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Research Article

EVALUATION OF *IN-VITRO* ANTIOXIDANT ACTIVITY OF SEEDS OF BLUE AND WHITE FLOWERED VARIETIES OF *CLITORIA TERNATEA* LINN.

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ABSTRACT

The seeds of Blue and White flowered varieties of *Clitoria ternatea* were studied for their *in-vitro* antioxidant potential. Pet Ether, Chloroform, and Methanol extracts seeds of Blue and White flowered varieties of *Clitoria ternatea* (CT) were studied for DPPH free radical scavenging assay, reducing power assay and hydroxyl radical scavenging assay. Pet Ether, Chloroform and Methanol extracts seeds of White flowered variety of CT were significantly inhibited the DPPH free radical at the concentrations ranging from 50-600 µg/ml, showed highest inhibition at 600 µg/ml i.e. 52.07%, 56.20% and 76.46% respectively. Pet Ether, Chloroform and Methanol extracts seeds of Blue flowered variety of CT were showed highest inhibition of DPPH free radical i.e. 46.44%, 54.03 % and 70.68% at 600µg/ml respectively. Methanol extracts of CT also showed significant reductive ability as well as hydroxyl radical scavenging activity. Methanol extract of seeds of white flowered variety of CT showed more significant antioxidant activity as compared to blue flowered variety of CT. The methanol extract of CT (MECT) showed better antioxidant activity when compared to control and these differences were statistically significant ($p < 0.001$). The seeds of *Clitoria ternatea* Linn. could be potential source of antioxidant.

Keywords: *Clitoria ternatea*, Antioxidant, DPPH, Reducing power, Hydroxyl.

INTRODUCTION

Oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation *in-vivo* and *in-vitro.* These ROS creates oxidative stress which results in numerous diseases and disorders such as cancer, cardiovascular disease, neural disorders, Alzheimer's disease mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, ageing, atherosclerosis. The compounds especially from natural sources are capable of providing protection against free radicals. This has attracted a great deal of research interest in natural antioxidants. It is necessary to screen out the medicinal plants for their antioxidant potential.[1]

Clitoria ternatea L. (CT) a perennial twining herb, found throughout India in tropical areas. CT is commonly known as 'Butterfly pea' belongs to Family: Fabaceae. CT has two flowered varieties one is white flowered variety and second blue flowered variety. CT has been traditionally used as a remedy for various disease like urinogenital disorder, bronchitis, purgative, diuretic, anthelmintic, rheumatism, demulcent, anticancer, antidote for animal stings.[2,3,4,5] CT has been used as an ingredient in 'Medhya Rasayana' a rejuvenating recipe used for treatment of neurological disorders.[6] CT has been scientifically studied for various pharmacological activities like antioxidant[7], local anesthetic[8], anthelmintic[9,10], antipyretic, anti-inflammatory, analgesic[11], anxiolytic, antidepressant, anticonvulsant, sedative[12], hypoglycemic[13], anticancer[14] also enhances acetylcholine content in rat hippocampus[15]. A wide range of chemical constituents are present in CT. The comparative evaluation of antioxidant activity of blue and white flowered varieties of CT has not been carried out yet. Aim of this present study is to explore the antioxidant potential both varieties of *Clitoria ternatea* L.

MATERIAL AND METHODS

Collection, authentication and extraction of plant material

The seeds of blue and white flowered varieties of *Clitoria ternatea* were collected from local habitat. The plant specimens were authenticated (Specimen No 9492, 9493) by Botany Department Rashtrasant Tukdoji Maharaj, Nagpur University, Nagpur. The seeds of Blue and White flowered varieties of *Clitoria ternatea* were dried at room temperature. The dried seeds were subjected to size reduction to get coarse powder by using grinder. This powder was packed into soxhlet apparatus and extracted with pet. ether (60-80^{0}c), chloroform, methanol[16]. The extracts were evaporated to dryness at 40^{o}C (White flowered variety yields 9.5%, 10%, 11.5% w/w respectively and Blue flowered variety yields 9%, 10.5%, 11% w/w respectively). The phytochemical screening of residues revealed the presence of sterols, alkaloids, saponins, glycosides, carbohydrates, proteins, phenolic compounds and tannins.[17,18]

