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Comparative studies of free radicals scavenging activity of *Clitoria ternatea* L. Extract

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ABSTRACT

Herbal medicine has gained global significance in terms of its medicinal and economic value. The present research has aims to compare the study of the scavenging activity of DPPH, ABTs, FRAP, and Superoxide anions of ethanolic extract of root, leaf, flower and seed tissue of Clitoria ternatea plant at five distinct concentrations such as 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml, respectively against positive control. The result shows that the increased concentration of the extract with increased the mean value for all parts and all selected antioxidants. The DPPH, ABTs, FRAP and superoxide activity exhibits maximum scavenging activity at the highest concentration i.e. 1mg/ml. The flower showed the maximum scavenging activity of all four selected antioxidants activity to leaf, root and seed at 1.00 mg/ml concentrations. And their inhibition percentage recorded highest for superoxide was 97.56% followed by seeds (90.23%), leaf (85.76%) and root (72.37%). The maximum potency of scavenging activity was found in superoxide antioxidants followed by ABTs, DPPH and FRAP for flower. Overall, the findings of the result concluded that flower has highest scavenging activity than other parts of the Clitoria ternatea. The presence of bioactive compounds as antioxidants revealed that C. ternatea could be used in the pharmacological industry.

Keywords: *Antioxident Clitoria ternatea, herbal Extraction, DPPH, ABTs, FRAP, Superoxide anion activity and medicinal plants*

INTRODUCTION

The butterfly pea flower (*Clitoria ternatea L.*), known as CT, belongs to the family *Fabaceae*. All parts of the plant (roots, seeds, and leaves) are used for medicinal purposes and are believed to possess various beneficial properties. The different varieties of *Clitoria ternatea* primarily rely on their composition of anthocyanidins/anthocyanins present in the flowers. The plants were found in mainly three genotypes in India. *C. ternatea* plant genotype was identified by the colour of flowers which is mainly found in blue, pink and white.The discovery of natural sources of antioxidants to prevent these diseases is proving helpful for human health. *C. ternatea* plants include improving cognitive function and reducing dementia, treating respiratory conditions such as asthma and bronchitis, reducing inflammation, and exhibiting laxative and diuretic actions [1] [2]. The compounds have the potential to address various diseases, including allergies, cough [3] arthritis [4], neuroprotective effects [5], hepatoprotective effects [6], diabetes [7] inflammatory conditions, cancer, and other life-threatening conditions [8].

Plant products have been recognized for their potential to provide a broad choice of health welfare due to their bioactive compounds having antioxidant properties.

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Free radicals scavenge and prevent cellular damage, which is how antioxidants mitigate the damaging effects of ROS. Antioxidants are molecules that protect the enzymes as well as cell organelles from oxidative damage, and they are used as a defense mechanism in removing or repairing damaged molecules. Natural antioxidant compounds have become very important. And medicinal plants are becoming important natural sources of antioxidant enzymes [9].

Clitoia ternatea or butterfly pea plant has gained attention for its antioxidant properties. *C. ternatea's* antioxidant scavenging activity and antioxidant enzymes have been assessed by using a number of assays. The comparative study of antioxidants activity of root, leaf, flower, and seed of CT was not done in before the research study. The scavenging methods are generally used for assessing the antioxidant activities of foods, food additives, and natural items [10]. The current research objectives were comparative analyzed for antioxidant activity present in the root, leaf, seed, and flower of *C. ternatea*.

