Screening Antianxiety and Antioxidant Profile of Stems and Leaves of Blue Variety of *Clitoria ternatea* L

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Kumar and Dhobi: Antianxiety and Antioxidant Profile of *Clitoria ternatea*

Previous studies from our laboratory demonstrated that the antianxiety and antioxidant activities of the roots of the blue variety of *Clitoria ternatea* were greater compared to the white variety. An attempt has been made to investigate the antianxiety and antioxidant activities of aerial parts of this plant using elevated plus maze and 2,2-diphenyl-1-picrylhydrazyl assay, respectively. Methanol extract of stems and leaves of the blue variety (50, 100 or 200 mg/kg, p.o.), diazepam (2 mg/kg, p.o.) and control (vehicle) were separately evaluated for antianxiety activity. The stem extract (200 mg/kg) and leaf extract (100 or 200 mg/kg) exhibited significant antianxiety activity with respect to control and standard. The remaining doses did not exhibit any antianxiety activity. Mice treated with the blue variety leaf extract, showed decreased number of entries from 6.20 to 4.60 when the dose was increased from 100 to 200 mg/kg indicating a possibility of decreased locomotor activity, which could be due to central nervous system depressant activity. In case of blue variety stem extract, the average number of entries increased from 3.20 to 5.80 as the dose was increased from 100

to 200 mg/kg. The IC_{50} values of ascorbic acid, blue variety stem extract and leaf extract were found to be 20.01, 264.32 and 251.13 µg/ml, respectively. None of the plant parts showed activity close to that of ascorbic acid. Finally, it can be concluded that aerial parts of blue variety of *C. ternatea* exhibited mild antianxiety and antioxidant activities.

Key words: Anxiety, antioxidant, *Clitoria ternatea*, diazepam, DPPH

Clitoria ternatea L. (Butterfly pea; Papilionaceae) has long tradition of use in the treatment of asthma, fever, inflammation and mental disorders[1-3]. The plant is mainly distributed in Andaman Islands at an altitude of 1500 m[4]. *C. ternatea* has been reported to contain flavonoids like norneolignans, kaempferol, quercetin, myricetin and triterpenoids like taraxerol, taraxerone[5-7]. The plant has been reported to possess anticonvulsant, antistress, antidepressant and tranquilizing properties[8,9].

Previous studies from our laboratory reported a greater antianxiety and antioxidant activities from the roots of the blue variety of *C. ternatea* compared to the white variety[10]. Preliminary phytochemical screening of methanol extract of roots, stems and leaves showed presence of steroids, triterpenoids, flavonoids and alkaloids. A survey of literature revealed that these phytoconstituents are responsible for antianxiety and antioxidant activities. Thus, it was planned to investigate antianxiety and antioxidant activities on stems and leaves of blue variety of *C. ternatea*. The stems and leaves of blue variety of *C. ternatea* were collected during the month of June 2010 from the cultivated plants at herbal garden and medicinal plants garden, Panjab University, Chandigarh. The samples were further authenticated at the Forest Research Institute, Dehradun, vide certificate number 765/2006-bot/15-1. The methanol extract of stems and leaves (2 kg each) of blue vareity of *C. ternatea* were extracted separately with methanol (Laboratory Grade; E Merck, Delhi, India) as per procedure described in our laboratory[10].

Anxiolytic activity was investigated using well established model, i.e., elevated plus maze (EPM) model[11]. Laboratory Animal Centre A-strain (LACA) mice were procured from the Central Animal House of Panjab University, Chandigarh. The animal model was approved by the Institutional Animal Ethics Committee of Panjab University, Chandigarh (IAEC/97, dated 24-03-2011). The experimental animals were divided into 3 groups of control, standard and test. The control group animals received only vehicle (2 % Tween 80, p.o.); the standard group animals received diazepam as standard drug (2 mg/kg, p.o.) for comparison and test group animals received the test substances (50, 100 and 200 mg/kg, p.o.). The results were expressed as mean±SD. The inter group variation was measured by one way analysis of variance (ANOVA) followed by Tukey's test.

