Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

EVALUATION OF ANTIOXIDANT POTENTIAL OF CLITORIA TERNATEA L.

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Received: 02 Mar 2013, Revised and Accepted: 25 Apr 2013

ABSTRACT

Clitoria ternatea L. (*C. ternatea var. ternatea* and *C. ternatea var. pilosa*) are herbaceous medicinal plant are used in India to treat liver problems. The objective of this study is to investigate the antioxidant activities to justify the use of the plant in folkloric medicine. Antioxidant activities of different fractions from different extracts (Leaves, Stem and Root) were evaluated by using antioxidant assay like DPPH, FRAP, Metal Chelating Ability, Reducing Power assay. Methanolic extract of *C. ternatea var. pilosa* root showed highest value 87.75±0.05% and 74.26±0.04% in DPPH and Ferrous ion chelating activity whereas its stem extract 0.588±0.2 and leaves extract 2.132±0.037 mg of AAE per 100 g in FRAP and Reducing power assay respectively. Almost all the fractions of white variety showed highest activity as compare to blue variety. The results obtained in this study showed that both variety of *C. ternatea* have antioxidant properties which provide a basis for the traditional use of plant and could be harnessed as drug formulation.

Keywords: Antioxidant activity, Chelating activity, Reducing power, *Clitoria ternatea*.

INTRODUCTION

Antioxidants are considered important nutraceuticals on account of their many health benefits and are widely used in the food industry as inhibitors of lipid peroxidation [1]. Antioxidant compounds are widely used compound to counter the free radicals mediate oxidative stress in the cell. These antioxidant compounds can be derived from natural and chemical sources. Natural sources are much safer to use due to less toxicity and side effects, so the production of antioxidant compounds from the natural sources such as plants and algae is in great demand [2]. The study done on medicinal plants and vegetables strongly support the idea that plants constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [3].Synthetic antioxidant like, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are carcinogenic [4]. There is an increasing interest in the investigation of naturally occurring antioxidants from plants. One of the plants that deserve attention is *Clitoria ternatea* L.belongs to family Fabaceae, commonly known as 'Gokarn'. It is a perennial climber having slender downy stem with leaves 5-7 leaflets elliptical to oblong in shape and flowers are usually solitary, bright blue or white.

The various plant parts of *Clitoria ternatea L.* used in various diseases in folk [5]. The comparative evaluation of antioxidant activity of blue and white flowered varieties of *Clitoria ternatea* has not been carried out yet. Aim of this present study is to explore the antioxidant potential of both varieties of *Clitoria ternatea* through DPPH, FRAP, Ferrous ion chelating ability, Reducing Power assay.

MATERIAL AND METHODS

The fresh and healthy leaves, stem and root of blue and white flowered varieties of *Clitoria ternatea* have been collected from local habitat. These materials were dried room temperature. The dried materials were subjected to size reduction to get coarse powder by using grinder. This powder was extracted with methanol in orbital shaker 3 hrs. Then filtered using Whatman no.1. Filtrate was vaporized in water bath at 45°C. Each extract was concentrated to a small volume and allowed to dry. After drying, the respective extracts were weighted and percentage extractives values were determined.

DPPH radical scavenging activity:

The antioxidant activities of plant extracts and the standard were assessed on the basis of the free radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method (Braca *et al.,* 2002)[6]. Equal volumes of methanolic solutions of DPPH (100µM) and crude extract containing (20-200µg/ml) were mixed together. The reaction mixture was shaken well and allowed to stand at room temperature for 30 minutes. The absorbance of the colored complex was measured at 516nm on double beam UV- spectrophotometer against methanol as blank. The L- ascorbic acid (100µg/ml) was used as standard and the percent scavenging effect was calculated by using the formula.

$$\%\text{Inhibition} = \frac{(Ac - AE/AS)}{Ac} \times 100$$

Where,

Ac is the Absorbance of Control (DPPH),

AE is the Absorbance of DPPH + plant extract,

As is the Absorbance of standard

Ferric Reducing Antioxidant Power

Antioxidant activity assays were performed as per the method described by Benzie and Strain (1996) [7]. 0.5 ml plant extract mixed with equal volume of distilled water. Add 3 ml of FRAP reagent (0.3M acetate buffer: TPTZ: Fecl3=10:1:1) was mixed well and incubate for 15 min. at 37°C. Absorbance was measured at 593 nm. The blank was prepared without plant extract. The results are expressed as ascorbic acid equivalent antioxidant capacity.

