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Comparative Evaluation of *in vitro* Antioxidant Activity of Root of Blue and White Flowered Varieties of *Clitoria ternatea* Linn.

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Abstract: Pet ether, chloroform and methanol extracts of roots of blue and white flowered varieties of *Clitoria ternatea* were studied for their antioxidant potential. DPPH free radical scavenging assay, reducing power assay, hydroxyl radical scavenging assay were studied for evaluation of antioxidant potential. Pet ether, chloroform and methanol extracts roots of blue and white flowered varieties of *Clitoria ternatea* (CT) significantly inhibited the DPPH free radical at the concentrations ranging from 50-600 μg mL^{-1}. Pet ether, chloroform and methanol extracts roots of blue flowered variety of CT showed highest inhibition i.e., 49.11, 35.42 and 70.67% at 600 μg mL^{-1}, respectively. Pet ether, chloroform and methanol extracts roots of white flowered variety of (CT) showed highest inhibition i.e., 54.48, 39.21 and 78.13% at 600 μg mL^{-1}, respectively. Methanol extracts of blue and white flowered varieties of CT showed a very powerful antioxidant activity in DPPH radical-scavenging assay. Methanol extracts of CT also showed significant reductive ability as well as hydroxyl radical scavenging activity. Methanol extract of white flowered variety of CT showed more significant antioxidant activity as compared to blue flowered variety of CT. All the concentrations of methanol extract of CT (MECT) antioxidant activity when compared to control and these differences were statistically significant ($p<0.001$). *Clitoria ternatea* Linn. could be considered as a potential source of natural antioxidants.

Key words: *Clitoria ternatea*, antioxidant, DPPH, reducing power, hydroxyl

INTRODUCTION

Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction. Reactive Oxygen Species (ROS) have been found to play an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease (Halliwell, 1997). Thus, present studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals (Hou *et al*., 2003).

Earliest description of curative properties of medicinal plants is found in Rig-Veda. Charaka, Samhita and Sushrusha Samhita which give extensive description on various medicinal herbs. Information on medicinal plants in India has been systematically organized (Satyavati *et al*., 1976; Satyavati *et al*., 1987).

Clitoria ternatea L. (CT) a perennial twining herb, found throughout India in tropical areas. CT is commonly known as Butterfly pea belong 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 (Kapoor, 2005; Parrotta, 2001; Prajapati *et al*., 2003; Khare, 2004). CT has been used as an ingredient in Medhya Rasayana a rejuvenating recipe used for treatment of neurological disorders (Kamkaen and Wilkinson, 2009). CT has been scientifically studied for various pharmacological activities like local anesthetic (Mukherjee *et al*., 2008), anthelmintic (Khadatkar *et al*., 2008; Nirmal *et al*., 2008), antipyretic, anti-inflammatory, analgesic (Devi *et al*., 2003), anxiolytic, antidepressant, anticonvulsant, sedative (Jain *et al*., 2003), hypoglycemic (Sharma and Majumdar, 1990), anticancer (Balachandran and Govindarajan, 2005) also enhances acetylcholine content in rat hippocampus (Rai *et al*., 2002). A wide range of chemical constituents are present in CT. Still the comparative evaluation of antioxidant activity of blue and white flowered variety of CT has not been carried out. Aim of this study to explore the antioxidant potential both varieties of *Clitoria ternatea* L.

MATERIALS AND METHODS

Plant material: The roots of blue and white flowered variety of *Clitoria ternatea* were collected from local habitat. The plant specimens were authenticated

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(Specimen No 9492, 9493) Botany Department Rashtrasant Tukdoji Maharaj, Nagpur University, Nagpur.

Extraction of plant material: The roots of blue and white flowered variety of *Clitoria ternatea* were cut into small pieces and dries at room temperature. The dried roots were subjected to size reduction to coarse powder by using dry grinder. This powder is packed into soxhlet apparatus and extracted with pet.ether (60-80°C), chloroform, methanol (Sharma *et al*., 2009). The extracts were evaporated to dryness at 40°C (White flowered variety yields 9, 11, 12% w/w, respectively and blue flowered variety yields 9, 11, 10% w/w, respectively). A phytochemical screening of residues revealed the presence of phenolic compounds, triterpenoid sterol and protein (Egwuche *et al*., 2011).

