

## Non- enzymic antioxidant activity of *Clitoria ternatea* leaf extracts *in vitro*

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### ABSTRACT

Non-enzymic antioxidants were analyzed in both blue flowered leaf and white flowered leaf of *Clitoria ternatea*. The *in vitro* model used in the study as alternatives to live animals, was goat liver slices. The liver slices exposed to different treatments were incubated for one hour at 37°C with very mild shaking. The non- enzymic activity was analyzed by using goat liver slices, both blue flowered leaf and white flowered leaf of *Clitoria ternatea* and H<sub>2</sub>O<sub>2</sub> as oxidant. The results showed that the white flowered leaves had higher content of all the non-enzymic antioxidants analyzed than the blue flowered ones.

**Key words:** *Clitoria ternatea*, Non-enzymic antioxidants, goat liver slices, H<sub>2</sub>O<sub>2</sub>.

### INTRODUCTION

Traditional herbal remedies are used as alternative medicine by a large proportion of people worldwide. Plants are the source of medication for preventive, curative, protective or promotive purposes (Sidhu *et al.*, 2007). Antioxidants act as a defense mechanism that protects against oxidative damage, and include compounds and repair enzymes to remove or repair damaged molecules. However, the natural antioxidant compounds become important (Malpure *et al.*, 2006). Antioxidants can prevent/retard the oxidation caused by free radicals and sufficient intake of antioxidants is supposed to protect against diseases (Celiktar *et al.*, 2007). These antioxidants include (endogenous) enzymic and the (predominantly exogenous) non-enzymic antioxidants. Medicinal plants are considered as potential sources of antioxidant compounds. There is an increasing interest in the investigation of naturally occurring antioxidants from plants

(Nickavar *et al.*, 2006). One of the plants that deserves attention is *Clitoria ternatea* (Sanskrit-Sankupushpam) belongs to the family *Fabaceae*, is widely used in traditional Indian system of medicine as a brain tonic (Gomez and Kalamani, 2003). *Clitoria ternatea* is a perennial twinning herb bearing blue or white flowers as shown in Figure 1. The present study is the new one concentrating on Non-enzymic antioxidant activity was analyze in both blue and white flowered leaf of *Clitoria ternatea* leaf extracts and also analyzed by using (goat liver slices presence and absence of oxidant H<sub>2</sub>O<sub>2</sub>) *in vitro* model.

### MATERIAL AND METHODS

#### Non-enzymic antioxidants in *clitoria ternatea* leaves

The non-enzymic antioxidants analyzed in the *Clitoria ternatea* leaves were ascorbic acid, á-tocopherol, total carotenoids, total phenols, flavonoids, reduced glutathione and chlorophyll.

### Estimation of ascorbic acid

Ascorbic acid, a scavenger of oxyradicals was estimated by the method of Roe and Keuther (1943). An accurate amount (1g) of leaves were homogenized in 4% TCA and made upto 10ml. Centrifuged and the supernatant obtained was treated with a pinch of activated charcoal, mixed vigorously and kept for 10 minutes. Centrifuged again to remove the charcoal residue and the supernatant obtained was used for the estimation.

Aliquots of 0.5 - 1.0ml of this supernatant were taken and 0.2 to 1.0ml of standard ascorbate were made up to 2.0ml with 4% TCA. 0.5ml of DNPH reagent was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37°C for 3 hours. The osazones formed were dissolved in 2.5ml of 85% sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. After incubation for 30 minutes at room temperature, the absorbance was read spectrophotometrically at 540nm. From the standard curve constructed on an electronic calculator set to the linear regression mode, the concentration of ascorbate in the samples were calculated and expressed as mg ascorbate/g leaf.

### Estimation of tocopherol

The method described by Rosenberg (1992) was followed for the estimation of tocopherol. An exact amount (2.5g) of the homogenized plant tissue (with 5ml 0.1N sulphuric acid) was allowed to stand overnight. Then the contents of the flask were shaken vigorously and filtered through Whatmann No.1 filter paper. Aliquots of the filtrate were used for the estimation. Into 3 stoppered centrifuge tubes, 1.5ml of plant tissue extract, 1.5ml of the standard and 1.5ml of water were pipetted out respectively. To all the tubes, 1.5ml of ethanol and 1.5ml of xylene were added, mixed well and centrifuged. 0.1ml of the xylene layer was transferred into another stoppered tube and 0.1ml of 2,2'-dipyridyl reagent was added to each tube and mixed. Pipetted out 1.5ml of the mixture into a spectrophotometer cuvette and the extinction was read at 460nm. 0.33ml of ferric chloride solution was added and mixed well, and after exactly 15 minutes, the absorbance of the red colour produced was read against a blank at 520nm. The

concentration of tocopherol in the sample was calculated using the formula,

$$\text{Tocopherols } (\mu\text{g}) = \frac{\text{Reading at 520nm} - \text{Reading at 450nm}}{\text{Reading of standard at 520 nm}} \times 0.29 \times 0.15$$

The results were expressed as  $\mu\text{g}$  tocopherol / g leaf.