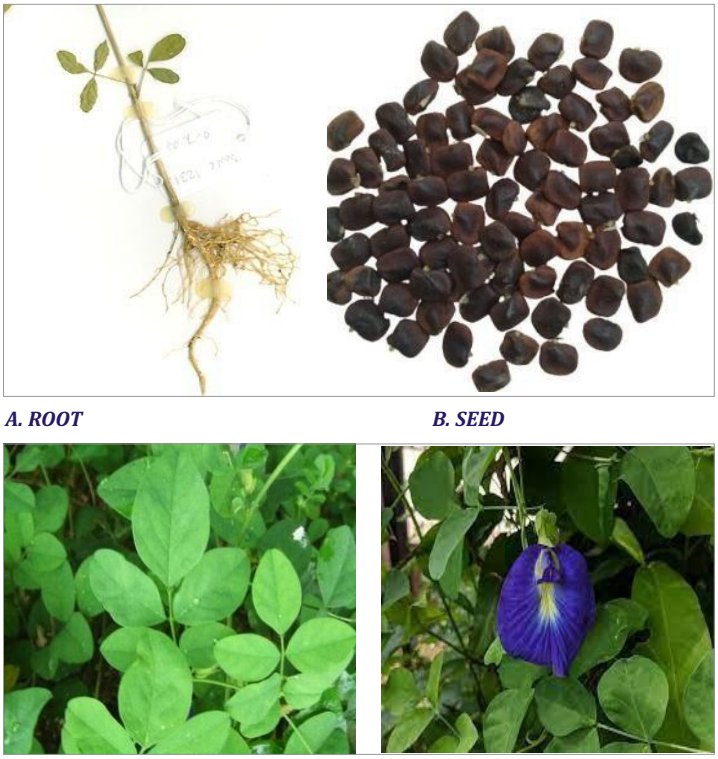
Materials and Methods

Chemicals Required: Ethanol, DPPH, Ascorbic acid, ABTs, Potassium Persulphate, Gallic acid, Acetone, Potassium Ferricyanide, $FeCl_3$, BHT, Guaiacol, Hydrogen peroxide, Sodium pyrophosphate, Phenazine Methosulphate (PMS), Nitroblue tetrazolium (NBT), NADH, Glacial acetic acids, Butanol, Guaiacol, Sorbitol, NaCl, Catechol, Tris-HCl

Collection of Plant Sample

From the nursery of the Horticulture Wing of the Central Public Works Department, located in Mehrauli, Delhi, the healthy and

diseases free plant parts of *Clitoria ternatea* were collected (Figure 1). All analytical work was performed in the well-developed laboratory of the National Institute of Food Technology Entrepreneur and Management (NIFTEM) at Sonipat, Haryana. The blue variety blooming plant's roots, leaves, seeds, and flowers were taken for further analysis as it was easily available than the white and pink genotype.



A. ROOT *B. SEED*

C. LEAF *D. FLOWER*

Figure 1: Different Parts of C. ternatea Plant

Extracts Preparation: From the nursery, the mature, robust, and fresh *Clitoria ternatea* leaves, roots, seeds, and flowers were collected. Five minutes were spent cleaning them in fresh water. After that, distilled water was used to rinse it. All parts such as leaves, roots, seeds, and flowers were crushed in to small pieces. They were stored separately in bowls.

The solvent used to prepare the extract was ethanol; one gram of fresh material was dissolved in 100 ml of a 60% ethanol solution [11] [15]. After being kept at room temperature for three hours, the flasks were put in a rotary shaker with a speed setting of 100 rpm. For the qualitative test of secondary metabolites, samples were treated with charcoal powder and left for 10 minutes to remove colour of the solvents. Extracts were then centrifuged separately in ethanol at 5000 rpm for 15 minutes. The extraction and analytical procedures were performed in an ice box or at 0^0C for preventing denaturation of enzymes. After that samples were kept in the refrigerator at 4^0C

Assay of DPPH Scavenging activity

Chan *et al.* [12] method was used for DPPH radical scavenging activity with the slight modification. The 0.5 g sample was homogenized in mortar-pestle with 50% ethanol in an ice box. The sample was centrifuged at 5000 rpm for 15 minutes and the final volume of the supernatant was made 4.0 ml which was adjusted with 50% ethanol. Finally, extracts of 1.0 ml aliquot and 1.0 ml DPPH solution (0.1mM) was added. For blank equal amount of ethanol and DPPH was used.

The mixture was shaken vigorously and was left to stand in dark for 30 min and the absorbance was read at 517 nm. DPPH radical scavenging assay was standardized against mg/ml of ascorbic acid and expressed as mg ascorbic acid/g of fresh weight of the sample and calculated by inhibition percentage.

% Inhibition = [(A control - A sample) / A control] x 100%

The extract's capacity to scavenge DPPH radicals was also quantified as IC50 values. The effective concentration of the extract (IC50) needed for 50% DPPH radical scavenging was determined by plotting the scavenging activity against sample concentration.