***In-vitro* antioxidant assay**

Chemicals: DPPH solution (200 μM) Phosphate buffer (0.2M, pH 6.6) (Potassium dihydrogen phosphate 0.2M): (NaOH 0.2M): Potassium ferricyanide (1%), TCA (10%), Ferric chloride (0.1%).

Dpph free radical scavenging assay: DPPH free radical scavenging assay was performed according to method of (Rajeshwar *et al*., 2005; Gill *et al*., 2011a). Percent inhibition of DPPH free radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenged } (\%) = \frac{(\text{A cont-A test})}{\text{A cont}} \times 100$$

Where, Acont is the absorbance of the control reaction. Atest is the absorbance in the presence of the sample of the extracts.

Determination of reducing power: The total reducing power of roots of blue and white flowered variety of *Clitoria ternatea* was determined according to the method of Oyaizu (Oyaizu, 1986; Wong and Kitts, 2003).

Hydroxyl radical scavenging assay: The hydroxyl radical scavenging activity of MECT was performed according to Halliwell and Londonkar. (Halliwell *et al*., 1987; Londonkar and Kamble, 2009)

The hydroxyl radical scavenging activity of MECT is reported as %inhibition of deoxyribose degradation and is calculated as follows:

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

where, A cont is the absorbance of control at 532 nm and A test is the absorbance of BMS samples at 532 nm.

Statistical analysis: All the values were expressed as Mean±Standard Error of Means (SEM). Statistical analysis was performed by one-way Analysis of Varience (ANOVA). Further comparison was done by Tukey`s multiple range tests using graphpad prism 4.0 software. A probability value of $p<0.05$ was considered to be statistically significant (Krishna *et al*., 2010).

RESULTS

DPPH radical scavenging assay: Pet ether, chloroform and methanol extracts roots blue flowered variety of CT showed significant inhibition of DPPH radical i.e., 49.11% (Fig. 1), 35.42% (Fig. 2) and 70.67% (Fig. 3) at 600 μg mL^{-1}. Pet ether, chloroform and methanol extracts roots of white flowered variety of (CT) showed highest inhibition of DPPH radical i.e., 54.48% (Fig. 4), 39.21% (Fig. 5) and 78.13% (Fig. 6) at 600 μg mL^{-1}. MECT of blue and white flowered varieties showed significant DPPH radical scavenging activity as compared to other extracts. The IC_{50} values of methanol extract of blue flowered is 492 μg mL^{-1}. The IC_{50} values of methanol extract of white flowered is 342 μg mL^{-1}. 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^{-1}, showing highest % inhibition i.e., 91.58% at 20 μg mL^{-1} (Fig. 7). The IC_{50} value obtained was found to be 9.38 μg mL^{-1}.

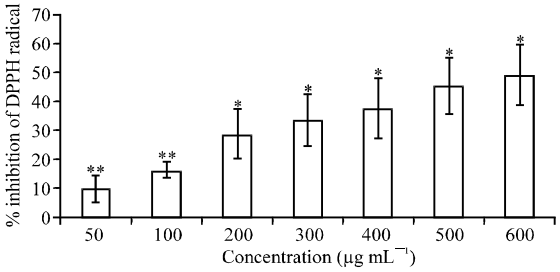

Fig. 1: DPPH free radical scavenging activity of Pet. ether extract of blue flowered variety of CT (PEECT). Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: $p<0.001$, when compared with control, n = 3. **Represents no statistical significance: $p>0.05$, n = 3

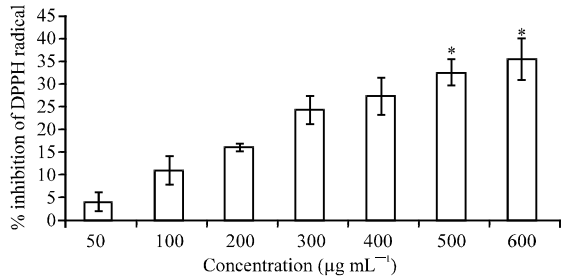

Fig. 2: DPPH free radical scavenging activity of chloroform extract of blue flowered variety of CT (CECT). Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, when compared with control, n = 3

