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Variation in Antioxidant Activity and Antioxidant Constituents of *Ocimum basilicum* Linn. with the Maturity of Plant Grown in Open Field and Inside Polyhouse Conditions

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ABSTRACT

The variation in antioxidant activity and antioxidant constituents of different plant growth stages i.e. in every 15 day's interval till maturity of plant of *Ocimum basilicum* Linn. grown in an open field and inside polyhouse conditions was studied. The correlation between antioxidant constituents and antioxidant activities was also studied. The result revealed that the 90 days old plant grown in an open field condition exhibited maximum antioxidant activity with the lowest IC_{50}/EC_{50} value by ABTS (0.064 mg/ml), DPPH (0.090 mg/ml), and FRAP (0.099 mg/ml) followed by 75 days and 60 days old plants, similar pattern was also observed in their antioxidant constituents. Similarly, the 90 days old plant grown inside polyhouse condition showed maximum antioxidant activity with antioxidant constituents followed by other plant growth stages in descending order. The chlorophyll content was found maximum in 15 days old plant(0.926 mg/100g) grown in an open field condition, whereas the maximum chlorophyll content (1.470 mg/100g) exhibited by 90 days old plant grown inside polyhouse condition. The correlation analysis revealed that the antioxidant constituents exhibited a significant negative correlation with the IC_{50}/EC_{50} value and the IC_{50} value of ABTS assay had a significantly positive correlation with the IC_{50} and EC_{50} value of DPPH and FRAP assay, respectively. Hence, the study revealed that the leaves extract of plant grown in open field conditions possesses a significantly higher antioxidant activity and antioxidant constituents than the plant grown inside polyhouse condition.

Keywords: Antioxidant constituents; Correlation; IC_{50} value; Open field; Polyhouse

1. INTRODUCTION

Oxidative stress is caused by an imbalance between the body's formation of reactive oxygen species (ROS) and its normal physiology. Free radical oxidation triggers cell membrane disintegration, DNA mutation, and membranous protein damage, as well as the induction or progression of several diseases, which include diabetes, liver injury, cardiovascular disorders, and even cancer[1]. Antioxidant biomolecules, such as ascorbic acid, polyphenols, thiol and other reducing agents, can detain or disrupt the oxidation of molecules by oxidising themselves. Antioxidants can thus improve life quality by preventing the onset of different degenerative diseases[2]. Free radicals react with important macromolecules, which leads to cell damage and homeostatic disruption. Free radicals target all kinds of molecules in the body. Among them, major targets are proteins, lipids and nucleic acids. Factors that stimulate the production of free radicals in the body can be internal, such as inflammation, or external, i.e., UV exposure, cigarette smoke and pollution.

Natural antioxidant molecules, especially plant phytochemicals like phenolic compounds, flavonoids, carotenoids, benzoic acid derivatives, proanthocyanidins, coumarins, stilbenes, and lignins, are being investigated to replace synthetic antioxidant compounds, having a variety of side effects. Medicinal plants contain a variety of phytochemical constituents that have antioxidant properties and help to prevent chronic disease progression[3-4].

Ocimum basilicum Linn. (Lamiaceae), commonly known as "Holy basil" or Sweet Basil, possesses various pharmacological activities[5-6]. It is an aromatic and annual herb distributed in tropical and hotter parts of India[7]. Many compounds like eucalyptol, α-terpineol, α-bergamotene, linalool, eugenol, β-elemene, α-guaiene, α- cariophylene, citral, and methyl chavicol have been identified from the essential oil of *O. basilicum*[8-9]. The plant extracts have been reported to possess various phyto-constituents such as polyphenols, triterpenoids, phenylpropanoids and steroids[5,8]. *O. basilicum* has been utilised since pre-historic times, in both Ayurveda and Unani system of medicines to treat various disease condition. The herb possesses immense ethnomedicinal properties and traditionally used for having antibacterial, antioxidant, antispasmodic, digestive, anti-inflammatory, carminative, stomachic and hypoglycemic activity. Traditionally, the herb used for the treatment of several ailments such as diabetes, muscle cramps, migraine headache, dizziness, fever, cough, dysentery, nausea, paralysis, and respiratory disorders[10-13].

