 2.5ml of upper layer solution was taken and mixed with 2.5ml freshly prepared 1% ferric chloride solution in distilled water and absorbance was

measured at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power[24].

Anti-lipid peroxidation activity[25, 26]

Rat liver homogenate 10% was prepared according to the method described by (Tripathi et. Al., 2001; Wade et.al., 1985). Perfused liver was isolated and 10% (w/v) homogenate was prepared using a tissue homogenizer under ice- cold (0-4ºC) condition .The homogenate was used to study in-vitro lipid peroxidation.

The mixtures containing 0.5ml of homogenate, 1ml of 0.15 ml KCl and 0.5ml of different concentrations of extracts were prepared. Lipid peroxidation was initiated by adding 100µl of 1mM ferric chloride .The reaction mixture were incubated for 30min.at 37 º C. After incubation the reaction mixture was stopped by adding 2ml of ice-cold 0.25N HCl containing 15% trichloroacetic acid (TCA) and 0.38% thiobarbituric acid (TBA) and 0.2ml of 0.05% butylated hydroxyl toluene (BHT). These reactions mixtures were heated for 60min at 80ºC, cooled and then centrifuged at 5000 rpm for 15min. The absorbance of the supernatant was measured at 532 nm against blank which contained all reagents except liver homogenate and drug. Identical experiments were performed to determine the normal (without drug & ferric chloride) and induced (without drug) lipid peroxidation leveling the tissue.

$$\% \text{ ALP} = \frac{\text{Ferric chloride O. D. - Sample O. D.}}{\text{Ferric chloride O. D. - Normal O. D.}} \times 100$$

Nitric oxide scavenging activity[27, 28]

Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated by the use of griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. (Mondal et. al., 2006; Sreejayan Rao. 1997). Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration of methanolic extract of flowers, leaves, stem, seeds and roots of the plant *C. ternatea* dissolved in standard phosphate buffer (0.025M, pH7.4) and the tubes were incubated at 25^{0}C for 5 hr. after 5 hr, 0.5 ml of incubation solution containing nitrite was pipetted and mixed with 0.5 ml Griess reagent and allow to stand for 5 min completing diazotization. The absorbance was measured at 546 nm against the blank and % scavenging activity was calculated according to the formula given below

$$\% \text{ NO Scavenging activity} = \frac{\text{Control Abs- sample A}}{\text{Control Abs.}} \times 100$$

Statistical Analysis

The results were reported as mean±SEM and analyzed for statistical significance using one-way ANOVA followed by Dunnet's test.

RESULTS AND DISSCUSSION

Analgesic activity

Among all the extracts, the methanolic extract of roots of *C. ternatea* showed the significant increased (P<0.01, P<0.05) in reaction time in hot plate (Table 1) and significant increased (P<0.01) in pain threshold in tail flick model (Table 2). Thermic painful stimuli are known to be selective to centrally active drugs. Prostaglandins and bradykinins are suggested to play an important role in analgesia[29, 30]. Flavonoids and tannins are reported to inhibit prostaglandin synthesis [31]. A number of flavonoids and tannins have been reported to produce analgesic activity [32]. As phytochemical tests showed the presence of flavonoids and tannins in the methanolic extract of root of *C. ternatea*, they may be exerting their action by suppressing the formation of prostaglandin and bradykinins or antagonizing their action.

Table 1: *Effect of methanolic extract on Hot plate test.*

Treatment (Dose mg/kg i.p.)	Latency to lick the paw (Sec)±SEM						
	Predrug reaction time	30 min	60 min	90 min	120min	150min	180min
Vehicle(2% DMF)	6.45±0.648	6.47±0.796	7.0±1.238	6.46±0.796	6.48±0.697	5.57±0.593	6.16±0.602
Pentazocine (50)	9.85±0.232**	11.46±0.732**	12.36±0.746**	14.52±0.862**	19.70±0.883**	15.97±0.929**	15.07±0.773**
MEF(50)	8.25±0.641	8.47±0.464*	9.04±0.384	11.37±0.312**	11.48±0.922**	12.57±0.839**	11.04±0.616**
MEL(50)	12.51±0.814**	15.39±0.377**	17.23±0.174**	18.26±0.892**	19.70±0.353**	17.70±0.645**	15.89±0.404**
MESt(50)	14.50±0.105**	10.26±0.194**	12.88±0.234**	13.36±0.212**	14.47±0.532**	13.92±0.100**	11.86±0.780**
MESe(50)	8.74±0.127*	12.64±0.409**	13.87±0.227**	15.71±0.573**	18.13±0.116**	15.80±0.932**	11.83±0.376**
MER(50)	14.85±0.34**	15.56±0.398**	18.36±0.209**	19.56±0.142**	20±0.167**	20±0.345**	19.42±0.84**