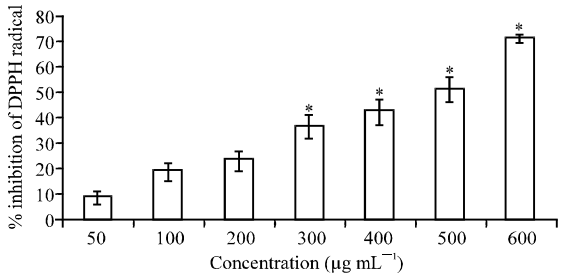

Fig. 3: DPPH free radical scavenging activity of methanol extract of blue flowered variety of CT(MECT). Values are expressed as a Mean±standard error of mean of the 3 observations. *Represents statistical significance: p< 0.001, when compared with control, n = 3

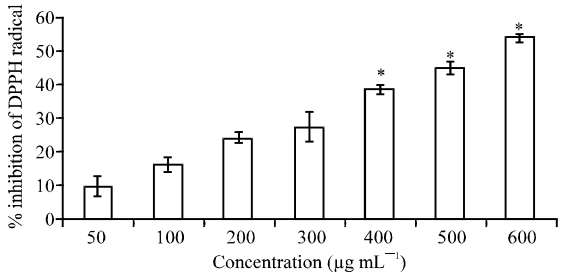

Fig. 4: DPPH free radical scavenging activity of pet ether extract of white flowered variety of CT (PEECT). Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, when compared with control, n = 3

Methanol extracts of both flowered varieties of CT were further used for reducing power assay and hydroxyl radical scavenging assay.

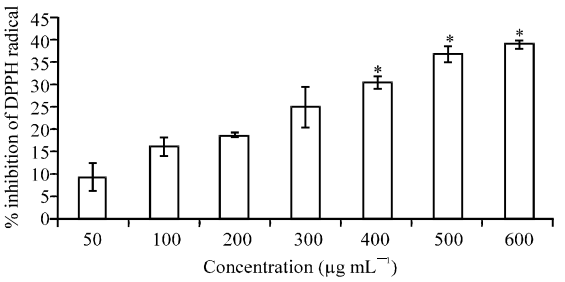

Fig. 5: DPPH free radical scavenging activity of choloform extract of white flowered variety of CT (CECT). Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, when compared with control, n = 3

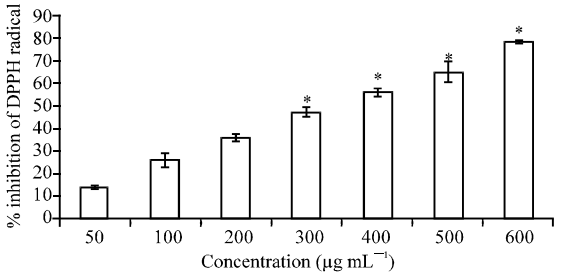

Fig. 6: DPPH free radical scavenging activity of methanol extract of white flowered variety of CT (MECT). Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, when compared with control, n = 3

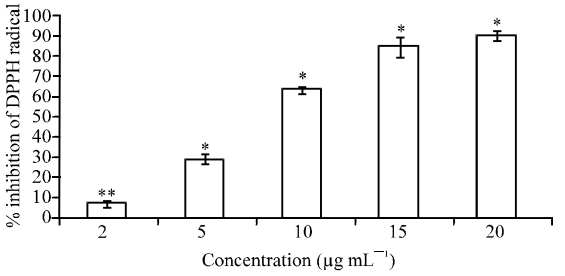

Fig. 7: DPPH Free radical scavenging activity of L-ascorbic acid: Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, when compared with control, n = 3. **Represents no statistical significance: p>0.05, n = 3.

