A study on the levels of nonenzymic antioxidants in the leaves and flowers of *Clitoria ternatea*

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Abstract

Antioxidants are substances that inhibit oxidation and thus prevent cell damage. The objective of the study was to determine the antioxidant activity of the leaves as well as blue and white flowers of *Clitoria ternatea*. They exhibited significant antioxidant activity and the sample from the blue flower bearing plant showed better scavenging activity. The results show that *Clitoria ternatea* can be used as a potential antioxidant and can be used to prevent oxidative damage.

Keywords:
Oxidative stress, Medicinal plants, *Clitoria ternatea*

Introduction

Antioxidants act as radical scavengers, inhibit lipid peroxidation and other free radical-mediated processes, and therefore they protect the human body from several diseases attributed to the reactions of radicals. Numerous substances have been suggested to act as antioxidants. Various phenolic antioxidants such as flavonoids, tannins, coumarins, xanthenes and, more recently, procyanidins have been shown to scavenge radicals in a dose-dependent manner and therefore are viewed as promising therapeutic drugs for free radical pathologies (Repetto and Llesuy, 2002).

Antioxidants and reactive oxygen species (ROS) have diverse roles in plant growth and in resistance to environmental stress. ROS are continuously produced in plants as byproducts of aerobic metabolism; some are highly toxic and rapidly detoxified by various cellular enzymic and non-enzymic mechanisms (Janardhanan and Jose, 2000).

Some antioxidants are themselves free radicals, donating electrons to stabilize and neutralize the dangerous free radicals. Other antioxidants work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage (Bray, 1999).

Medicinal plants are plants which may have medicinal properties. Many of our present medicines are derived from research on medicinal plants. Plant possesses efficient antioxidant defense systems to scavenge the ROS and protect the plants from destructive reactions. A regulated balance between oxygen radical production and their destruction is required to maintain metabolic pathway efficiency and functions.
under both optimal and stress conditions (Pandhair and Sekhon, 2006)

*Clitoria ternatea* is a twining evergreen perennial herb with compound leaves. The stems are pubescent and spindly. The flowers are in various shades of blue or pure white. The fruits are pods resembling thin peas. The plants belong to the family *Fabaceae*. The roots, seeds and flowers have medicinal value and are used to treat various ailments (Gomez and Kalamani, 2003).

In the present study the levels of some non-enzymic antioxidants namely ascorbic acid, reduced glutathione and total carotenoids were estimated in leaves and flowers of two varieties of *Clitoria ternatea*, one bearing blue flowers and the other bearing white flowers.

**Materials and Methods**

**Sample Preparation**

The flowers and leaves of *C. ternatea* (both white and blue variety) were collected from plants maintained in the campus and were washed thoroughly with distilled water and dried completely and used for the experiments. Crude aqueous extracts of the plants were used for the assay. The non-enzymic antioxidants were estimated as follows:

**Estimation of Ascorbic Acid :** (Roe and Kuether, 1953)

The sample was homogenized and centrifuged. To the supernatant a pinch of activated charcoal was added and centrifuged. To the supernatant obtained 0.5ml of DNPH was added and drops of 10% thiourea and then it was incubated at 37°C for 3 hours. Osazones formed which was dissolved in 2.5ml of 85% sulphuric acid in cold. The tubes were incubated for 30 minutes at room temperature and the absorbance was read spectrophotometrically at 540nm.

**Estimation of Reduced Glutathione :** (Moron *et al*, 1979)

To 0.1ml of supernatant added 0.9 ml of 0.2 M sodium phosphate buffer pH 8.0 and 2ml of freshly prepared DTNB solution. The intensity of the yellow colour formed was read at 412nm in a spectrophotometer after 10minutes. A standard curve of reduced glutathione was prepared using concentrations ranging from 2-10 nanomoles of reduced glutathione in 5% TCA.

**Estimation of Total Carotenoids :** (Zakaria *et al*, 1979)

The sample was extracted using alcoholic KOH, it was saponified and then extracted using petroleum ether. The petroleum ether layer containing the carotenoid pigment was retained and the aqueous phase was extracted until it became colourless. To the petroleum ether extract small quantities of anhydrous Na₂SO₄ was added. Noted the final volume of petroleum ether extract. Measured the absorbance at 450nm.

**Results and Discussion**

The antioxidant activity of leaves and flowers of *Clitoria ternatea* was determined based on their ability to scavenge free radicals. The results obtained for the levels of some non enzymic antioxidants of the two types of leaves and flowers are tabulated below in table 1.

The levels of ascorbic acid and reduced glutathione were found to be high in blue flowers. The blue leaves possessed higher levels of total carotenoids compared to other samples.
The leaves of and fruits of Iranian Conifers, was evaluated for their antioxidant activity. Methanol extract of fruits of *Cupressus semipervirens* cv. Cereiformis showed the highest antioxidant activity while the methanol extract of leaves of *Cupressus semipervirens* var. *semipervirens* possessed the lowest antioxidant activity. The findings showed that most of the tested extracts were showing strong antioxidant activity even higher than -tocopherol (Asili et al, 2007).

A study was conducted to evaluate the antioxidative activity of extracts from different parts of *Morinda citrifolia* L., including leaf, fruit and root. Methanol and ethyl acetate were used as solvents and antioxidative effects measured by a ferric thiocyanate method (FTC) and thiobarbituric acid test (TBA). The methanol extract of *Morinda citrifolia* L. root exhibited high antioxidative activity; the ethyl acetate extract of all parts of *Morinda citrifolia* L. exhibited significant antioxidative activity, which is comparable to that of both á-tocopherols. Roots showed the highest activity of the parts tested (Hamid, et al, 2001).

The antioxidant properties of 90% ethanol extracts of leaves, and 90% methanol extracts of stem bark, pulp and flowers from Indian Laburnum (*Cassia fistula* L.) were investigated. The antioxidant activity power was in the decreasing order of stem bark, leaves, flowers and pulp and was well correlated with the total polyphenolic content of the extracts; the stem bark had more antioxidant activity in terms of reducing power, inhibition of peroxidation, O₂⁻ and DPPH radical scavenging ability (Becker et al, 2002).

All these studies support our present finding that different parts of the plant possess different levels of antioxidants.

**Conclusion**

Antioxidants are found in varying amounts in foods such as vegetables, fruits, grain cereals, legumes and nuts. The above results show that the leaves and flowers of *Clitoria ternatea* have potent antioxidant activity. The flowers and leaves of the blue flower bearing plant were found to possess higher antioxidant activity. Hence they can be used to treat free radical mediated disorders and to combat oxidative stress.
Table 1: Levels of non-enzymic antioxidants in leaves and flowers of
*Clitoria ternatea*

| S. No. | Sample       | Ascorbic acid (mg) | Reduced glutathione (n moles/g) | Total carotenoids (mg) |
|--------|--------------|--------------------|-------------------------------|------------------------|
| 1.     | Blue flower  | 5.94               | 25.68                         | 26.58                  |
| 2.     | Blue leaf    | 3.39               | 22.05                         | 33.90                  |
| 3.     | White flower | 3.55               | 16.56                         | 16.13                  |
| 4.     | White leaf   | 2.46               | 19.85                         | 22.14                  |

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