In-vitro antioxidant assay

DPPH free radical scavenging assay

10 mg extracts of seeds of blue and white flowered varieties of *Clitoria ternatea* were dissolved in 10 ml of 90% methanol solution to obtain 1000 µg/ml sample solutions. 1000 µg/ml solution of each extract was series diluted into concentration ranging from 50-600 µg/ml (i.e. 50, 100, 150, 200, 300, 400, 500 and 600 µg/ml). 200µM solution of DPPH in methanol was prepared and 1.5 ml of this solution was added to 1.5 ml of each extract solution at different concentrations (50-600µg/ml). Ascorbic acid was used as the standard control, with concentrations ranging from 2-20µg/ml (i.e. 2, 5, 10, 15 and 20µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. The absorbance of DPPH solution decreases when kept in contact with antioxidant test sample and free radical scavenging activity is inversely proportional to the absorbance of DPPH solution[19,20,27]. Percent inhibition of DPPH free radical scavenging activity was calculated using the following formula,

$$\text{DPPH Radical Scavenged (\%)} = \frac{(\text{Acont} - \text{Atest})}{\text{Acont}} \times 100$$

Where Acont is the absorbance of the control reaction.

Atest is the absorbance in the presence of the sample of the extracts.

Hydroxyl radical scavenging activity

Stock solutions of EDTA (1 mM), FeCl3 (10 mM), ascorbic acid (1 mM), H2O2 (10 mM) and deoxyribose (10 mM) were prepared in distilled water. The assay was performed by adding 0.1 ml of EDTA, 0.01 ml of FeCl3, 0.1 ml of H2O2, 0.36 ml of deoxyribose, 1 ml of methanol extracts of seeds of Blue and White flowered varieties of CT (Concentrations used were 50, 100, 200, 300, 400, 500, 600 µg/ml), 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence.

The mixture was then incubated at 37°C for 1 hr. 1 ml portion of the incubated mixture was mixed with 1 ml of 10% TCA and 1ml of 0.5% TBA to develop the pink chromogen measured at 532 nm[23]. The hydroxyl radical scavenging activity of MECT is reported as % inhibition of deoxyribose degradation and is calculated as follows:

$$\% \text{ Inhibition} = (A \text{ cont} - A \text{ test})/A \text{ cont} \times 100$$

Determination of reducing power

The total reducing power of methanol extracts seeds of blue and white flowered varieties of *Clitoria ternatea* were determined according to the method of Oyaizu. (Oaizu1986; Wong 2003) Different concentrations of extracts of *Clitoria ternatea* (50, 100, 200, 300, 400 500 and 600 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50ºC for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 × g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm using a UV-Visible spectrophotometer[21,22]. Increasing absorbance at 700 nm was interpreted as increasing reducing activity. Ascorbic acid was used as the standard control with concentrations 10, 20, 40, 60, 80 and 100 µg/ml.

RESULT AND DISCUSSION

DPPH radical scavenging assay

DPPH is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products. The free radical scavenging activities of test compounds were examined based on their ability to bleach the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Thus, the absorbance of DPPH solution decreases when kept in contact with antioxidant test sample and free radical scavenging activity is inversely proportional to the absorbance of DPPH solution.

Pet ether, chloroform and methanol extracts seeds blue flowered variety of CT were showed significant inhibition of DPPH radical i.e. 46.44%, 54.03 % and 70.68% at 600µg/ml respectively. (Figure No. 1, 2 and 3).

The MECT of Blue and White flowered varieties showed significant DPPH radical scavenging activity. The IC50 values of methanol extract of blue flowered variety is 410 µg/ml. The IC50 values of methanol extract of White flowered is 325µg/ml. The Methanol extract of White flowered variety showed significant antioxidant activity as compared to all other extracts of CT. Ascorbic acid was used as reference standard for the DPPH free radical scavenging assay; it significantly inhibits DPPH free radical at the concentrations ranging from 2-20 µg/ml, showing highest % inhibition i.e. 92.43% at 20µg/ml (Figure No. 7). The IC50 value obtained was found to be 8.20µg/ml.

