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Comparative Analysis of Enzymatic and Antioxidant Properties in Two Varieties of *Clitoria ternatea*

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Abstract: ***The goal of the present study was to compare the enzymatic antioxidants viz., superoxide dismutase, catalase, lipid peroxidation, polyphenol oxidase, ascorbic acid oxidase and antioxidant properties in leaves and roots white flowered and purple flowered varieties of Clitoria ternatea. white flowered leaves showed higher enzymatic activity as compared to Clitoria ternatea purple flowered variety. In case of roots the purple flowered variety showed higher lipid peroxidation, superoxide dismutase and polyphenol oxidase as compared to white flowered variety. Whereas white flowered variety roots showed higher catalase and ascorbic acid oxidase activity as compared to purple flowered variety. Clitoria ternatea white flowered leaves and roots showed higher antioxidant activity as compared to purple flowered leaves and roots.***

Keywords: *Clitoria ternatea*, enzymatic properties and antioxidants.

1. Introduction

Clitoriaternatea is a member of leguminoseae (Fabaceae). It is commonly called *Clitoriaternatea*, blue pea, butterfly pea, aparajita (India), kordofan pea (Sudan), cunha (Brazil) or pokindang (Philippines), is a vigorous, twinning summer growing, perennial legume of Old World Origin. The taxonomy, nomenclature and distribution of *Clitoriaternatea* have been reviewed by Fantz (1977).It is a deep rooted, tall slender and are a climbing legume. *Clitoriaternatea*is self-pollinated however segregating genotype have been identified indicating partial out crossing probably exists. *Clitoriaternatea* is widely used in traditional Indian systems of medicine as a brain tonic and is believed to promote memory and intelligence. The study conducted on rats revealed that *Clitoriaternatea* root extracts increase rat brain acetyl choline content and acetyl choline esterase activity in a similar fashion to the standard cerebro drug pritinol (Taranalli and Cheeramkuzhy, 2003). The plant is considered as a good brain tonic and is useful for throat and eye infection, skin diseases, urinary troubles even in cattle, ulcer and antidotal properties (Malabodi and Nataraja, 2001) besides its medicinal property *Clitoriaternatea* is also a good source of phytochemical substances. It contains antifungal proteins and has been shown to be homologous to plant defenses (ct-AMP1) (Thevissen*et al*., 2000).

2. Materials and Methods

The plants of two *Clitoriaternatea* varieties (purple and white flowered) were procured from the local nursery, Civil lines, Allahabad.

Extraction of sample

The leaves and roots of two *Clitoriaternatea* varieties were washed under tap water separately. The fresh sample was used for antioxidant enzyme analysis. The samples used for enzyme analysis were homogenized by using different buffers.

Lipid peroxidation was measured by estimating the end product malondialdehyde as per method of Heath and Packer (1968).Antioxidant activity was determined through DPPH free radical scavenging activity method given by (Yen and Duh, 1994).The superoxide dismutase activity was assayed the method of Bauchamp and Fridovich (1971).The method followed was given by Hosetti and Frost (1994).The polyphenol oxidase activity was assayed by measuring the increase in absorbance at 420 nm with the oxidation of catechol as substrate according to the method given by Liu et al. (2005).The ascorbic acid oxidase activity was assayed the method of (Bruning and Mohr, 1972).

3. Results and Discussion

In case of leaves the maximum lipid peroxidation was found to be 22.26µM/g in white flowered and 21.55µM/g in purple flowered respectively, in case of roots the two varieties of *Clitoriaternatea* under study, purple flowered shown the maximum lipid peroxidation 27.84 µM/g followed by white flowered 26.20 µM/g The results of present study are in accordance to Becana *et al*. (1986) who observed the lipid peroxidation in leaves of *Medicagosativa* which was 119.6 nmol MDA/g dry wt and in roots was 26.2 nmol MDA/g dry wt.

The maximum SOD activity was obtained 8.09 U/mg of protein in leaves of white flowered followed by in leaves of purple flowered 6.86 U/mg of protein respectively. Whereas in case of roots of two varieties of *Clitoriaternatea* the maximum activity 1.54U/mg of protein in purple flowered and 1.05 U/mg of protein in white flowered were found. The observations made on the parameter are in agreement with those of Padmaja *et al*. (2011) who also observed that 7.234 U/mg of protein in leaves of *Sesbaniagrandiflora*

The maximum catalase activity was obtained 71.50 U/mg of protein in leaves of white flowered followed by in leaves of purple flowered 62.94 U/mg of protein respectively. Whereas in case of roots of two varieties of *Clitoriaternatea* the maximum activity 14.78 U/mg of protein in white

flowered and 13.55 U/mg of protein in purple flowered were found. The observations made on the parameter are in agreement with those of Padmaja *et al*. (2011) who also observed that the catalase activity 76.06 U/mg protein in leaves of *Sesbania grandiflora.*

The maximum polyphenpl oxidase activity was obtained 12.71 µmol/g in leaves of white flowered followed by in leaves of purple flowered 11.85 µmol/g respectively. Whereas in case of roots of two varieties of *Clitoriaternatea* the maximum activity 15.16 µmol/g in purple flowered and 14.14 µmol/g in white flowered were found. The results of present study are in accordance with those of Gomathi*et al*. (2013) who observed that the activity of polyphenol oxidase in whole parts of *Evolvulusalsinoides* was 6.78 µmol/g.