The EPM model was used in present investigations because it is cost effective, easy to operate, less time consuming, and require no preliminary training to the mice and do not cause much discomfort to the animals while handling. The model is principally based on the phobia due to height[12]. The percentage yields of methanol extract of blue variety stems and blue variety leaves were found to be 8.3 and 16.9 % w/w, respectively. It is evident from Table 1 that blue variety stems and blue variety leaves possessed mild anxiolytic activity. The blue variety stems showed mild anxiolytic activity, which was dose dependent.

TABLE 1: ANXIOLYTIC ACTIVITY OF STEMS AND LEAVES OF *C. TERNATEA* BLUE VARIETY USING EPM MODEL

Group	Dose (mg/kg)	Mean^n number of entries in open arm±SD	Mean^n time spent in open arms (s)±SD
Control	-	1.60±0.54^a	3.66±0.73^a
Diazepam	2	10.60±1.14*	24.96±4.37*
Stems	50	1.50±0.57^a	3.20±0.34^a
	100	3.20±1.30^a	7.64±1.89^a
	200	5.80±1.48^{*a}	10.08±2.51^{*a}
Leaves	50	2.70±0.95^a	4.47±0.54^a
	100	6.20±1.14^{*a}	12.52±3.95^{*a}
	200	4.60±2.16^{*a}	11.06±1.29^{*a}

n=6; the data is expressed as mean±SD; $p<0.05$ vs. control*; vs. diazepam^a; one way ANOVA followed by Tukey's test

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Blue variety stems showed it's best mild response at dose 200 mg/kg and average time spent in open arms was increased from 3.66 s in control group to 10.08 s in treated animals. The activity of stems and leaves of blue variety at a dose of 50, 100 and 200 mg/kg was significant with respect to diazepam. In comparison to control, methanol extract of stems and leaves showed significant activity except 50 mg/kg dose and 100 mg/kg of blue variety stems. A dip in activity at a higher dose in case of blue variety leaves extract showed the CNS depressant activity. The behavioural changes were also observed in the treated animals. The animals treated with 100 mg/kg dose of blue variety leaves showed an average number of entries 6.20 in open arms. The number of entries decreased when the dose was increased from 100 to 200 mg/kg indicating a possibility of decreased locomotor activity, which could further be related to CNS depressant activity. In case of blue variety stems, the average number of entries increased from 3.20 to 5.80 as the dose was increased from 100 to 200 mg/kg. The antioxidant activity was investigated using well established *in vitro* DPPH assay[13]. The IC_{50} values of ascorbic acid (Analytical Grade; Hi-media Laboratories Pvt. Ltd, Mumabi, India) blue variety stems and blue variety leaves were found to be 20.01, 264.32 and 251.13 µg/ml, respectively (Tables 2-4). None of the plant parts showed activity close to the ascorbic acid (IC_{50}=20.01 µg/ml) and confirmed mild antioxidant activity. From the results, it can be concluded that aerial parts of blue variety of *C. ternatea* exhibited mild antianxiety and antioxidant activities.

TABLE 2: PERCENT INHIBITION OF DPPH AT DIFFERENT CONCENTRATIONS OF ASCORBIC ACID

Concentration (µg/ml)	Absorbance (mean±SD)	Percent inhibition
12	0.2507±0.005	6.07
15	0.1961±0.003	26.52
18	0.1542±0.004	42.22
21	0.1237±0.004	53.65
24	0.0728±0.002	72.72
27	0.0365±0.002	86.32
30	0.0072±0.0005	97.30

TABLE 3: PERCENT INHIBITION OF DPPH BY METHANOL EXTRACT OF BLUE VARIETY STEMS OF *C. TERNATEA*

Concentration (µg/ml)	Absorbance (mean±SD)	Percent inhibition
50	0.2120±0.001	15.06
150	0.1650±0.003	33.80
250	0.1260±0.001	49.51
350	0.0952±0.002	61.85
450	0.0456±0.003	81.73
550	0.0229±0.002	90.82

TABLE 4: PERCENT INHIBITION OF DPPH BY METHANOL EXTRACT OF BLUE VARIETY LEAVES OF *C. TERNATEA*

Concentration (µg/ml)	Absorbance (mean±SD)	Percent inhibition
50	0.2281±0.003	22.34
150	0.1770±0.002	39.73
250	0.1462±0.005	50.22
350	0.1090±0.002	62.8
450	0.0700±0.003	76.16
550	0.0431±0.002	85.32

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Conflict of interest:

The authors declare that they have no conflict of interest.

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