Methanol extracts seeds of both flowered varieties of CT were further used for Reducing power assay and hydroxyl radicals scavenging assay.

Reducing power assay

For the measurements of the reductive ability, we investigated the Fe3+-Fe2+ transformation in the presence of methanol extracts using the method of Oyaizu. The reducing capacity of a compound may serve as a significant indicator of its extract found to be significant ($p < 0.001$) (Figures 8-10). The antioxidant activity has been reported to be concomitant with development of reducing power. The reducing power of methanol extracts both flowered varieties of CT were found to be increase with increasing amount of extracts concentration. All the concentrations of methanol extracts of both flowered varieties of CT were showed significant activity when compared to control and these differences were statistically significant ($p < 0.001$).

Hydroxyl radical scavenging assay

MECT of white flowered variety of CT showed significant hydroxyl radical scavenging activity as compared to blue variety. The IC50 value of MECT of White flowered variety was 292.5 µg/ml. The IC50 value of MECT of Blue flowered variety of CT was 360µg/ml. Ascorbic acid was used as standard drug for assay. IC50 value of Ascorbic acid was 32.5µg/ml. (Figure No.11, 12, 13).

There is abundant evidence that oxidative stress imposed by reactive oxygen species plays an important role in many chronic and degenerative diseases, such as atherosclerosis, ischemic heart disease, cancer, diabetes mellitus, neurodegenerative diseases and ageing. [24]

Free radicals are known to play a definite role in wide variety of pathological manifestations of pain, inflammation, cancer, diabetes and hepatic damage etc. antioxidant fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanism.[26]

Natural antioxidants present from plant origin protect against these radicals and are therefore important tools in obtaining and preserving good health. Strong epidemiological evidence suggests that regular consumption of fruits and vegetables, which are a rich source of the antioxidants which can reduce cancer and coronary heart diseases.[25]

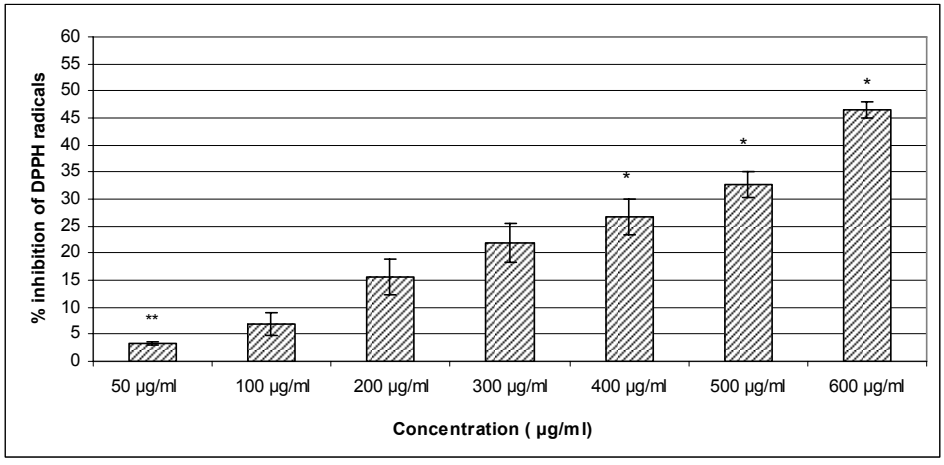

Fig. 1: DPPH free radical scavenging activity of Pet. ether extract of seeds of blue flowered variety of CT (PEECT)

* Represents statistical significance: $p < 0.001$, when compared with control.