*All the values are expressed as mean ± SEM; n=6, **P<0.01, *P<0.05 significant compared to control. MEF, MEL, MESt, MESe and MER are methanol extract of flowers, leaves, stems, seeds and roots of Clitoria ternatea respectively.*

Table 2. *Effect of methanolic extract on tail immersion method.*

Treatment (Dose mg/kg i.p.)	Reaction time (Sec)±SEM						
	Predrug reaction time	30 min	60 min	90 min	120min	150min	180min
Vehicle(2% DMF)	5.34±0.648	5.65±0.796	5.89±1.238	6.21±0.796	6.13±0.697	6.67±0.593	7.34±0.602
Pentazocine (50)	8.68±0.232**	9.14±0.732**	13.98±0.746**	16.28±0.862**	19.82±0.883**	19.34±0.929**	17.23±0.773**
MEF(50)	6.37±0.641	7.24±0.464	8.87±0.384**	11.56±0.312**	13.42±0.922**	13.76±0.839**	10.46±0.616**
MEL(50)	8.29±0.814	9.49±0.377**	14.45±0.174**	16.93±0.892**	18.32±0.353**	16.12±0.645**	14.66±0.404**
MESt(50)	9.23±0.105**	11.45±0.194**	12.23±0.234**	14.89±0.212**	15.11±0.532**	12.42±0.100**	11.16±0.780**
MESe(50)	8.23±0.127**	10.28±0.480**	12.66±0.227**	16.42±0.573**	16.52±0.116**	14.80±0.932**	10.46±0.376**
MER(50)	12.25±0.34**	14.72±0.398**	16.89±0.209**	18.32±0.142**	18.87±0.167**	16.17±0.345**	17.11±0.84**

*All the values are expressed as mean ± SEM; n=6, **P<0.01, significant compared to control.*

All the extracts of *C. ternatea* at dose of 50 mg/kg, i.p., significantly attenuated the number of writhing and stretching induced by intraperitoneal 0.6% acetic acid (Table 3). Methanolic extract of root of *C. ternatea* showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts as well as the standard drug paracetamol. Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response [33]. The effect of the extracts in reducing writhing may be an indication that they depressed the production of noxious substances

Table 3. *Effect of methanolic extract on Acetic Acid-induced writhing in mice.*

Treatment (mg/kg)	Number of writhing
Vehicle (2% DMF)	65.66±0.431
Paracetamol (50)	42.06±0.287**
MEF(50)	52.06±0.223**
MEL(50)	39.00±0.722**
MESt(50)	45.36±0.189**
MESe(50)	42.34±0.396**
MER(50)	27.8±0.654**

*All values are expressed as mean±SEM (n=6), *P<0.01 significant as compared to control. MEF, MEL, MESt, MESe and MER are methanol extract of flowers, leaves, stems, seeds and roots of Clitoria ternatea respectively.*

Antioxidant activity

DPPH radical activity of MEF, MEL, MESt, MESe and MER of *C. ternatea* expressed as IC_{50} values 81.55, 62.48, 57.32, 73.19, 45.29 ìg/ml respectively as shown in Table 4 compared with ascorbic acid having IC_{50} value 44.91 ìg/ml. in DPPH test the ability of compound to act as donor for hydrogen atoms or electrons was measured spectrophotometrically. Results of % inhibition of all extracts and ascorbic acid are shown in Table 5.