### Estimation of total carotenoids

The method described by Zakaria *et al.* (1979) was followed for the estimation of total carotenoids. An exact amount (0.5g) of the sample was homogenized and saponified for about 30 minutes in a shaking water bath at 37°C with a specific volume of 12% alcoholic KOH. The saponified extract was transferred into a separating funnel containing 10 to 15ml of petroleum ether (40-60°C) and mixed well. The lower aqueous phase was transferred to another separating funnel and collected the upper petroleum ether containing the carotenoid pigment. The extraction was repeated until the aqueous phase was colourless. To the petroleum ether extract, a small quantity of anhydrous sodium sulphate was added to remove turbidity. The absorbance of the extract at 450nm was noted in a spectrophotometer using petroleum ether as blank.

The amount of total carotenoids was calculated using the formula,

$$\text{Amount of total carotenoids} = \frac{A_{450} \times \text{Volume of the sample} \times 100 \times 4}{\text{Weight of the sample}}$$

The total carotenoids were expressed as mg/g leaf.

### Estimation of total phenols

Total phenols were assayed by the method proposed by Mallick and Singh (1980) in plant tissue. An accurate amount (0.5g) of the leaves were homogenized in 10X volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The residue was reextracted with 80% ethanol. The supernatants were centrifuged, pooled and evaporated to dryness. The residue was dissolved in a known volume of distilled water and 0.5ml of Folin-Ciocalteu reagent was added to it. After 3 minutes, 2.0ml of 20% sodium carbonate

solution was added, mixed thoroughly and placed in a boiling water bath for exactly 1 minute, cooled and measured the absorbance at 650 nm in a spectrophotometer. Standard catechol solution (0.2-1ml) corresponding to 2.0-10 µg concentrations were added with Folin-Ciocalteu reagent and sodium carbonate. A standard curve was constructed using an electronic calculator on the linear regression mode, using which the concentrations of phenols in the samples were read. The values are expressed as mg phenols/g leaf.

#### Estimation of flavonoids

Flavonoids were extracted and estimated by the method of Cameron *et al.* (1943).

Leaves (0.5g) were extracted first with MeOH:H<sub>2</sub>O (2:1) and secondly with MeOH:H<sub>2</sub>O (1:1). The two extracts were then combined and evaporated to about 1/3 of the original volume or until most of the MeOH had been removed. The resultant aqueous extract was cleared of low polarity contaminants such as fats, terpenes, chlorophylls and xanthophylls by extraction with hexane or chloroform. This was repeated several times and the extracts combined. The solvent extracted aqueous layer containing the bulk of the flavonoids was then concentrated and used for the assay. For estimation an aliquot of the extract was pipetted out and evaporated to dryness. 4.0ml of vanillin reagent was added and heated for 15 minutes in a boiling water bath. The standard was also treated in the same manner. The optical density was read at 340nm. The values are expressed as mg flavonoids/g leaf.

#### Estimation of reduced glutathione

Estimation of reduced glutathione was done according to the procedure described by Moron *et al.* (1979). An exact amount (0.5g) of the plant sample was homogenized with 2.5ml of 5% TCA. The precipitated protein was centrifuged at 1000 rpm for 10 minutes. 0.1 ml of the supernatant was taken for the estimation. For estimation an accurate aliquot (0.1 ml) of the supernatant was made up to 1.0 ml with 0.2M sodium phosphate buffer. Freshly prepared DTNB solution (2.0 ml) was added and the intensity of the yellow colour formed was read at 412nm in a spectrophotometer after 10 minutes. A standard curve of GSH was prepared

between the concentration ranges of 2 to 10 nmoles. The values are expressed as nmoles GSH/g leaf.