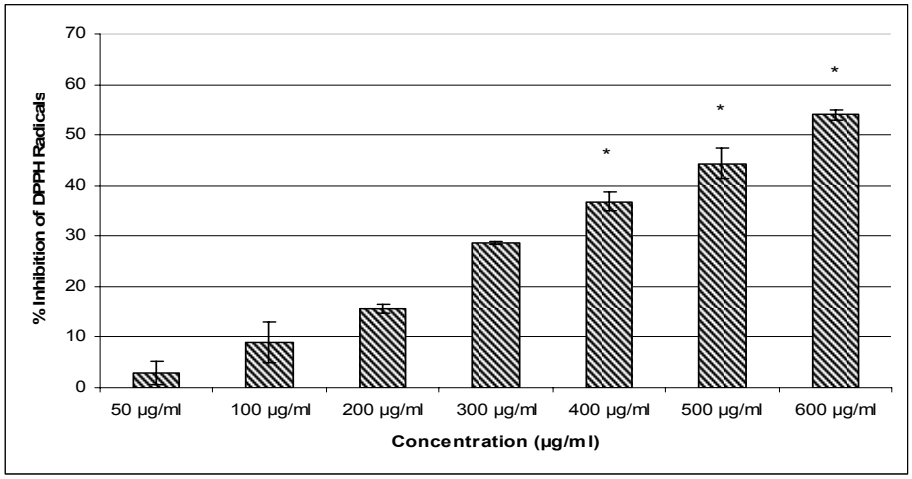

Fig. 2: DPPH free radical scavenging activity of Chloroform extract of seeds of blue flowered variety of CT (CECT)

* Represents statistical significance: $p < 0.001$, when compared with control.

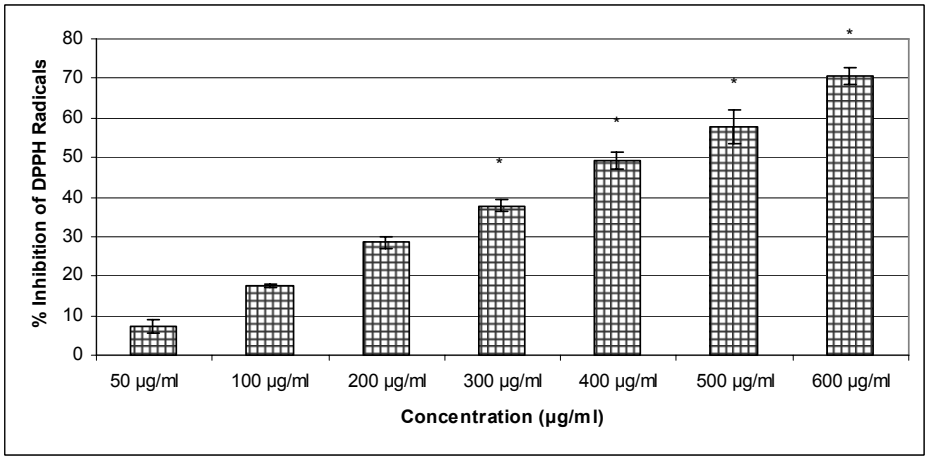

Fig. 3: DPPH free radical scavenging activity of Methanol extract of blue flowered variety of CT (MECT)

* Represents statistical significance: $p < 0.001$, when compared with control.

Pet Ether, Chloroform, and Methanol extracts seeds of White flowered variety of (CT) showed highest inhibition of DPPH radical i.e. 52.07%, 56.20% and 76.46% at 600µg/ml respectively (Figure No.4, 5 and 6).