ABTs radical scavenging assay:

Stratil *et al.* [13] method used for the procedure for measuring ABTs (2, 2'-azino-bis -3- ethyl benzothiazoline 6-sulphonic acid) was determined. 0.5 g of fresh sample was homogenized in mortar-pestle with 2.0 ml of phosphate buffer and centrifuged at 5,000 rpm for 10 minutes at 4^0C and the supernatant was only used for the assay. Adjust the volume to 4.0 ml with phosphate buffer. For the ABTs solution, made mixture of ABTs (7mM) and potassium persulfate (4.95mM) in 1:1 ratio (v/v) was allowed to stand overnight at room temperature in the dark to form radical cations ABTs+. In the reaction mixture, 1.0 ml of extract was mixed with 3.9 ml ABTS solution and 0.1 ml phosphate buffer (P^H7.4), and a decrease in the absorbance was measured at 745 nm. The blank was prepared in the same procedure without the use of extract. ABTs assay was standardized against mg/ml of Gallic acid and calculated by inhibition percentage by using the following formula:

% Inhibition =[(A control - A sample) / A control] x 100%

The extract's ABT radical scavenging activity was represented as IC50 values. A graph that shows the sample concentration needed to scavenge 50% of the free radicals produced by 50% ABT was used to compute the IC50 values which expressed in percentage value.

Assay of Ferric Reducing Antioxidant Power (FRAP)

FRAP activity was determined by the modified method of Athukorala *et al*. [14].0.5g of fresh sample was homogenized in mortar-pestle with 4.0 ml of 70% acetone. After centrifugation at 5,000 rpm for 10 min, the final volume of the supernatant was made upto 10.0 ml with 70% acetone. In the reaction mixture, 1.0 ml of extract was mixed with 2.5 ml phosphate buffer and 2.5 ml potassium ferricyanide, incubated for 20 min at 50^0C, and then added 2.5 ml $FeCl_3$. After that again centrifuge for 10 minutes at 3000 rpm. The upper layer of solution was pull out and 2.5 ml was mixed with 2.5 ml distilled water and 0.5 ml $Fecl_3$. Absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

The ferric reducing antioxidant power assay was calibrated against mg per ml of BHT (Butylated Hydroxy Toluene). The extract's of FRAP radical scavenging activity was quantified in terms of IC50 values. A graph that shows the sample concentration needed to scavenge 50% of the FRAP free radicals was used to calculate the IC50 values. The inhibition percentage was also calculated by using the following formula:

% Inhibition =[(A control - A sample) / A control] x 100%

Statistical analysis

The results of all data were statistically analyzed by analysis of variance at least significant difference at 0.05 levels and performed by Microsoft Excel.

Results and Discussion

Table 1.0: DPPH activity of plant parts of Clitoriaternatea on different concentrations

Concentration(mg/ml)	Inhibition % (Unit: mg Ascorbic acid /g Fresh wt.)			
	Root	Leaf	Flower	Seed
0.2	25.53±0.61	35.80±0.72	53.57±1.13	23.28±0.57
0.4	28.96±0.67	43.16±0.74	58.96±1.27	27.50±1.09
0.6	35.77±0.55	53.93±0.83	68.40±0.63	34.50±0.69
0.8	39.90±0.59	65.26±1.01	79.33±0.66	38.87±1.13
1.0	48.8±0.72	79.73±1.15	88.43±0.69	48.20±1.06
Mean	35.79	55.57	69.74	34.47
C.D.	1.973	2.89	2.91	2.997
SE(m)	0.618	0.91	0.912	0.939
SE(d)	0.874	1.28	1.29	1.328
C.V.	3.002	2.82	2.265	4.718
P value at 5%	0.0091	0.0061	0.0046	0.0098

Values are presented as mean±S.D, n=4.(Experiments were made as 3 parallel)

Ethanol based extract of root, leaf, flower and seed tissue of *C. ternatea* plant was taken at five distinct concentrations like 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml, respectively, and observed by inhibition percentage value for all antioxidant activity like DPPH, ABTs, FRAP and Superoxide. It was used to analyze the inhibition % or IC50 value of the scavenging activity of free radicals. These activities were recorded as per Table 1.0 and Figure 2 below:

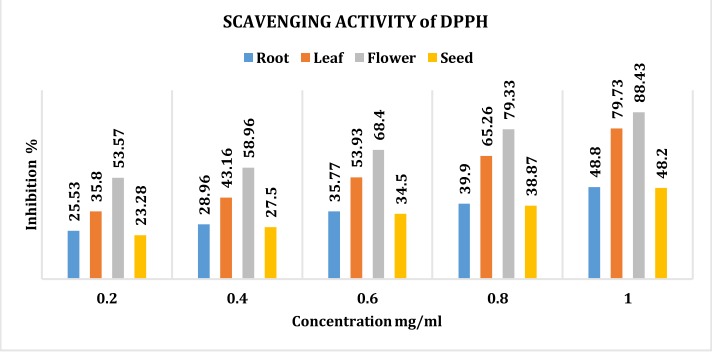


Figure 2: DPPH Scavenging Activity in parts of C. ternatea at different concentration