Reducing power assay: For the measurements of the reductive ability, the Fe^{3+}-Fe^{2+} transformation in the presence of methanol and aqueous extracts

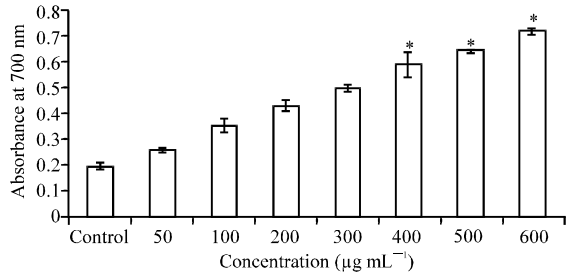

Fig. 8: Reducing power of methanol extract of blue flowered variety of CT at 700 nm. Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, n = 3

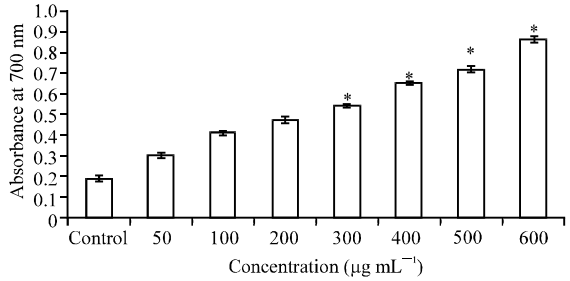

Fig. 9: Reducing power of methanol extract of white flowered variety of CT at 700 nm. Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, n = 3

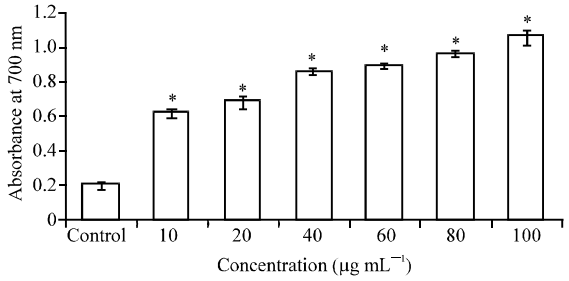

Fig. 10: Reducing power of L-ascorbic acid at 700 nm. Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, n = 3

using the method of oyaizu was studied. The reducing capacity of a compound may serve as a significant indicator of its extract found to be significant (p<0.001). The antioxidant activity has been reported to be concomitant with development of reducing power. The reducing power of methanol extracts both

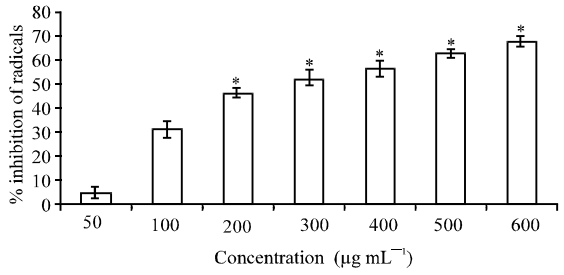

Fig. 11: Hydroxyl radical scavenging activity of methanol extract of blue flowered variety of CT (MECT). Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, when compared with control, n = 3

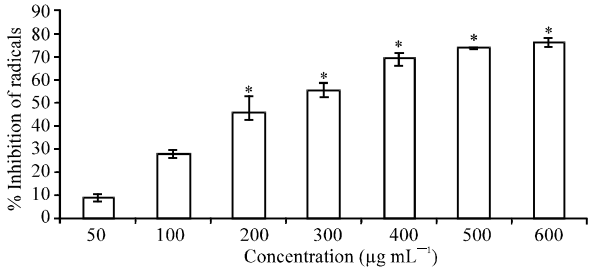

Fig. 12: Hydroxyl radical scavenging activity of methanol extract of white flowered variety of CT (MECT). Values are expressed as a Mean±Standard error of mean of the 3 observations. *Represents statistical significance: p<0.001, when compared with control, n = 3

zlowered 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) (Fig. 8-10). Methanol extract white flowered variety of CT showed significant reducing power as compared to methanol extract blue flowered variety of CT.