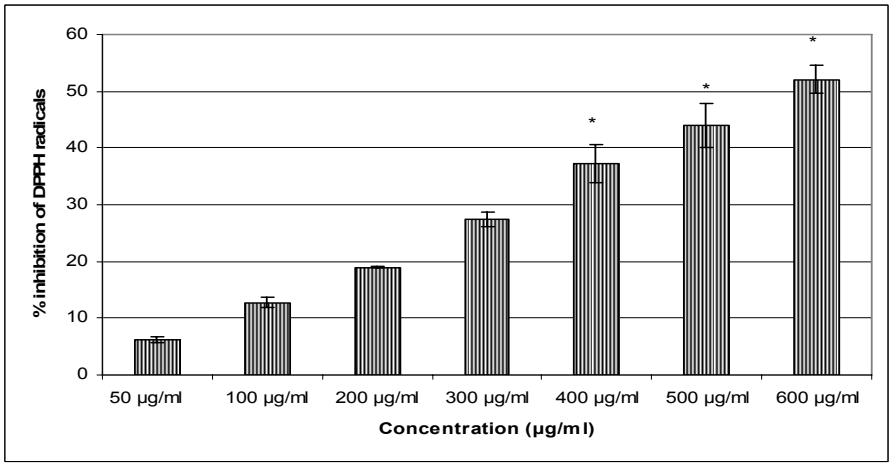

Fig.4: DPPH free radical scavenging activity of Pet. ether extract of white flowered variety of CT (PEECT)

* Represents statistical significance: $p < 0.001$, when compared with control.

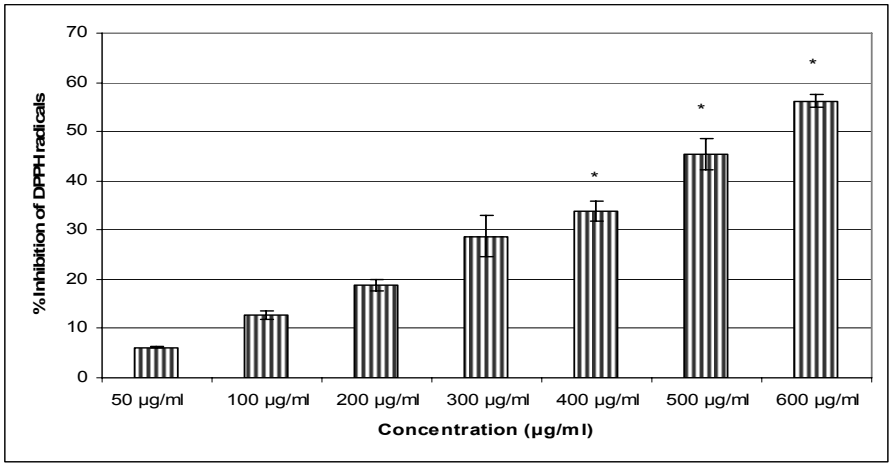

Fig. 5: DPPH free radical scavenging activity of Chloroform extract of white flowered variety of CT (CECT)

* Represents statistical significance: $p < 0.001$, when compared with control.

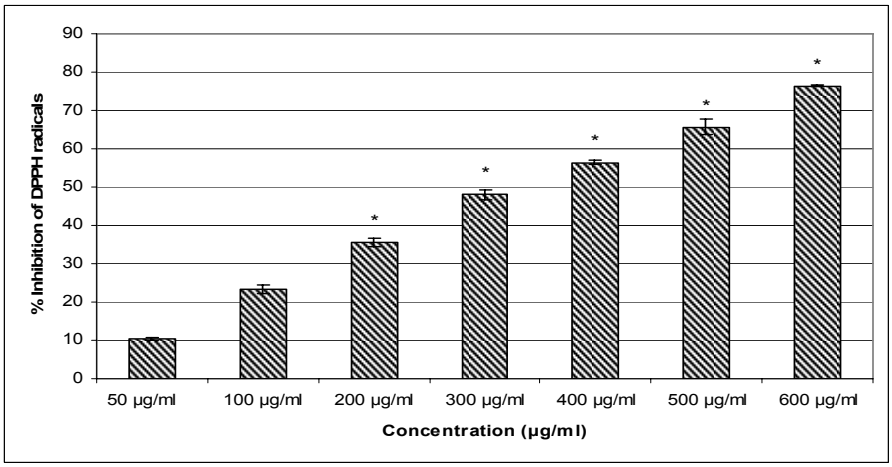

Fig. 6: DPPH free radical scavenging activity of Methanol extract of white flowered variety of CT (MECT)

* Represents statistical significance: $p < 0.001$, when compared with control.