Table 4. *IC_{50} of methanolic extract of Clitoria ternatea.*

Test material	IC_{50} value (µg/ml)		
	DPPH	Nitric oxide	Lipid peroxidation
MEF	81.55	73.98	74.66
MEL	62.48	59.22	51.23
MESt	57.32	67.43	76.21
MESe	73.19	62.98	67.26
MER	45.29	52.83	59.14
Ascorbic acid	44.91	43.80	52.28

Table 5. *DPPH scavenging activity of Clitoria ternatea*

Concentration (µg)	% Inhibition					
	MEF	MEL	MESt	MESe	MER	Ascorbic acid
25	37.35±0.0123	20.63±0.047	39.39±0.016	42.23±0.004	59.58±0.056	62.98±0.078
50	39.45±0.12	38.49±0.021	46.89±0.042	47.67±0.015	70.51±0.054	73.57±0.082
100	43.78±0.044	28.92±0.042	49.16±0.064	53.94±0.008	73.28±0.028	77.8 1±0.032
200	44.39±0.054	31.49±0.068	53.87±0.084	55.57±0.058	76.99±0.042	80.37±0.036
400	49.98±0.032	35.38±.060	61.47±0.018	61.95±0.024	84.20±0.066	89.23±0.046

Increased absorbance of the reaction mixture indicates increased reducing power. Reducing power of MEF, MEL, MESt, MESe and MER of *C. ternatea* increased with concentration as shown in Table 6.

Table 6. *Reducing power assay of Clitoria ternatea*

Concentration (µg)	Absorbance at 700 nm.					
	MEF	MEL	MESt	MESe	MER	Ascorbic acid
25	0.209±0.17	0.278±0.18	0.152±0.46	0.186±0.94	0.214±0.16	0.27 16±0.17
50	0.367±0.33	0.314±0.45	0.229±0.52	0.265±0.84	0.396±0.15	0.4476±0.14
100	0.412±0.20	0.384±0.19	0.263±0.26	0.296±0.56	0.446±0.34	0.5516±0.14
200	0.482±0.15	0.413±0.13	0.304±0.45	0.319±0.24	0.519±0.29	0.67 ±0.118
400	0.521±0.31	0.429±0.33	0.319±0.15	0.376±0.23	0.567±0.20	0.72 1±0.11

In the inhibition of lipid peroxidation the IC_{50} values of MEF, MEL, MESt, MESe and MER of *C. ternatea* 74.66, 51.53, 76.21, 66.26, 59.14 ìg/ml respectively as compared to ascorbic acid having IC_{50} values 52.28 ìg/ml. Root extract of *C. ternatea* may inhibit the initiation of lipid peroxidation by scavenging the free radicals that form alkylperoxyl and alkoxyl radicals or can donate hydrogen atom to alkylperoxyl and alkoxyl radicals and thus stop chain propagations. Results of % ALP of all extracts and ascorbic acid are shown in Table 7.

Table 7. *Anti-lipid peroxidation of Clitoria ternatea.*

Concentration (µg)	% ALP					
	MEF	MEL	MESt	MESe	MER	Ascorbic acid
25	23.14±0.52	28.56±0.14	26.45±00.19	39.12±0.56	52.94±0.48	60.27±0.53
50	25.98±0.27	35.59±0.35	28.19±0.26	43.27±0.21	58.93±0.85	67.23±0.75
100	34.67±0.04	43.92±0.84	34.72±0.71	45.87±0.18	63.63±0.35	69.29±0.83
200	45.89±0.32	49.76±0.56	36.76±0.24	53.92±0.95	67 .16±0.28	71.98±0.780
400	58.49±0.72	54.67±0.23	48.56±0.35	59.41±0.22	79.27±0.35	82.67±0.19

Nitric oxide scavenging effect of MEF, MEL, MESt, MESe and MER of *C. ternatea* and ascorbic acid shows IC_{50} values 73.98, 59.22, 67.43, 62.98, 52.83, 43.80 ìg/ml respectively. Nitric oxide is an important chemical mediator generated by endothelial cells macrophages; neurons etc are involved in regulation of various physiological processes. Oxygen reacts with excess nitric oxide to generate nitrite and peroxinitrite anions which acts as free radicals. In the present study extract competes with oxygen to react with nitric oxide and thus inhibits generation of anions. Results of % inhibition of all extracts and ascorbic acid are shown in Table 8.