The result showed that DPPH scavenging activity of free radicals were found in increasing order with increase in concentration of leaf, flower, root, and seeds of CT plant. The maximum inhibition percentage was observed in flowers with all concentrations followed by leaf, root, and seeds respectively. The flower with the highest DPPH scavenging activity was measured 88.43% at a concentration of 1.0 mg/ml and its mean value was 69.74 % observed in the CT extract. However, flower followed leaf (79.73%), root (48.8%), and seed (48.2%) respectively at 1.0 concentration. (DPPH scavenging activity flower> leaf> root> seed).

The ABTs activity was the extract contents' capacity to transfer an electron to the preformed radical, which lowers absorbance. The observations were recorded in Table 2 below and inhibition percentage were plotted at figure 3. *Clitoria ternatea* activities of ethanolic extract against ABTs free radicals showed concentration-dependent effects and maximum inhibition percentage found in flower at 1mg/ml concentration i.e. 96.7 mg GAE/gFW followed by leaf- 85.76 mg GAE/gFW, root- 68.73 mg GAE/gFW and seed 68.5 mg GAE/gFW. Although the potency of the CT extract was less than that of the positive control (GAE), these assays demonstrated the extract's ability to scavenge free radicals. As per result, the increasing order of ABTs activity in extracts were recorded with increasing concentration. (ABTs activity Flower > leaf > root > seed).

Table 2: ABTs activity in plant parts of C.ternatea at different concentrations

Concentration(mg/ml)	Inhibition %(Unit: (GAE) mg/gFresh wt.)			
	Root	Leaf	Flower	Seed
0.2	33.26±1.13	55.46±1.24	65.36±1.09	35.20±1.18
0.4	46.83±0.63	58.87±0.55	78.87±0.60	48.40±0.60
0.6	53.63±0.54	64.20±0.60	85.59±0.66	55.30±0.68
0.8	59.17±1.09	73.43±1.12	89.53±0.69	59.20±1.12
1.0	68.73±0.60	85.40±1.15	96.70±1.21	68.50±0.52
Mean	52.32	67.54	83.21	53.32
C.D.	2.69	3.14	2.823	2.739
SE(m)	0.843	0.92	0.884	0.858
SE(d)	1.192	1.39	1.251	1.214
C.V.	2.79	2.58	1.841	2.788
P <0.05	0.0112	0.0048	0.00384	0.0062

Value are presented as mean±S.D,n=4. (Experiments were made as 3 parallel)

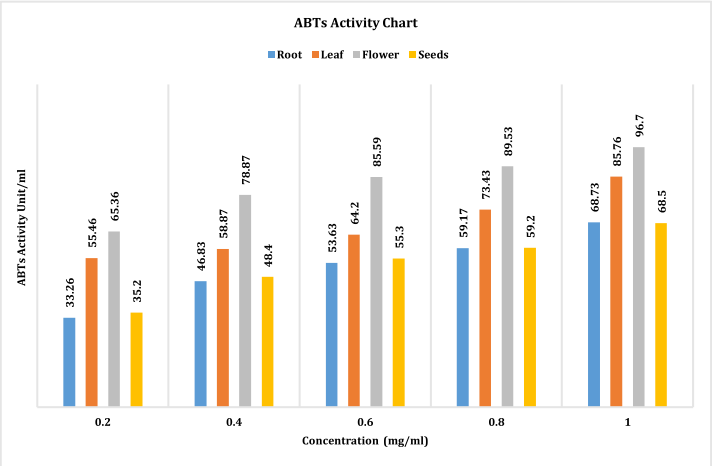


Figure 3: ABT activity at different concentrations in C. ternatea

The inhibition percentage of FRAP activity was evaluated on the different concentration i.e. 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml of roots, seeds, flowers, and leaves of CT plant. The observations were recorded as per Table 3 and the inhibition percentage was plotted in Figure 4 below:

Table 3: FRAP activity on different concentration in Plant tissue of CT

Concentration(mg/ml)	**Inhibition %** (mg BHT/g F.W.)			
	Root	**Leaf**	**Flowers**	**Seeds**
0.2	22.70±0.63	37.43±0.61	42.70±0.46	32.67±0.66
0.4	29.87±0.61	41.80±0.53	59.87±0.55	39.77±1.09
0.6	37.41±0.87	53.80±1.21	67.43±0.56	47.43±0.67
0.8	43.40±0.74	61.37±0.62	73.26±1.18	53.33±0.56
1.0	56.67±1.18	69.87±0.60	86.63±0.63	66.67±0.69
Mean	38.01	52.85	65.98	47.97
C.D.	2.66	2. 41	2.321	2.42
SE(m)	0.834	0.75	0.727	0.77
SE(d)	1.179	1.06	1.028	1.07
C.V.	3.798	2.47	1.909	2.73
P<0.05	0.009	0.006	0.005	0.007

Values are presented as mean±S.D, n=4.(Experiments were made as 3 parallel)

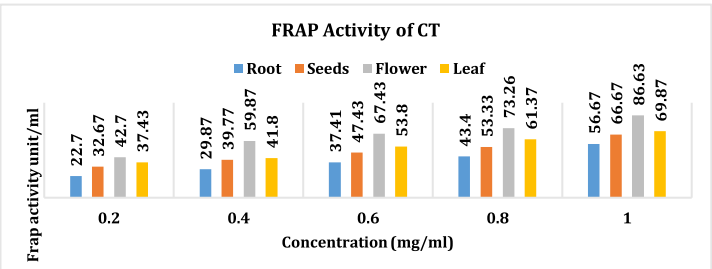


Figure 4:FRAP activity at different concentration in C.ternatea

Table 3 shows that gradually increased in concentration of the ethanolic extract of all parts, the mean value of FRAP scavenging was also increased. The study revealed that the concentration of 1mg/ml, the flower showed the maximum FRAP activity i.e. (86.63±0.63) followed by leaf (69.87±0.60), seeds (66.67±0.69) and root (56.67±1.18) [16]. The same trend was observed in all other concentrations (Figure 4.) (FRAP activity Flower > leaf > seed > root).

Superoxide radical activity was evaluated through inhibition percentage on the different concentration of leaf, root, flowers, and seeds of CT plant. The standard used for superoxide scavenging activity was ascorbic acid in mg/gm. The results were recorded at Table 4 and Figure 5. The result shows that at 1mg/ml concentration, superoxide scavenging % was maximum in flower 97.56% followed seed 90.23%, leaf 85.76 %, and root 72.37%. The same trend was found in all other concentration (Figure 5). (Superoxide activity Flower > Seed > leaf > root) [16].

Table 4:Superoxide activity on different concentration in Plant tissue of C. ternatea

Concentration(mg/ml)	**Inhibition %** (Unit: mg ascorbic acid/g FW)			
	Root	**Leaf**	**Flower**	**Seeds**
0.2	52.67±0.64	59.47±0.55	62.37±0.76	59.57±0.25
0.4	55.87±0.66	66.23±0.54	69.07±0.69	67.11±0.16
0.6	61.47±0.64	71.43±0.64	76.70±0.52	72.15±0.56
0.8	69.43±0.78	79.60±0.65	89.86±0.60	82.33±0.34
1.0	72.37±1.18	85.76±0.47	97.56±0.52	90.23±0.47
Mean	62.36	72.42	79.11	74.28
C.D.	2.557	1.81	2.004	2.23
SE(m)	0.801	0.56	0.628	0.71
SE(d)	1.133	0.84	0.888	0.94
C.V.	2.225	1.35	1.374	2.14
P <0.05	0.005	0.004	0.004	0.004

Values are presented as mean±S.D, n=4.(Experiments were made as 3 parallel)

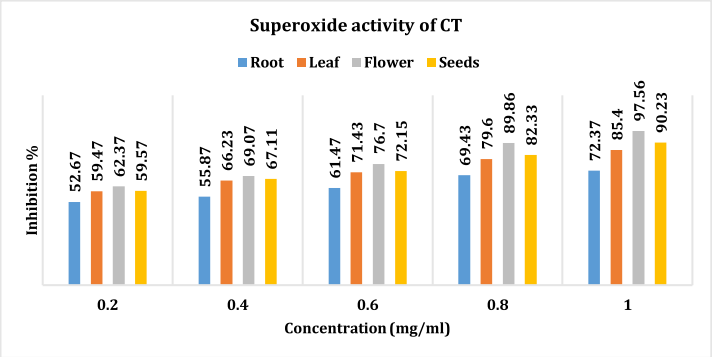


Figure 5: Superoxide activity at different concentrations in C. ternatea

Table 5: Comparative study of different antioxidants of Inhibition % at 1.0 mg/ml concentration