Ferrous ion-chelating ability assay:

The method proposed by Decker and Welch (1990)[8] was used to determine the ferrous ion-chelating ability of plant extract. 2 ml of methanolic plant extract (100µg/ml) was mixed with 0.1 ml of 2 mM FeCl2 and 0.2 ml of 5 mM ferrozine solutions and allowed to react for 10 minutes at room temperature. The absorbance was measured at 562 nm on Spectrophotometer. The $FeCl_2$ and ferrozine used as a control. Distilled water instead of ferrozine solution was used as a blank. The percentage inhibition of the ferrous ion was calculated by comparing the results of test with that L-ascorbic acid (100µg/ml) of the control using the formula.

$$\%\text{ scavenging activity (Ferrous ion chelating ability)} = \frac{(Ac - AE/AS) \times 100}{Ac}$$

Where,

Ac is the Absorbance of Control reaction.

AE is the Absorbance of plant extract.

As is the Absorbance of standard.

Reducing power

The reducing power of the methanolic extract was determined according to the method given Oyaizu (1986)[9]. One ml of the fruit extract containing (10-100µ/ml) in 1ml of the deionised water mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml potassium ferrocyanide (1%). The mixture was incubated at 500C for 20 minutes. 2.5ml of TCA (10%) and centrifuged at 3000 rpm. The upper layer of the solution was mixed with 2.5ml distilled water and FeCl3 (0.5ml, 0.1%). The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated the higher reducing power. The absorbance compared with the standard ascorbic acid (100µg/ml).

RESULTS AND DISCUSSION

DPPH free radical scavenging activity

The essence of DPPH assay is that the antioxidant react with the stable free radical 1,1,-Diphenyl-2-picrylhydrazyl (deep violet color) and converts it to 1,1-Diphenyl-2-picrylhydrazine with a yellow color. The degree of discoloration indicates the scavenging potential of the sample. Hence the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. Determination of DPPH scavenging activity in plants, methanol and aqueous extract was done in triplicates. Five different concentrations of samples extracts were tested to identify the significance of extract concentration on the scavenging activity. Water and DPPH mixture was used as negative control[10].

The changes in the free radical scavenging ability of blue and white varieties of *C.ternatea* on the basis of percent inhibition of methanolic extracts presented in [Fig.1]. From the graph, it can be observed that, percentage of DPPH scavenging activity for all plant extracts increased with the concentration of plant extract used. This statement is true for all methanol extract with concentration 100, 200, 300, 400 & 500 µg/ml. It is evident from the figure, that the methanolic extract of white flower root has the highest i.e.87.24±0.02% and that of blue flower leaves has the lowest i.e.(72.75±0.05%) free radical scavenging potential among the studied blue and white varieties. The order of antioxidant activity in *C.ternatea* varieties can be given as WR>WST>BR>BST>WL>BL.

