

## Antianxiety and antioxidant profile of blue and white variety of *Clitoria ternatea* L

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### ABSTRACT

*Clitoria ternatea* L. (Butterfly pea; Papilionaceae) has been traditionally used in the treatment of mental disorders. But the plant has not been systematically investigated to validate its traditional claims. Thus, it was planned to compare blue and white variety of *C. ternatea* for antianxiety and antioxidant activities to validate traditional claims. The antianxiety activity was evaluated using elevated plus maze whereas antioxidant activity was evaluated using DPPH assay. Properly identified roots of both the varieties were separately extracted with methanol using maceration method. The methanol extract of roots of both the varieties (50, 100 or 200 mg/kg, *p.o.*) was subjected to antianxiety activity. The efficacy of both the varieties was statistically compared with the standard antianxiety drug, diazepam (2 mg/kg, *p.o.*). The root of blue and white variety showed best antianxiety activity at dose 100 mg/kg and animals spent 19.4 and 16.98 sec in open arms, respectively, in comparison to control animals (3.66 sec spent in open arms). Maximum *in vitro* antioxidant activity was observed in blue variety root with IC<sub>50</sub> of 193.07 µg/ml, very closely followed by white variety root (IC<sub>50</sub> = 220.66 µg/ml). The present studies have validated traditional claims of *C. ternatea* for its antianxiety activity. Finally, it can be concluded that the both the varieties of *C. ternatea* possess close biological profile with blue variety being more potent antianxiety and antioxidant activities.

**KEYWORDS:** Anxiety, Antioxidant, *Clitoria ternatea*, Diazepam.

### 1. INTRODUCTION

In the Ayurvedic system of medicine, many herbal plants are used to treat various ailments. Drugs mentioned in Ayurvedic system of medicine are group of herbal medicines, used to improve mental abilities<sup>18</sup>. These herbal drugs include extracts from *Clitoria ternatea*, *Celastrus panniculatus*, *Acorus calamus*, *Centella asiatica*, *Withania somnifera*, *Guduchi* and *Areca*<sup>1</sup>. Recently, large number of side effects like liver damage and mutagenesis are associated with the use of chemicals obtained from synthetic sources<sup>6</sup>. Thus, researchers are exploring natural resources to find out newer and safer natural potent agents<sup>2</sup>.

*Clitoria ternatea* L. (Family – Papilionaceae) is a well-known plant commonly called butterfly pea and Shankpushpi. There are two known varieties of *C. ternatea*, blue-flowered and white-flowered<sup>5</sup>. But the plant has not been systematically investigated to validate its traditional claims. Thus, it was planned to explore antianxiety and antioxidant activities of both the varieties of *C. ternatea* to validate traditional claims<sup>7</sup>.

### 2. MATERIALS AND METHODS

**Plant material:** The roots of blue and white 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 identity of plants was confirmed on the basis of detailed study of taxonomic characters and by comparison with authentic samples available at Museum-cum-Herbarium of University Institute of

Pharmaceutical Sciences, Centre of Advanced Study, Panjab University, Chandigarh. The samples were further confirmed by Forest Research Institute, Dehradun vide certificate number 765/2006-Bot/15-1. The plant specimens of blue and white variety have been deposited in Museum-cum-Herbarium of University Institute of Pharmaceutical Sciences, Centre of Advanced Study, Panjab University, Chandigarh, India, under the voucher number 1474 and 1475, respectively<sup>8</sup>.

**Solvents, chemicals and reagents:** The solvents, chemicals and reagents of laboratory grade were obtained from E.Merck (India) Ltd., Sigma-Aldrich Chemicals Pvt. Ltd. and S.D. Fine Chem. Ltd. Distilled water was used wherever water is mentioned<sup>19</sup>.