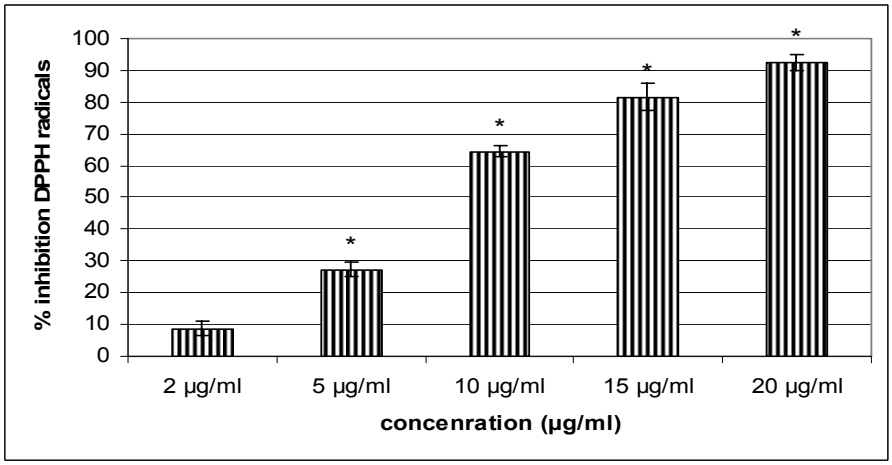

Fig. 7: DPPH Free radical scavenging activity of L-ascorbic acid

* Represents statistical significance: $p < 0.001$, when compared with control.

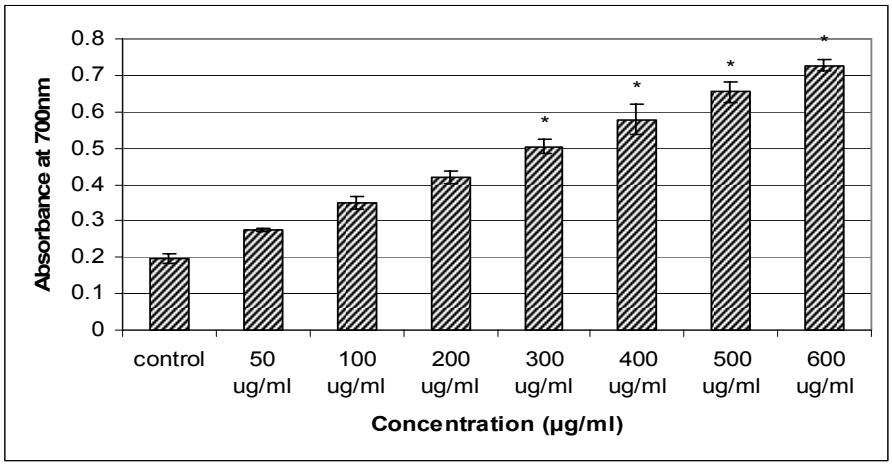

Fig. 8: Reducing power of Methanol extract of blue flowered variety of CT at 700 nm

* Represents statistical significance: $p < 0.001$.

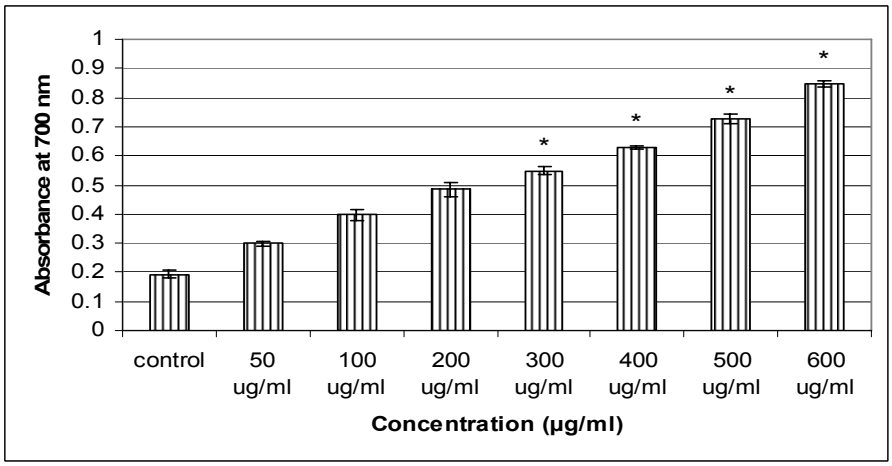

Fig. 9: Reducing power of Methanol extract of white flowered variety of CT at 700 nm

* Represents statistical significance: $p < 0.001$.