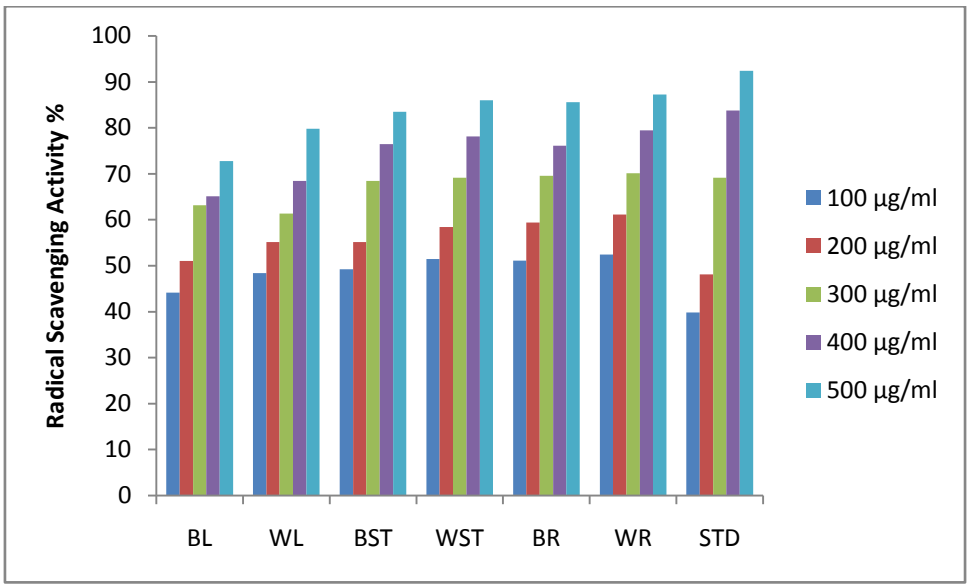

Fig. 1: Radical scavenging activity by DPPH method.

BL= Blue variety leaves, BST= Blue variety stem, BR= Blue variety root, WL= White varity leaves, WST= White variety stem, WR= White variety root, STD= Ascorbic Acid.

Peteros and Uy (2010)[11] studied the antioxidant screening of four medicinal plants using methanolic extracts of different concentration. The values of DPPH results (10.8-98.3%) were somewhat similar to the present work. The results of the DPPH free radical scavenging assay suggest that root of white flower *C.ternatea* have potent antioxidant activity of scavenging free radicals. These species could be used as a potent source for the cancer chemo protective therapy. Jain et al. (2010) [12] evaluated in-vitro stem extract of *C. ternatea* for antioxidant property, the DPPH results in acetone and methanol extract (65.12 &47.96%) obtained by them were lesser than present studied methanolic extract. Shekhawat et al. (2010)[13]assessed free radical scavenging activity of crude extract of some medicinal plants using ethanolic extract. The aerial parts of C.ternatea values 99.66% (100-500 ug/ml) were slightly greater than present study. Sini et al. (2010) [14] worked on antioxidant activity of certain medicinal plants using DPPH assay. There results of *Clitoria ternatea* (Root) 82.87±0.246% were lesser than present study.

In present work the DPPH of WR is more in white flower variety than the blue flower variety. Above previous studies showing the results from only blue variety of *C.ternatea*. In present work we taken both varieties and results indicates that DPPH activity in white variety of *C.ternatea* always greater than blue variety.

Ferric Reducing Antioxidant Power

Ferric Reducing Antioxidant Power (FRAP) is a simple inexpensive assay and may offer index of antioxidant activity. The FRAP assay measure the reducing potential of antioxidant to react on ferric tripyridyltriazine (Fe 3+ TPTZ)complex and produce blue color of ferrous form which can be detected at absorbance 593 nm (Benzie and Strain,1996) [7]. The ferric reducing antioxidant activity of *C.ternatea* in methanolic extract presented in [Fig. 2]. It is clear from result that FRAP activity of white variety stem is the highest among all the studied parts of blue and white variety.

The highest absorbance of FRAP was observed in WST at 500 µg/ml and the lowest was that in BL at 100 µg/ml i.e. 0.588±0.2 and 0.203±0.01 mg of AAE/100g DW respectively. Katalinic et al. (2006) [15] screened seventy medicinal plant extracts for antioxidant capacity and total phenols. The FRAP values ranged from 0.06 to 25 Mm/L. according to their antioxidant capacity, the medicinal plant extracts were divided into five groups: a)very low FRAP(<1Mm/L) n=9; b) low FRAP(1-5 Mm/L), n=37; c) good FRAP (5-10 Mm/L), n=15; d)high FRAP(10-20 Mm/L),n=8; e)very high FRAP(>20 Mm/L),n=1. The best result were obtained for *Melissae folium* which showed a very high FRAP (>20 Mm/L) values. Surveswaran et al. (2007) [16] evaluated one hundred thirty two medicinal plants for the antioxidant activities of leaves. Following

are some of these plants with their antioxidant activity. *Aloe littoralis* Baker 8.68, *Murraya exotica* L.1.80, *Vitex negundo1*.44 and *Viola serpens* Wall.ex. Ging 0.91 µmol/g DW. Bhaskarrao et al. (2011) [17]evaluated antioxidants of some Indian medicinal plants. In that, the FRAP results *C.ternatea* (Leaves) 1320 FRAP Units observed. Buddepu et al. (2011) [18] studied in-vitro antimicrobial and free radical scavenger assay of two medicinal plants *C. ternatea* and *Cardiospermum halicacabum*. They observed in methanolic extraction higher FRAP value (11.3µM) than other solvent extraction (Hexane and Chloroform). Chanu et al. (2012) [19] observed FRAP value in *Parkia javanica* and *Phlogacanthus thyrsiflorus* 8.24±0.19 and 41.81±0.68 mg of ascorbic acid equivalent/gm respectively which was higher than the present study.