The maximum ascorbic acid oxidase activity was obtained 0.494 U/mg of protein in leaves of white flowered followed by in leaves of purple flowered 0.438 U/mg of protein respectively. Whereas in case of roots of two varieties of *Clitoria ternatea* the maximum activity 0.378 u/mg of protein in white flowered and 0.298 U/mg of protein in purple flowered were found. Similarly results were observed by Padmaja *et al.* (2011) which was 0.374 U/mg protein in leaves of *Sesbania grandiflora*. Accordingly Singh (2012) observed 6.392 U/mg protein activity of ascorbic acid oxidase in roots of *Glycyrrhizaglabra*.

It was observed from the Table 2 that methanolic extract of the roots and leaves shows an increase in scavenging activity of DPPH on increasing concentration .the percentage inhibition of DPPH highest in roots of white flowered varieties of *Clitoriaternatea* 57.10 at 400 µg/ml concentration as compare to roots of purple flowered verities of *Clitoriaternatea* 44.95 % at 400 µg/ml concentration the percentage inhibition found in minimum leaves of purple flowered varieties of *Clitoriaternatea* 10.83 % at 100µg/ml concentration followed by 54.28%,73.38% and 89.95% at200, 300, 400 µg/ml concentration respectively.

The lower IC50 represent the higher antioxidant activity of leaf and root extracts. Patil and Patil (2011) observed the IC50 value of methanolic extracts of roots of blue flowered varieties of *Clitoriaternatea* which was 492 µg/ml and in white flowered varieties of *Clitoriaternatea* which was 342 µg/ml. Accordingly Rabeta *et al*. (2013) also observed the % inhibition of DPPH scavenging in leaves of *Clitoriaternatea* which was 64.67 % at 25 µg/ml, 264% at 50 µg/ml, 408.67 % at 100 µg/ml and 472% at 125 µg/ml respectively.

Table 1: Enzymatic analysis in leaves and roots of PF (Purple flowered) and WF (White flowered) varieties of *Clitoria ternatea*

Enzymatic analysis	Leaves		Roots	
	PF	WF	PF	WF
Lipid peroxidation (nmol MDA/g dry wt)	21.54±0.41	22.25±0.083	27.84±0.21	26.20±0.21
Superoxide Dismutase (U/mg protein)	6.86±0.44	8.09±0.84	1.54±0.077	1.05±0.035
Catalase (U/mg protein)	62.94±0.06	71.50±0.72	13.55±0.30	14.78±0.10
Polyphenol oxidase (U/mg protein)	11.85±0.15	12.71±0.16	15.16±0.16	14.14±0.14
Ascorbic acid oxidase (U/mg protein)	0.43±0.05	0.49±0.07	0.29±0.01	0.38±0.02

The data have been reported as mean ± standard deviation (n=3).Students T-test were used for determination of statistical significance. p< 0.05 were regarded as significant.

Table 2: Antioxidant activity in leaves and roots of two varieties of *Clitoriaternatea*

Concentration (µg/ml)	**% Inhibition of DPPH radicals**			
	Leaves of purpled flowered *Clitoriaternatea*	Leaves of white flowered *Clitoriaternatea*	Roots of purpled flowered *Clitoriaternatea*	Roots of white flowered *Clitoriaternatea*
100	10.83±0.35	12.95±0.03	19.54± 0.31	26.29± 0.54
200	54.28±0.09	52.79±0.1	23.73± 0.45	35.57± 1.0
300	73.38±0.26	81.64±0.1	35.88± 0.58	45.92 ±0.28
400	89.95±0.26	94.30±0.27	44.95±2.41	57.10 ± 1.52

The data have been reported as mean ± standard deviation (n=3).Students T-test were used for determination of statistical significance. p< 0.05 were regarded as significant.

Table 3: IC_{50} value in ethanolic extracts of two varieties of *Clitoriaternatea*

S. No.	Leaves and roots of two varieties of *Clitoriaternatea*	IC_{50} (µg/ml)
1.	Purple flowered leaves	174.4
2.	White flowered leaves	189.3
3.	Purple flowered roots	424.8
4.	White flowered roots	378.3

The data have been reported as mean ± standard deviation (n=3).Students T-test were used for determination of statistical significance. p< 0.05 were regarded as significant.

4. Conclusion

Clitoria ternatea white flowered leaves and roots showed higher antioxidant activity as compared to purple flowered leaves and roots.

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