#### Estimation of chlorophyll

The estimation of chlorophyll was done according to the procedure described by Witham *et al.* (1971). An accurate amount (1g) of leaves were extracted with 20ml of 80% acetone, centrifuged (5000rpm for 5 minutes) and transferred the supernatant to a 100ml volumetric flask. This procedure was repeated until the residue was colourless. The supernatant was made upto 100 ml with 80% acetone. The absorbance of the solution was read at 645nm and 663nm against 80% acetone blank. The amount of chlorophyll present in the extract was calculated using the following formula:

$$\text{Total chlorophyll} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

where V was the final volume of the extract and W was the fresh weight of the leaves taken for extraction. The results are expressed as mg chlorophyll/g leaf.

#### Antioxidant status *in vitro*

*In vitro* testing encompasses the use of ultra sensitive probes to test (or to study) the single effect or action of a substance in an isolated environment eliminating interference from other biological phenomena which contribute to a reduction in the number of animals (Robinson, 1991). The *in vitro* model used in the study as alternatives to live animals was goat liver slices. The effect of the exposure of the leaf extracts in the presence or absence of the oxidants was followed in precision-cut goat liver slices, which simulated the *in vivo* environment.

Fresh goat liver was obtained from a local slaughter house and transported to the laboratory on ice. The liver was washed with isotonic KCl and processed for the assays. Liver was the organ of choice because it is the metabolic organ and is responsible for the metabolic clearance of many xenobiotics (Tingle and Helsby, 2006). Very thin slices (~1mm thick) were cut from the liver using a sterile scalpel. The slices were taken in sterile Hank's balanced salt solution (HBSS) at a proportion of

0.25g in 1ml, in broad, flat-bottomed flasks. The standard oxidant  $H_2O_2$  was used at a final concentration of 200 $\mu$ M. The plant extract (20  $\mu$ l of the plant extract corresponding to 20mg) was used to study the antioxidant effect on the cells. The liver slices exposed to different treatments were incubated for one hour at 37°C with very mild shaking.

The following groups were set up for every assay.

1. Untreated (negative) control
2.  $H_2O_2$  treated (positive) control
3. Group treated with white flowered leaf extract
4. Group treated  $H_2O_2$  and white flowered leaf extract
5. Group treated with blue flowered leaf extract
6. Group treated with  $H_2O_2$  and blue flowered leaf extract

After the addition of the respective agents, the tissue slices were incubated at 37°C for one hour with mild shaking. After the incubation period, the tissue was homogenized in a Teflon homogenizer with HBSS. The estimation of various parameters indicative of antioxidant potential were carried out in the homogenate as explained below.

#### **Effect of leaf extract treatment on the levels of non-enzymic antioxidants in liver slices subjected to oxidant stress**

The non-enzymic antioxidants determined in the liver slice homogenate were ascorbic acid, tocopherol, vitamin A and reduced glutathione. The procedures adopted for the determination of vitamin C, vitamin E and reduced glutathione were the same as those for *Clitoria ternatea* leaf analysis. An aliquot of the tissue homogenate was used instead of leaf tissue in the assay.

#### **Estimation of vitamin A**

Vitamin A was estimated by the method of Bayfield and Cole (1980). To 1.0ml homogenate, 1ml of saponification mixture was added. The tubes were gently refluxed for 20 minutes at 60°C. The tubes were cooled at room temperature, 20ml of water was added and mixed well. Vitamin A was extracted twice with 10ml portions of petroleum ether (40-60°). The extracts were pooled, washed thoroughly with water, separating the layers using a separating funnel. When the petroleum ether fraction was clear, a pinch of sodium sulphate

(anhydrous) was added to remove the excess moisture. The volume of the extract was noted and 1.0ml of it was evaporated to dryness at 60°C. The dried residue was dissolved in 1.0ml of chloroform. Aliquots of the standard were pipetted out into a series of clean, dry test tubes in the concentration range of 0-7.5  $\mu$ g. The volumes in all the test tubes were made up to 1.0ml with chloroform. From a fast delivery pipette, 2ml of TCA reagent was added rapidly, mixing with the contents of the tube. The absorbance was recorded immediately at 620nm in a spectrophotometer. The procedure was repeated for the sample tubes. A standard graph was constructed on an electronic calculator set to the linear regression mode, using which the concentration in the samples were read off and expressed as  $\mu$ g/g tissue.

## **RESULTS**

### **Levels of non-enzymic antioxidants in *Clitoria ternatea* leaves**

The major representatives of the non-enzymic antioxidants, namely ascorbate, tocopherol, total carotenoids, reduced glutathione, total phenols, flavonoids and total chlorophyll were estimated in blue and white flowered leaves. The concentrations of non-enzymic antioxidants observed in the leaves of *Clitoria ternatea* are presented in Table 1.