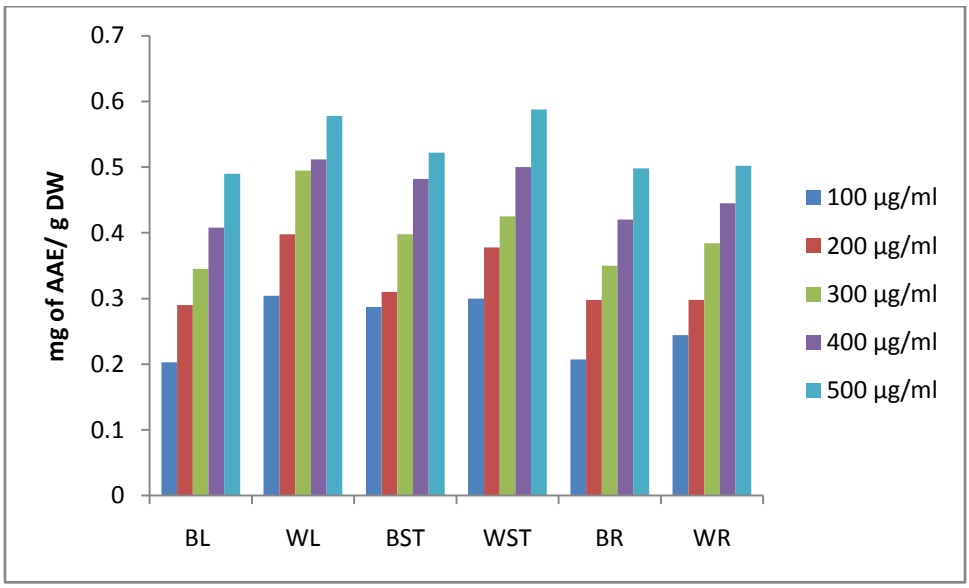

Fig. 2: Antioxidant activity in *Clitoria ternatea* by FRAP method [mg AAE/gm DW]

BL= Blue variety leaves, BST= Blue variety stem, BR= Blue variety root, WL= White varity leaves, WST= White variety stem, WR= White variety root.

In present work various plant parts of both (blue and white variety) of *C.ternatea* has been studied instead of leaves. [17,18]. The results showing present study the FRAP activity is always greater in white variety than blue variety. Among the plant part stem of white flower showing greater FRAP activity.

Ferrous ion-chelating ability assay

One of the assay of antioxidative action is chelation of transition metal, thus preventing catalysis of hydroperoxide decomposition and fenotype reactions (Gordon, 1990) [20]. The complex formation is disrupted with the result that the red color reduction, therefore allows the estimation of chelating activity of the coexisting chelator. In the presence of chelating agents, The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. The relatively mild iron (II) chelating activity of the plant extract is of great significance, because it has been proposed that the transition metal ions contribute to the oxidative damage in neurodegenerative disorders, like Alzheimer's and Parkinson's diseases and one of the lines of treatments currently under investigation is selective low affinity binding of transition metals (Bush 2003 [21]; Vardarajan et al.2000 [22]). The chelating effect of the test sample on ferrous ions is shown in graph 3. The percent inhibition activity in white variety (Root) at 5oo µg/ml is 74.26±0.04 % which is highest among all samples and that of lowest has been recorded in blue variety (Stem) at 100µg/ml concentration i.e.20.24±0.21%. It is observed that inhibition percentage values go on increasing with continuous increase in concentration of methanolic plant extracts in the assay mixture. Comparison of the aforementioned results obtained from free radical scavenging activities indicated that the ferrous ion chelating effect of methanolic extracts did not correlate with the results from DPPH and FRAP assay. This discrepancy in the antioxidant assay may be due to different mechanisms involved in antioxidant assay.