Table 8. *Nitric oxide scavenging activity of Clitoria ternatea*

Concentration (µg)	% Inhibition					
	MEF	MEL	MESt	MESe	MER	Ascorbic acid
25	23.87±0.011	31.83±0.056	34.72±0.038	13.65±0.073	45.1 1±0.023	41.98±0.084
50	28.92±0.015	37.28±0.036	38.19±0.044	17.93±0.068	49.27±0.052	52.73±0.023
100	35.83±0.086	41.90±0.084	42.93±0.045	21.48±0.011	56.27±0.034	67.22±0.044
200	42.98±0.044	46.38±0.038	45.36±0.084	22.58±0.052	63.9 1±0.056	69.44±0.011
400	54.38±0.075	53.63±0.045	57.48±0.068	28.52±0.086	71.56±0.075	73.28±0.015

Among all the methanolic extracts of flowers, leaves, stem, seeds and roots of the plant *C. ternatea*, methanolic extract of root posses significant antioxidant activity. The antioxidant and free radical scavenging activity of *C. ternatea* root might be due to the presence of phenolic compounds in methanolic extract of root[34].

REFERENCES

1. Kirtikar KR, et al., In Indian Medicinal plants. Vol. I, 2nd Edn. Delhi : Sri Satguru Publications, 1991, p 806.
2. Anonymous, In The Wealth of India, a Dictionary of Indian Raw Materials and Industrial Products, Vol. II, Council of Scientific and Industrial Research, New Delhi, 2005, p 71.
3. Rai KS, et al. Indian J Physiopharmac. 2001; 45 (3): 305.
4. Devi BP, et al. Fitoterapia. 2003; 74 (4): 345.
5. Jain NN, et al. Pharmacol Biochem Behav. 2003; 75 (3): 529.
6. Kazuma K, et al. Phytochemistry. 2003; 64 (6): 1133.
7. Kazuma K, et al. Phytochemistry. 2003; 62 (2): 229.

8. Terahara N, et al. Phytochemistry. 1990; 29 (3): 949.
9. Mahajan SG, et al. Int J Pharmacol Biol Sci. 2007; 1(3): 59.
10. Seth UK, et al. In Selected Topics in Experimental Pharmacology. Ist Edn., 1972, p126.
11. Woolfe G, et al. J Pharmacol Exp Ther. 1944; 80: 300.
12. Ghogare PB, et al. *Dhaka Univ J Pharm Sci.*, 2007; 6(1): 55.
13. Nirmal SA, et al. Natural Prod Res. 2007 ; 21(12) :1042.
14. Kamble BB, et al. Adv Pharmacol Toxicol. 2008; 9(2): 133.
15. Borgi W, et al. Fitoterapia. 2007; 78: 16.
16. Kudahewa DD, et al. Phcog Mag. 2008; 4 (13): 37.
17. Puchchakayala G. et al. Phcog Mag. 2008; 4(13): 32.
18. Koster R, et al. Federation Proc. 1958; 18:412.
19. Hendershot LC, et al. J Pharmacol Exp Ther. 1959; 125: 237.
20. Chindambra MJ. Agric Food Chem. 2002; 50: 5909.
21. Blios MS, Nature 1958; 81:1199.
22. Oyaizu M, Japanion J. Nutri. 1986; 44: 307.
23. Mau A. J. Agric. Food Chem. 2002; 50: 6072.
24. Gulcin I, et al. Phytomedicine. 2006;13: 343.
25. Tripathi YB, et al. Indian J Exp Biol. 2001; 39: 798.
26. Wade et al. Biochem. Medicine. 1985; 33: 291.
27. Mondal S, et al. Ind J Experiment Bio. 2006; 44: 39.
28. Geetha A, et al. Adv Pharmacol Toxicol. 2008; 9(3) : 21.
29. Dray A, et al. Neurosci. 1993; 16: 99.
30. Vinegar R, et al. J Pharmacol Exp Ther. 1969; 166: 96.
31. Alcarez MJ, et al. J Ethanopharmacol. 1987; 21: 209.
32. Hosseinzadeh H, et al. Phytomed. 2002; 9: 135.
33. Bartolini A, et al. British J Pharmacol. 1987; 92: 711.
34. Mruthunjaya K, et al. Phcog Mag. 2008; 4: 42.