**Preparation of extracts:** The plant material was dried under shade. Moderately coarse powdered drug (100 g) of root two varieties of *C. ternatea* was extracted separately with methanol three times (200, 200 and 100 ml) for 48 h. The extracts were filtered and concentrated under reduced pressure to obtain 11.5 and 9.6 g of blue and white variety root extract respectively. A portion of these extracts was used for the preparation of doses for pharmacological investigation. A small portion of dried methanol extract was subjected to phytochemical screening. All doses were prepared by suspending the extract in aqueous solution of Tween 80 and the concentration of the test solution was so adjusted that each animal received a uniform volume of 10 ml/kg. All doses were administered orally and the dose schedule was so adjusted that a uniform time interval elapsed

between the administration of dose and a time when the animal was subjected to test.

#### **In vivo antianxiety activity:**

**Animals:** Albino Laca mice of either sex were used for antianxiety activity. The animals were procured from the Central Animal House of Panjab University, Chandigarh. The animals were allowed a standard pellet diet and water *ad libitum*. The animals were acclimatized to laboratory conditions daily for one hour for continuous seven days before the start of experiment. All the experiments were carried out from 9 to 11 a.m. Groups of five mice (20-30 g) were used in all sets of experiments. The animals were overnight fasted before use. The doses were administered orally with the help of an oral cannula fitted on a tuberculin syringe. The animal model was approved by the Institutional Animal Ethical Committee of Panjab University, Chandigarh (IAEC/97, dated 24-03-2011). The studies were carried out in accordance with the guidelines given by the Indian Council for Medical Research and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India) and the Institutional Animal Ethical Committee approved the study.

**Experimental protocol:** Diazepam (Almopse) was used in this study. Dose of standard drug was prepared by suspending the standard material in a vehicle in such a concentration as to administer a volume ranging 0.2 to 0.3 ml. The diazepam was suspended in 1 to 3 percent aqueous solution of Tween 80 for oral administration. The experimental animals were divided into 3 groups of control, standard and test. The control group animals received only vehicle; the standard group animals received a standard drug for comparison and test group animals received the test material (extracts).

**Assessment of anxiolytic activity:** Anxiolytic activity was assessed by using elevated plus maze (EPM) test<sup>14, 16</sup>. The animal was placed in the center of plus maze with its head facing open arm. The behavior of animals was noted for next 5 min for the number of entries and time spent in open arm. The animal shows less number of entries and spent less time in open arms in anxious state. An anxiolytic drug will increase both the number of entries and time spent in open arm.

**Statistical analysis:** Results were expressed as mean  $\pm$  S.D. The variation between groups was estimated by one way analysis of variance (ANOVA) followed by Tukey's test<sup>20</sup>. Statistical significant was considered at  $p < 0.05$ . The statistical analysis was done using the Jandel Sigma Stat statistical software version 2.

#### **In vitro antioxidant activity**

**Assessment of antioxidant activity:** The antioxidant activity was evaluated using *in vitro* DPPH assay<sup>11,12</sup>. In DPPH assay, 1 ml of 10 mg/ml solution of plant extract

was added to 1 ml of methanolic solution of DPPH (100  $\mu$ M), and the volume was made up to 4 ml with methanol. The reaction mixture was shaken and absorbance was measured at 517 nm after 20 min. Ascorbic acid (1 mg/ml) was used as a positive control. Methanol solution of respective test material was used as a blank. Methanol with DPPH solution was used as a negative control. The degree of discoloration was the measure of the scavenging efficacy of the test substance. The activity was expressed as IC<sub>50</sub> value, and was calculated by linear regression of plots where the abscissa represented the concentration of test substance and the ordinate the percent antioxidant activity from average of three separate tests and the percent scavenging was calculated from the equation:

$$\text{Percent scavenging} = (\text{Ab}_{\text{Scontrol}} - \text{Ab}_{\text{Stest}} / \text{Ab}_{\text{Scontrol}}) \times 100$$

### **3. RESULTS**

**Assessment of antianxiety activity:** Methanol root extract of blue and white varieties showed best activity at dose 100 mg/kg and animals spent 19.4 and 16.98 sec in open arms, respectively, in comparison to control animals (3.66 sec spent in open arms) as shown in table 1. The activity of root of two varieties at all the tested doses was significant with respect to diazepam. In comparison to control all extracts showed significant activity except 50 mg/kg dose. A dip in activity at a higher dose in case of methanol root extract of blue and white varieties showed the CNS depressant activity. The behavioural changes were also observed in the treated animals. The animals treated with 100 mg/kg dose of root extract of blue variety and white variety showed an average number of entries 6.8, 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.