From these results, it can be observed that both the varieties of leaves are good sources of non-enzymic antioxidants. The white flowered leaves had higher content of all the non-enzymic antioxidants analyzed than the blue flowered ones.

### **Effect of *Clitoria ternatea* leaves on the antioxidant status of cells exposed to oxidative stress *in vitro***

To minimize the number of animals needed for testing, alternative methods, such as *in vitro* and computer technology, and use of non-mammalian organisms, plays an increasingly important role in research, which incorporates reduction, refinement and replacement of animals for a particular study (Goldberg and Hartung, 2006). The standard oxidant used to induce the oxidative stress was  $H_2O_2$ . The activities of non-enzymic antioxidants were recorded in precision-cut goat liver slices

**Table 1: Levels of non-enzymic antioxidants in *Clitoria ternatea* leaves**

Parameter	Blue flowered leaf	White flowered leaf
Ascorbic acid (mg/g)	3.91 ± 0.032	5.56 ± 0.015 <sup>a</sup>
Tocopherol (mg/g)	4.73 ± 0.126	8.55 ± 0.057 <sup>a</sup>
Total carotenoids (mg/g)	8.65 ± 0.086	9.54 ± 0.061 <sup>a</sup>
Reducedglutathione(nmoles/g)	33.00 ± 1.000	39.00 ± 1.000 <sup>a</sup>
Total phenol (mg/g)	13.59 ± 0.537	16.30 ± 0.304 <sup>a</sup>
Flavonoids (mg/g)	16.00 ± 0.577	24.00 ± 0.579 <sup>a</sup>
Total chlorophyll (mg/g)	1.18 ± 0.020	1.37 ± 0.025 <sup>a</sup>

Values are mean ± SD of triplicates.

a - Statistically significant (P<0.05) compared to blue flowered leaves.

**Table 2: Effect of *Clitoria ternatea* leaves on the levels of vitamin C in H<sub>2</sub>O<sub>2</sub> - induced oxidative stress in goat liver slices**

Sample	Vitamin C Levels (mg/g tissue)	
	Without H <sub>2</sub> O <sub>2</sub>	With H <sub>2</sub> O <sub>2</sub>
Control	0.133 ± 0.020	0.127 ± 0.004 <sup>a</sup>
Blue flowered leaf	0.301 ± 0.020 <sup>a</sup>	0.147 ± 0.004 <sup>a b c</sup>
White flowered leaf	0.890 ± 0.036 <sup>a</sup>	0.264 ± 0.02 <sup>a b c</sup>

LSD (5%) = 0.034

Values are mean ± SD (n = 3)

a - Statistically significant (P<0.05) compared to untreated control group.

b - Statistically significant (P<0.05) compared to H<sub>2</sub>O<sub>2</sub> treated group.

c - Statistically significant (P<0.05) compared to respective plant group.

**Table 3: Effect of *Clitoria ternatea* leaves on the levels of vitamin E in H<sub>2</sub>O<sub>2</sub> - induced oxidative stress in goat liver slices**

Sample	Vitamin E Levels (mg/g tissue)	
	Without H <sub>2</sub> O <sub>2</sub>	With H <sub>2</sub> O <sub>2</sub>
Control	1.20 ± 0.05	1.05 ± 0.08 <sup>a</sup>
Blue flowered leaf	2.40 ± 0.13 <sup>a</sup>	1.95 ± 0.89 <sup>a b c</sup>
White flowered leaf	2.95 ± 0.07 <sup>a</sup>	2.25 ± 0.05 <sup>a b c</sup>

LSD (5%) = 0.632

Values are mean ± SD (n = 3)

a - Statistically significant (P<0.05) compared to untreated control group.

b - Statistically significant (P<0.05) compared to H<sub>2</sub>O<sub>2</sub> treated group.

c - Statistically significant (P<0.05) compared to respective plant group.

exposed to the standard oxidant ( $H_2O_2$ ) in the presence and the absence of ethanolic leaf extracts of *Clitoria ternatea*. Following a one hour exposure, the non-enzymic antioxidants were estimated in the tissue slices.