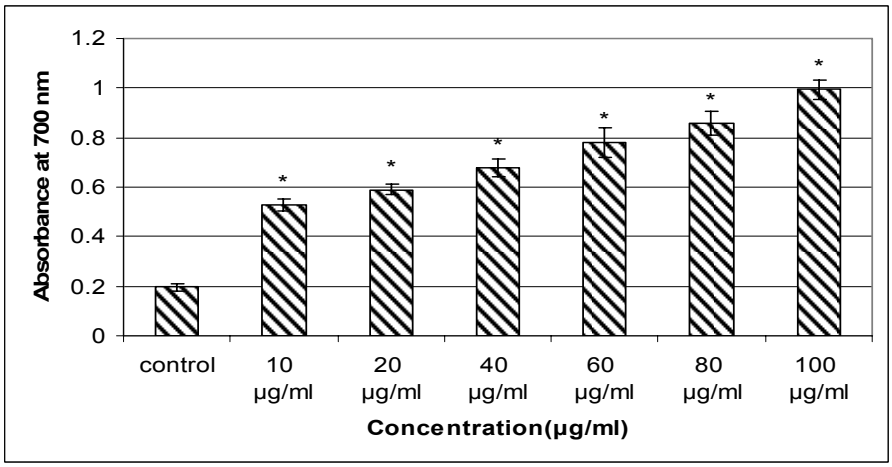

Fig. 10: Reducing power of L-ascorbic acid at 700 nm.

* Represents statistical significance: $p < 0.001$.

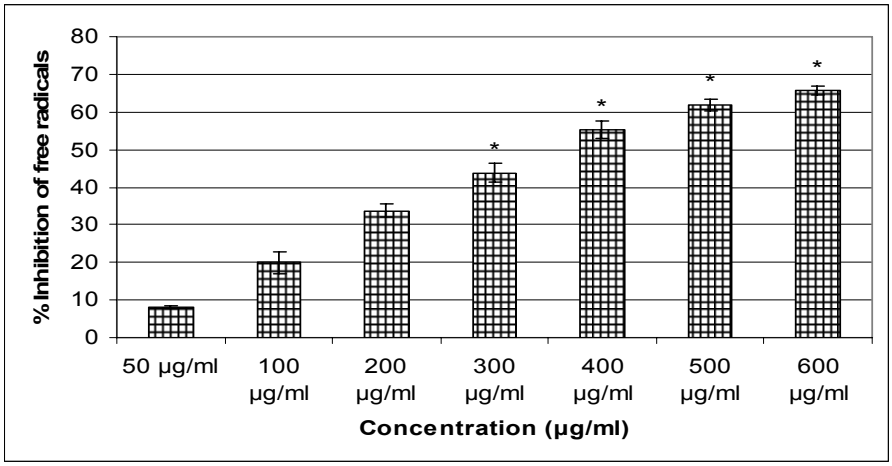

Fig. 11: Hydroxyl free radical scavenging activity of Methanol extract of blue flowered variety of CT (MECT)

* Represents statistical significance: $p < 0.001$, when compared with control.