Hydroxyl Radical scavenging assay: The hydroxyl radical scavenging activity of MECT is reported as %inhibition of deoxyribose degradation. Methanol extract white flowered variety of CT showed significant hydroxyl radical scavenging activity as compared to blue flowered variety of CT. Methanol extract white flowered variety of CT showed 75.50% (Fig. 11) and methanol extract blue flowered variety of CT showed 67.37% (Fig. 12) inhibition of hydroxyl radical at 600 µg mL^{-1}. Ascorbic acid was

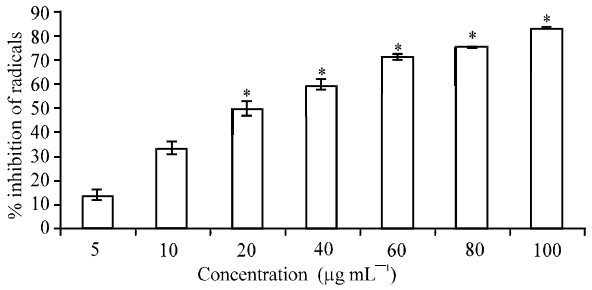

Fig. 13: Hydroxyl radical scavenging activity of L-Ascorbic acid. Values are expressed as a mean±standard error of mean of the 3 observations. *Represents statistical significance: $p<0.001$, when compared with control, n = 3

used as reference standard for this assay. It significantly inhibits hydroxyl radical at the concentrations ranging from 5-100 μg mL^{-1}, showing highest %inhibition i.e., 83.16% at 100 μg mL^{-1} (Fig. 13).

DISCUSSION

It has been studied that damages caused by free radical induced oxidative stress is the major causative agent of many disorders including cancer, tissue injury and rheumatoid arthritis (Govindarajan *et al*., 2005) neurodegenerative diseases, aging (Khlifi *et al*., 2006). When oxidative stress reaches a certain limit, a defence mechanism become insufficient led to decrease intracellular concentration of GSH and antioxidant enzymes (Bashandy and AlWasel, 2011). Oxidative stress major pathological factor for many disease. The chronic consumption of high fructose diet contribute to excessive formation of reactive oxygen species. This leads to induce oxidative stress (Bakoma *et al*., 2011). The human body counteract oxidative stress by producing antioxidants which are either natuarllu produced *in situ*, or externally supplied through food and supplements (Gill *et al*., 2011b).

Flower of CT was previously used for gel preparation (Kamkaen and Wilkinson, 2009), CT has been traditionally used as a remedy for various disease like urinogenital disorder, bronchitis, purgative, diuretic, anthelmintic, rheumatism, demulcent, anticancer and antidote for animal stings. Root of the CT also used in preparation of medhya rasayana, still antioxidant activity in comparisonof both varieties was not carried out yet. So this was attempt to explore antioxidant potential.

DPPH is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products. DPPH is one of a few stable and commercially available organic nitrogen radicals and has a UV-vis absorption maximum at 517 nm. Upon reduction, the solution color fades; the reaction progress is conveniently monitored by a spectrophotometer. 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 (Blois, 1958). The methanol extract of white flowered variety showed significant antioxidant activity as compared to all other extracts of CT. Methanol extracts of Both flowered varieties of CT were further used for Reducing power assay and hydroxyl radical scavenging assay.

The reducing power assay resembles the redox titration in classical chemical analysis. The degree of the color change is proportional to the antioxidant concentrations. The reaction end point is reached when color change stops. The antioxidant activity has been reported to be concomitant with development of reducing power. The reducing power of MECT was found to be increase with increasing amount of extract concentration. All the concentrations of MECT showed significant activities when compared to control and these differences were statistically significant ($p<0.001$).

The potentially reactive hydroxyl radical can cause oxidative damage to DNA, lipids and proteins. The effect of *Clitoria ternatea* on inhibition of free radical was significant (Jornot *et al*., 1998).

In recent years, antioxidants derived from natural resources, mainly from plants, have been intensively used to prevent oxidative damages (Ali *et al*., 2008). Polyphenolic compounds like flavonoids, Tannins, Phenolic acids commonly found in the plant have been reported to have multiple biological effects, including antioxidant activity (Rahman *et al*., 2011). 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 posses antioxidant activity etc. Natural antioxidants have also some advantages over synthetic ones. The methanol extract of white flowered variety showed significant antioxidant activity in DPPH assay, reducing power assay and hydroxyl radical scavenging assay. Root of *Clitoria ternatea* would be good source for antioxidants.

CONCLUSION

The present study showed prominent antioxidant activity of *Clitoria ternatea* Linn. Methanolic extract of white flowered variety of root 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 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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