Ho et al. (2012) [23] studied antioxidant activities of leaf extracts from 18 indigenous tree species in Taiwan. They observed ferrous ion chelating in methanolic extract of tree species within range of 88.1µg/ml to 1000µg/ml. Chan et al. (2009) [24] studied ferrous ion chelating activity from the fresh, air, and freeze dried leaves of *Alpinia zerumbet* (11±2.0 mg GAE/g, *Etlingera elatior* (17 ±4.2 mg GAE/g),*Curcuma longa* (2.9 ±0.1 mg GAE/g) and *Kaempferia galangal* (0.7±0.1 mg GAE/g). Lin and Chang (2005) [25] *Brassica oleracea* L. var italic fresh and precooked samples have the highest ferrous ion chelating ability, at around 90%; the extract from precooked +cooked samples have a ferrous ion chelating power of 82.5% ; the extract from cooked broccoli had the lowest ferrous ion chelating power at 79.0%. Among the plant parts roots of both varieties showing higher activity in 500µg/ml than other parts of *C.ternatea*.

Reducing power

The reducing power assay measures the electron-donating capacity of an antioxidant. The yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducer causes the conversion of the Fe 3+/ferricyanide complex used in this method to the ferrous form may serve as a significant indicator of its antioxidant capacity. The degree of color change is proportional to the antioxidant concentration. The reaction end point is reached when color change stop. The antioxidant activity has been reported to be concomitant with development of reducing power. In *C.ternatea*, the reducing power of methanolic extract is found to be increase with increasing amount of extract concentration as shown in fig.4. Similar results were obtained by Patil and Patil (2011) [26] in root of blue and white flowered varieties of *C.ternatea* L. In present study, white variety (Leaves) has shown the highest reducing power at all concentration of its extracts i.e.100 µg/ml, 0.430 ±0.0034; 200 µg/ml, 1.013±0.0069; 300 µg/ml, 1.642±0.004; 400 µg/ml, 1.862±0.002 and 500 µg/ml, 2.132±0.0037 mg of AAE per 100 g and blue flower (Stem) shown lowest reducing power i.e.. 100 µg/ml, 0.198 ±0.0053; 200 µg/ml, 0.748±0.0078; 300 µg/ml, 1.321±0.034; 400 µg/ml, 1.523±0.022 and 500 µg/ml, 1.809±0.0017 mg of AAE per 100 g. The high level absorbance indicates the strong reducing power. As white variety leaves has shown the highest absorbance reading, it possess the highest reducing power.

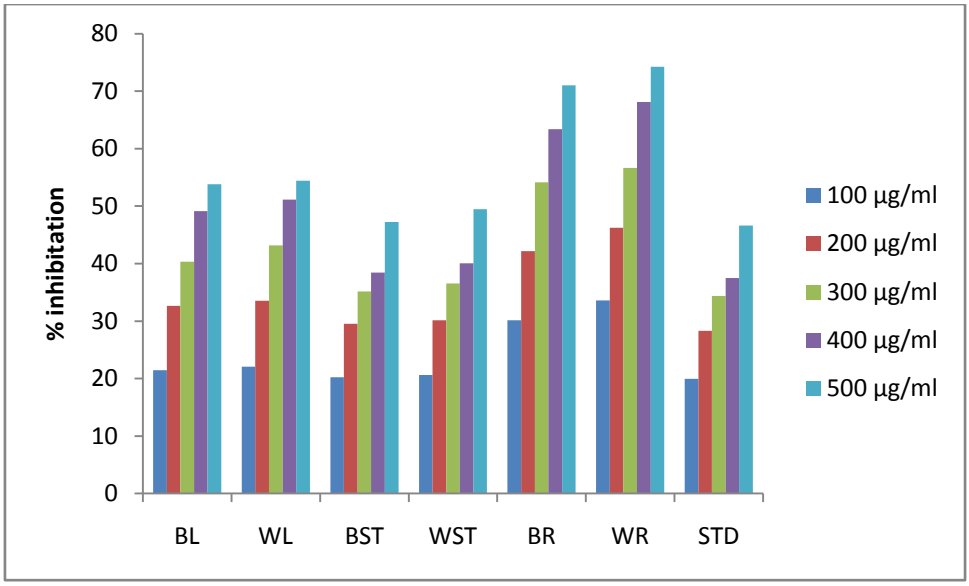

Fig. 3: Ferrous ion chelating activity in *C. ternatea*.