#### Ascorbic acid

The levels of vitamin C observed in the different treatment groups are presented in Table 2. The levels of vitamin C in the liver slices increased significantly upon exposure to the leaf extracts alone, in the absence of oxidative stress. When the slices were stressed with  $H_2O_2$ , the vitamin C levels depleted significantly ( $P < 0.05$ ). The effects of the leaves on vitamin C levels were more pronounced

in the slices treated with the white flowered leaves than the blue flowered leaves.

#### Vitamin E

Vitamin E, a major lipid soluble antioxidant, is the most effective chain breaking antioxidant within the cell membrane, where it protects membrane lipids from lipid peroxidation. The levels of vitamin E observed in the various treatment groups are listed in Table 3. The levels of vitamin E in the liver slices after exposure to the leaf extracts showed a significant increase when compared to the untreated group. The oxidant treatment decreased the levels of vitamin E compared to untreated control. This depletion was counteracted

**Table 4: Effect of *Clitoria ternatea* leaves on the levels of vitamin A in  $H_2O_2$  - induced oxidative stress in goat liver slices**

Sample	Vitamin A Levels (mg/g tissue)	
	Without $H_2O_2$	With $H_2O_2$
Control	43.83 $\pm$ 0.021	37.00 $\pm$ 1.00 <sup>a</sup>
Blue flowered leaf	62.07 $\pm$ 0.90 <sup>a</sup>	46.27 $\pm$ 1.10 <sup>a b c</sup>
White flowered leaf	71.13 $\pm$ 1.03 <sup>a</sup>	55.59 $\pm$ 0.55 <sup>a b c</sup>

LSD (5%) = 1.528

Values are mean  $\pm$  SD (n=3)

- a - Statistically significant ( $P < 0.05$ ) compared to untreated control group.
- b - Significant ( $P < 0.05$ ) compared to  $H_2O_2$  treated group.
- c - Statistically significant ( $P < 0.05$ ) compared to respective plant group.

**Table 5: Effect of *clitoria ternatea* leaves on the levels of reduced glutathione in  $H_2O_2$  - induced oxidative stress in goat liver slices**

Sample	GHS Levels (nmoles/g tissue)	
	Without $H_2O_2$	With $H_2O_2$
Control	266.13 $\pm$ 1.40	246.40 $\pm$ 0.31 <sup>a</sup>
Blue flowered leaf	384.53 $\pm$ 0.21 <sup>a</sup>	269.33 $\pm$ 0.16 <sup>a b c</sup>
White flowered leaf	481.30 $\pm$ 1.70 <sup>a</sup>	362.13 $\pm$ 0.55 <sup>a b c</sup>

LSD (5%) = 0.727

Values are mean  $\pm$  SD (n = 3)

- a - Statistically significant ( $P < 0.05$ ) compared to untreated control group.
- b - Statistically significant ( $P < 0.05$ ) compared to  $H_2O_2$  treated group.
- c - Statistically significant ( $P < 0.05$ ) compared to respective plant group



**Fig. 1: Blue and white flowered leaves of *Clitoria ternatea***

by the co-treatment with the leaf extracts. The levels were found to be higher in the white flowered leaf treated group, than the blue one .

#### **Vitamin A**

The levels of vitamin A in the goat liver slices exposed to  $H_2O_2$  in the presence and absence of *Clitoria ternatea* leaf extracts are represented in Table 4. Among the two, the liver slices treated with the white flowered leaves showed significant increase in vitamin A levels than the ones treated with blue flowered leaves.

#### **Reduced glutathione (GSH)**

Reduced glutathione levels were estimated in the liver slices after quick acidification of the reaction mixture to prevent aerial oxidation of the compound. The levels of the same are listed in Table 5. Once again the results showed that the impact of white flowered leaves was higher than that of blue flowered leaves

### **DISCUSSION**

#### **Non-enzymic antioxidants in *Clitoria ternatea* leaves**

Cervantes *et al.* (2007) have found that the aqueous extracts of *Cissus quadrangularis* showed high levels of ascorbic acid. These

antioxidant properties were also shown to depend on the concentration of the sample (Serteser *et al.*, 2008). Different varieties of *Cyanora scolymus* (Artichoke) exhibited differences in polyphenolic content (Wang *et al.*, 2003). *Gladiolus tepal* flower senescence showed decreased level of ascorbate and peroxidase activity when exposed to  $H_2O_2$  oxidant (Hossain *et al.*, 2006). In very recent studies, *Zizyphus jujuba* (Pawlowska *et al.*, 2009), *Teucrium polium*. L (Sharfifar *et al.*, 2009), *Whilst rush crimps* (Conforti *et al.*, 2009) and raspberries (Wange *et al.*, 2009) have been shown to possess higher level of phenols and flavonoid content. The results showed that the white flowered leaves possessed higher levels of the non-enzymic antioxidants and revealed that the white flowered leaves of *Clitoria ternatea* showed the maximum antioxidant content when compared to blue flowered leaves.