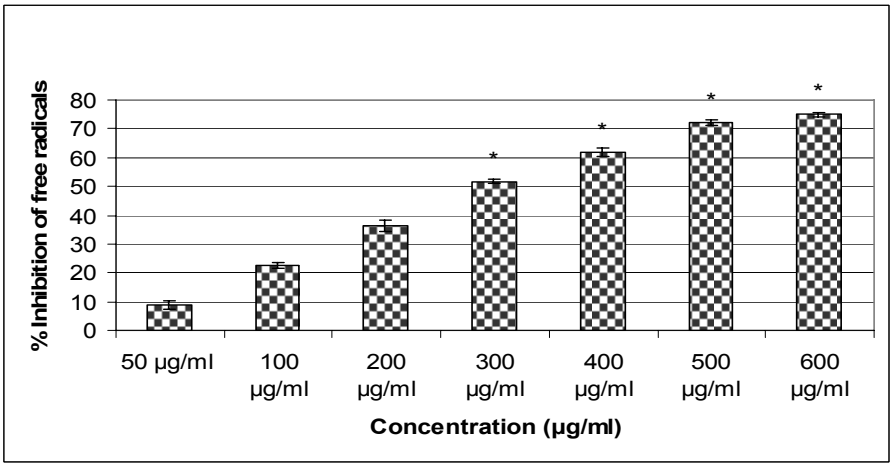

Fig. 12: Hydroxyl free radical scavenging activity of Methanol extract of white flowered variety of CT (MECT)

* Represents statistical significance: $p < 0.001$, when compared with control.

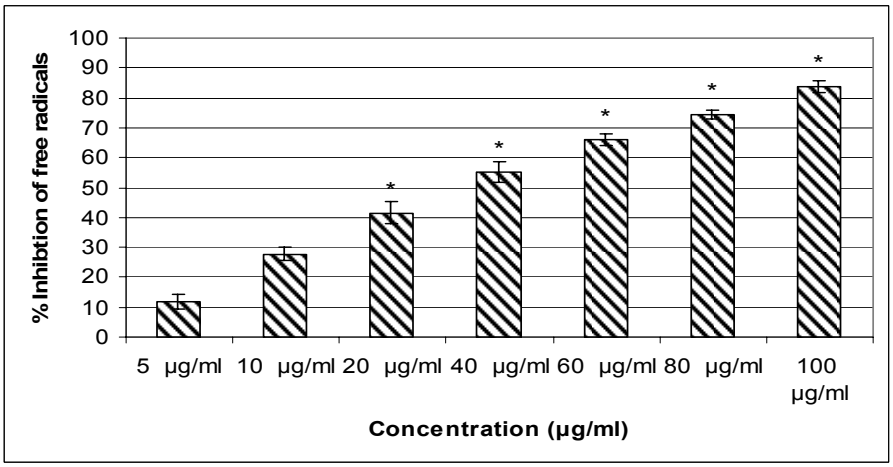

Fig. 13: Hydroxyl free radical scavenging activity of L-Ascorbic acid

* Represents statistical significance: $p < 0.001$, when compared with control, $n = 3$

In recent years, antioxidants derived from natural resources, mainly from plants, have been intensively used to prevent oxidative damages. Natural antioxidants have also some advantages over synthetic ones. They can be obtained easily and economically and have slight or negligible side effects Many plants have been announced to possess antioxidant activity etc. Natural antioxidants have also some advantages over synthetic ones. DPPH is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products. The present study shows prominent antioxidant activity of *Clitoria ternatea* Linn. Methanolic extract of seeds of white flowered variety of CT showed good antioxidant activity as compared to Blue flowered variety of CT. The White flowered variety may be the good source of natural antioxidant. Antioxidant activity may be due to phenolic compounds in *Clitoria ternatea* extract. However, the components responsible for the antioxidant activity of MECT are not clear. Therefore, further work is necessary to isolate and characterize those constituents.

CONCLUSION

In the present study seeds of *Clitoria ternatea* Linn. shows prominent antioxidant activity. Methanolic extract of white flowered variety of seed of CT showed better antioxidant activity as compared to Blue flowered variety of CT. The White flowered variety of CT may be the good source of natural antioxidant. Antioxidant activity may be due to presence of phenolic compounds in MECT. However, the exact components responsible for the antioxidant activity of MECT are not clear. Therefore, further work is necessary to isolate and characterize those constituents.

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