BL= Blue variety leaves, BST= Blue variety stem, BR= Blue variety root, WL= White varity leaves, WST= White variety stem, WR= White variety root, STD= Ascorbic Acid.

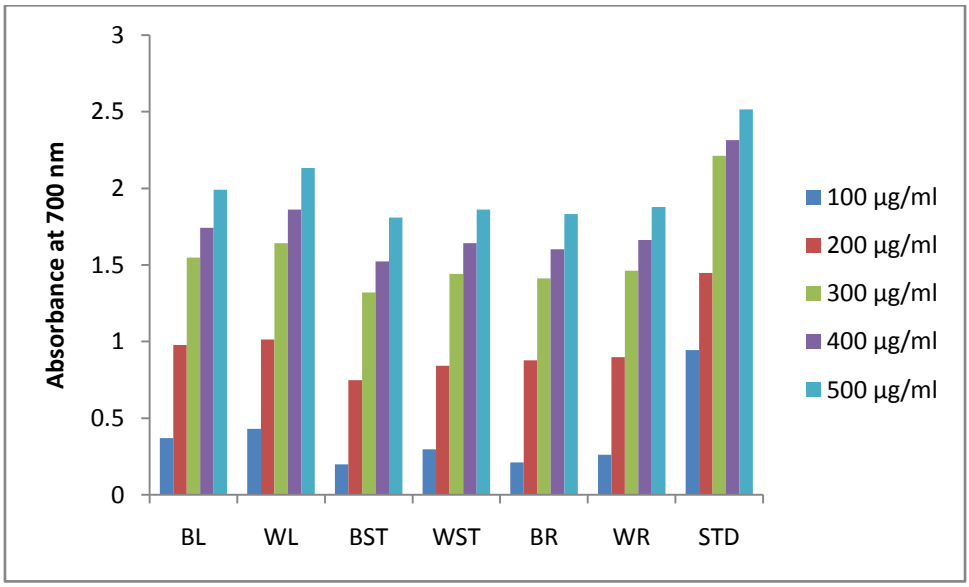

Fig. 4: Reducing power in *C.ternatea*.

BL= Blue variety leaves, BST= Blue variety stem, BR= Blue variety root, WL= White varity leaves, WST= White variety stem, WR= White variety root, STD= Ascorbic Acid.

Bhaskar Rao et al.(2009) [27] evaluated the antioxidant potential of *C.ternatea* by using aqueous extract. The reducing power was144-340 AscAE. Lu et al.(2010) [28] have reported the 53.59±2.19 mg/ml as the reducing power from green tea. Bursal and Koksal(2011) [29] evaluated the reducing power and radical scavenging activities of water and ethanol extracts from *Rhus coriaria* L. They found that the reducing power of the extracts and standard antioxidants was decreased in the order of BHA>trolox>BHT>tocopherol>water extract>ethanol extract, in presence of 30µg/ml test sample. They observed that reducing content of water extract were higher than those of the ethanol extract. In present work the leaves of white variety shows highest reducing power. The stem and root of both varieties found slightly different reducing power.

DPPH, FRAP, Metal chelating assay and reducing power assay mainly carried out for studying free radical scavenging activity. As per most of the assay white variety root has shown highest antioxidant capacity. While by FRAP assay white variety stem has highest and reducing power assay white variety leaves has highest antioxidant power. Similarly as per DPPH, Reducing power and Metal chelating assay blue variety stem has lowest antioxidant capacity but that the lowest in blue variety leaves as per FRAP assay. All this information indicates that all these assay help differently in scavenging free radical ions. Each assay has specificity of free radicals, therefore, these entire assay are essential to study any species.

CONCLUSION

The finding of the present study suggested that methanolic extract of *Clitoria ternatea* var.*pilosa* (White variety) could be a potential source of antioxidants and could have greater importance as therapeutically agent in preventing or slowing oxidative stress related degenerative diseases. However further studies are

necessary to examine underlying mechanisms of antioxidant effect and to isolate the active compounds responsible for these pharmacological activities.

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