**Assessment of antioxidant activity:** The antioxidant activity of each test material was expressed as IC<sub>50</sub> value (concentration showing 50 % scavenging of DPPH radical, Table 2). The IC<sub>50</sub> values were obtained from a linear regression of plots (Figure 1-3). The results showed that roots of two varieties showed antioxidant activity in DPPH assay and the highest activity was observed in blue variety root with IC<sub>50</sub> of 193.07  $\mu$ g/ml, very closely followed by white variety root (IC<sub>50</sub> = 220.66  $\mu$ g/ml). None of the plant showed activity close to the ascorbic acid (IC<sub>50</sub> = 20.01  $\mu$ g/ml).

### **DISCUSSION**

Now a days, there is an increased occurrence of various disease like cardiovascular disease, neurological disorders, cancer, diabetes and autoimmune disease due to presence of free radicals<sup>3</sup>. These free radicals are the unpaired electrons may lead

to lipid peroxidation and direct inhibition of mitochondrial respiration<sup>10</sup>. The average life expectancy has increased in modern times increasing world's population in old age.

A number of side effects like liver damage, cardiac problems and mutagenesis are associated with the use of antianxiety drugs obtained from synthetic sources. Thus, researchers are exploring natural resources to find out newer and safer natural anxiolytic agents.

Anxiolytic activity of methanol extract of blue and white variety of *C. ternatea* roots was evaluated employing widely used model, i.e., EPM. The EPM model was chosen since these are effective, cheap, simple, less time consuming, and require no preliminary training to the mice and do not cause much discomfort to the animals while handling<sup>13,17</sup>. The models are principally based on the observations that exposure of animals to approach-avoidance conflict which is manifested as an exploratory-cum-fear drive.

The above results indicated that both varieties of *C. ternatea* possess antianxiety and antioxidant activities. The CNS depressant activity was also observed at higher dose levels. It is evident from the

above results that antianxiety and antioxidant activity was more in root of the blue variety in comparison to root of the white variety. The traditional claim of *C. ternatea* being used as anxiolytic has been scientifically validated. The two varieties possess close biological profile with blue variety being more potent.

It is suggested that methanol extract of methanol extract of both the varieties of *C. ternatea* may be act via binding to benzodiazepine receptors as agonist<sup>4</sup> increase ascorbic acid level in brain<sup>22</sup>, inhibition of  $\gamma$ -amino butyric acid transmission<sup>21</sup> and monoamine oxidase inhibition. Preliminary phytochemical studies showed presence of flavonoids and triterpenoids in methanol extract of both the varieties of *C. ternatea*. The results of present investigations are in agreement with the available literature where flavonoids – kaempferol<sup>9</sup> and apigenin<sup>15</sup> and triterpenoids –  $\alpha$ ,  $\beta$ -amyrin have been reported to exhibit antianxiety activity. Further, it is concluded that triterpenoids or flavonoids may be responsible for antianxiety activity of both varieties of *C. ternatea*. It can be finally concluded that these phytoconstituent(s) may be isolated using column chromatography to develop anxiolytic agent.