	Root	Leaf	Flower	Seed
DPPH	48.8±0.72	79.73±1.15	88.43±0.69	48.20±1.06
ABTs	68.73±0.60	85.40±1.15	96.70±1.21	68.50±0.52
FRAP	56.67±1.18	69.87±0.60	86.63±0.63	66.67±0.69
Superoxide	72.37±1.18	85.76±0.47	97.56±0.52	90.23±0.47

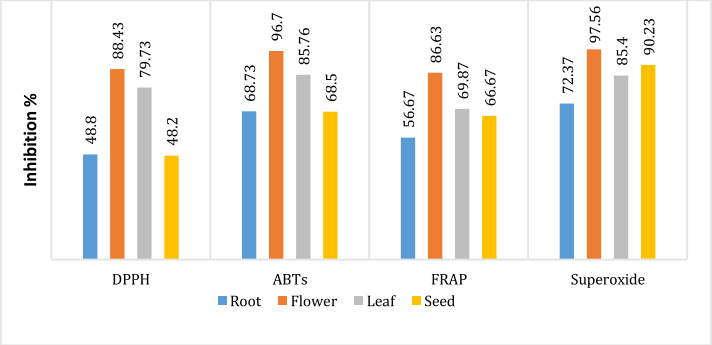


Figure 6: Inhibition % of scavenging activity at 1.0 mg/ml concentration of C.T plant

The comparative study of all antioxidants such as DPPH, ABTs, FRAP and superoxide, findings of above results in Table 5 observed that the superoxide scavenging activity showed maximum in root (72.37 mg/ml) at 1.0 mg/mg in comparison to other antioxidants activity like DPPH, ABTs and FRAP. However, the activities of ABTs and super anions were showed highest scavenging activities in leaves and found value (85.40 and 85.76) in comparison to DPPH and FRAP activities at 1mg/ml concentration. While, ABTS and superoxide anion activities observed highest in flowers and found approximate same value (97.56±0.52 and 96.70±1.21) at 1mg/ml in comparison to other selected antioxidants like DPPH and FRAP. The results of superoxide scavenging activity was observed highest in seed (90.23±0.47) than FRAP, ABTs and DPPH activity. The results of DPPH and superoxide activity were observed 48.20±1.06 and 90.23±0.47 in seeds whereas the FRAP shows 66.67±0.69 at 1mg/ml. Overall, among all selected antioxidant parameters, superoxide scavenging activity was observed highest in all part of *C. ternatea* plant (Table 5). Superoxide anion was showed more scavenging activity than ABTs activity for all parts like root, leaf flower and seed (Superoxide > ABTs) of CT plant.

Conclusion

2,2 diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), ferrous ion chelating power, 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging are the examples of synthetic antioxidant assays which are used for especially assay of free radicals in the laboratory. The maximum scavenging activity was observed at 1mg/ml concentration of all antioxidants.

The result shows the increasing trend after increase in superoxide activity on increase of concentration of the extract, the mean value of superoxide is also increased. The study revealed that on a concentration of 1mg/ml, the flower showed the maximum superoxide activity and their inhibition percentage was 97.56% followed by seeds (90.23%), leaf Overall, according to results of current research, *C. ternatea* flowers showed high antioxidant contents at 1.0mg/ml concentration, as per the given Table 5 (Figure 6) below:

By employing these complementary assays, the study intended to comprehensively characterize the antioxidant potential of *C. ternatea* and shed light on the mechanisms under lying its scavenging free radical molecules. Herbal medicine of Ayurvedic plants is spread all over world which is used for the removal of chronic and acute diseases. It has no side effect on the body and increase the boost up the immunity. *C. ternatea* plant has also keep good potential of phytochemicals or inactive ingredients which is safely used for the cure of many diseases. Presence of bioactive compounds as antioxidants revealed that it could be used in the natural colourants, pharmacological, bioengineering, ayurvedic, insecticidal, therapeutic which are extracted from various plant parts of *Clitoria ternatea.*

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Conflict of interest: No Conflict of intrest

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