#### **Non-enzymic antioxidants analyzed in liver**

The non-enzymic antioxidants analyzed in the liver slices exposed *in vitro* to  $H_2O_2$  and / or *Clitoria ternatea* leaf extracts were vitamins C, E, A and reduced glutathione.

#### **Vitamin C**

The level of vitamin C was significantly reduced by the treatment with  $H_2O_2$ . This reduction was reversed by the presence of the leaf extracts of *Clitoria ternatea*. Ascorbic acid is a terminal water soluble antioxidant that protects lipids against peroxidation (Maneesh *et al.*, 2005). It readily oxidizes the dehydro ascorbic acid and interferes with the process of LPO by scavenging the superoxide anion (Jariyapongskul *et al.*, 2002). Vitamin C supplementation in humans increases plasma ascorbate and improves the resistance of plasma lipids to LPO (Polidori *et al.*, 2004, Linster and Van Schaftingen, 2007). Vitamin C is required for the optimal activity of important biosynthetic enzymes and is therefore essential for various metabolic pathways in the body (Carr and Frei, 1999). Thus the effect was more pronounced in the slices treated with the white flowered leaves than the blue flowered leaves.

#### **Vitamin E**

The present study may be due to the consumption of vitamin E to quench the free radical

generated by H<sub>2</sub>O<sub>2</sub>. The levels of vitamin E were restored by the ethanolic extracts of *Clitoria ternatea* leaves. Vitamin E, a major lipid soluble antioxidant, is the most effective in preventing chain breaking within cell membrane, where it protects membrane fatty acids from lipid peroxidation (Brown and Goodman, 1998). *Hippophae rhamnoides* improved the levels of vitamin E to overcome the oxidative stress induced by nicotine in rats (Gumustekin *et al.*, 2003). Thus *Clitoria ternatea* leaf extracts can render protection to the membranes by increasing the levels of vitamin E, the major antioxidant present in the membrane.

### Vitamin A

Retinol and retinyl esters are precursors of retinoic acid, the most active form of vitamin A and a ligand for retinoid receptors. Retinoic acid plays an important role in controlling cell growth, cell differentiation and apoptosis as well as carcinogenesis, and is of potential clinical interest in cancer chemoprevention and treatment (Altucci and Gronemeyer, 2001). Carotenoids, such as  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, lutein and cryptoxanthine, have been shown to have antioxidant properties (Donaldson, 2004). Vitamin A also prevents hepatic injury caused by H<sub>2</sub>O<sub>2</sub> treatment (Noyan *et al.*, 2006). There was a reduction in plasma vitamin A during oxidant intoxication both in  $\alpha$ -tocopherol acetate supplemented and unsupplemented humans (MacDonald-Wicks and Garg, 2003). *Clitoria ternatea* leaf extracts very effectively increased the levels, reiterating the antioxidant effect of the leaf extracts. Among the two leaf extracts, the white flowered leaf extract caused a more significant

increase in vitamin A levels in the liver slices than the blue flowered leaves.

### Reduced glutathione

GSH maintains functional and structural integrity of cells and is often involved in the defense against tissue injury from administered or metabolically generated toxic agents (Ortman *et al.*, 2000). Reduced glutathione levels decreased in alloxan treated rats, which was efficiently counteracted by the administration of *Boerhavia diffusa* leaf extracts (Satheesh and Pari, 2004). Administration of the flower extract of *Punica granatum* (pomegranate) (Kaur *et al.*, 2006), melatonin (Dakshayani *et al.*, 2005) and curcumin (Marotta *et al.*, 2003) increased the levels of glutathione which had been depleted by severe liver injury in experimental rats. Thus the effect was more pronounced in the slices treated with the white flowered leaves than the blue flowered leaves.

In the present study, the use of liver slices were subjected to oxidative stress using H<sub>2</sub>O<sub>2</sub> and the effect of the co-administration of the leaf extracts was monitored on the antioxidant status. Our results show that the *Clitoria ternatea* leaf extracts as reflected by the beneficial alterations in the non-enzymic antioxidants. Among the two, the white flowered leaves rendered slightly higher induction of non-enzymic antioxidants than the blue flowered leaves.

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