**Table.1. Anxiolytic activity of methanol extracts of blue and white variety using EPM model.**

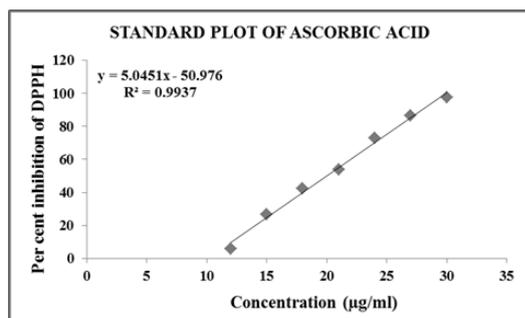
Group	Dose (mg/kg)	Mean <sup>n</sup> number of entries in open arm $\pm$ S.D	Mean <sup>n</sup> time spent in open arms (sec) $\pm$ S.D
Control	-	1.60 $\pm$ 0.54 <sup>a</sup>	3.66 $\pm$ 0.73 <sup>a</sup>
Diazepam	2	10.60 $\pm$ 1.14 <sup>*</sup>	24.96 $\pm$ 4.37 <sup>*</sup>
Blue variety root	50	3.60 $\pm$ 1.14 <sup>a</sup>	6.06 $\pm$ 2.33 <sup>a</sup>
	100	6.80 $\pm$ 1.92 <sup>*a</sup>	19.40 $\pm$ 1.78 <sup>*a</sup>
	200	6.00 $\pm$ 1.22 <sup>*a</sup>	11.10 $\pm$ 1.67 <sup>*a</sup>
White variety root	50	4.50 $\pm$ 1.29 <sup>a</sup>	7.07 $\pm$ 1.29 <sup>a</sup>
	100	6.80 $\pm$ 1.92 <sup>*a</sup>	16.98 $\pm$ 2.22 <sup>*a</sup>
	200	4.60 $\pm$ 1.14 <sup>*a</sup>	10.34 $\pm$ 1.23 <sup>*a</sup>

n=6; the data is expressed as Mean  $\pm$  S.D.; P<0.05 vs control<sup>\*</sup>; vs diazepam<sup>a</sup>; one way ANOVA followed by Tukey's test.

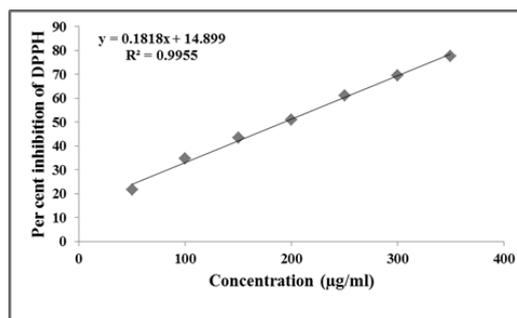
**Table.2. Antioxidant activity of methanol extracts of blue and white variety using DPPH assay**

Test sample	IC <sub>50</sub> ( $\mu$ g/ml)
Ascorbic acid	20.01
Blue variety root	193.07
White variety root	220.66

n=3



**Figure.1. Free radical scavenging activity of standard ascorbic acid**



**Figure.2. Free radical scavenging activity of methanolic extract of blue variety root of *C. ternatea***

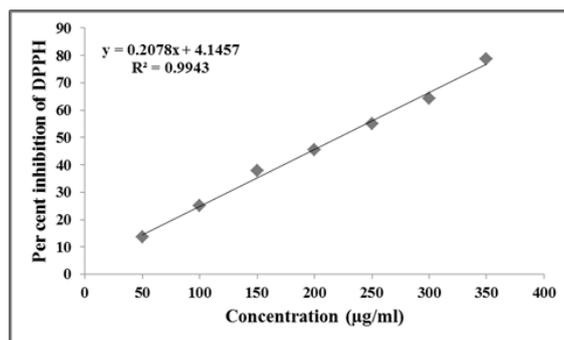


Figure.3. Free radical scavenging activity of methanolic extract of white variety root of *C. ternatea*

#### 4. CONCLUSION

The present studies have validated traditional claims of *C. ternatea* for its antianxiety activity. Finally, it can be concluded that the both the varieties of *C. ternatea* possess close biological profile with blue variety being more potent antianxiety and antioxidant activities.

#### 5. ACKNOWLEDGEMENT

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**Conflict of interest statement:** We declare that we have no conflict of interest.

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