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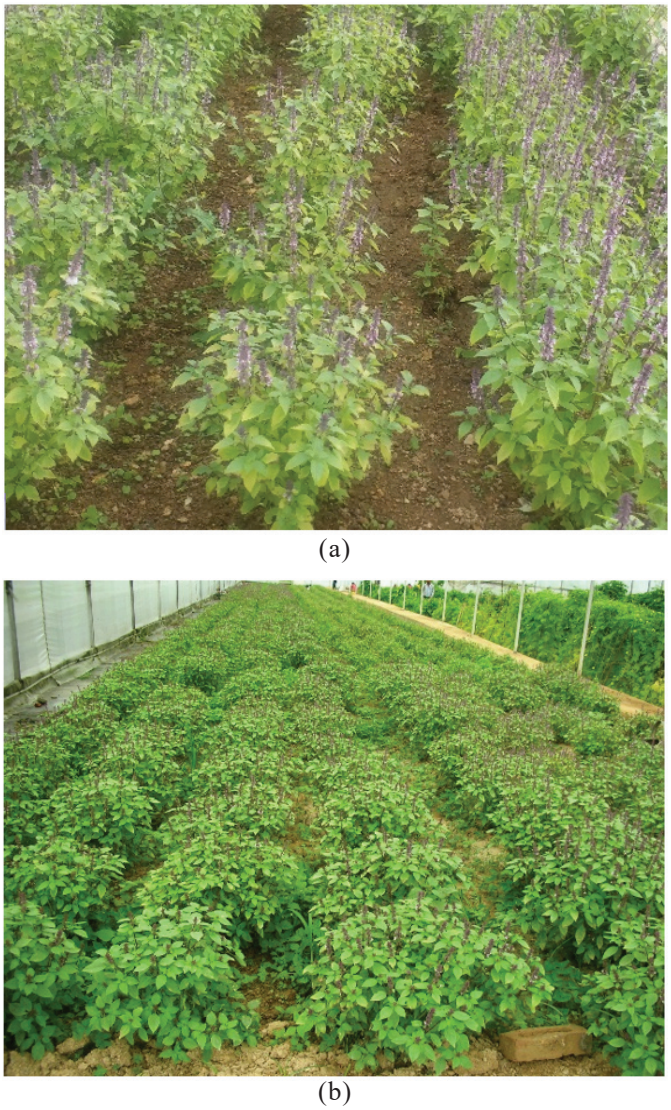
The aim of the present study was to evaluated the variation in the *in vitro* antioxidant potential and antioxidant constituentsof aqueous extract of leaves of *Ocimum basilicum* Linn. grown in an open field and inside polyhouse conditions with the development of plant till maturity in every 15 days interval. Further, the correlation analysis was established between the antioxidant activity and antioxidant constituents.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

The *Ocimum basilicum* plants were grown inside the polyhouse and open field conditions at DIBER (Defence Institute of Bio-Energy Research, DRDO) field station, Pithoragarh (Uttarakhand), India. The leaves (100 g) were collected in every 15 day's interval till the maturity of the plant from both the growing conditions. The plant leaves were washed properly and a finely chopped. The chopped parts were then dried in a hot air oven at 40˚C. After drying, the plant samples were powdered and stored in airtight containers for the present study. 10 gm of dried leaves powderwas extracted with 100 ml of water by cold maceration process and kept in the dark for 24 hrs., with occasional shaking. The resultant solution was concentrated to dryness. The leaves extract (100 mg/10 ml) was then used to evaluate of antioxidant activity and antioxidant constituents. (Fig. 1)



(a)

(b)

Figure 1. *Ocimum basilicum* Linn. plant grown in (a) an open field and (b) inside polyhouse conditions.

2.2 Chemicals

Ethanol, ascorbic acid, ferric chloride ($FeCl_3$), sodium dihydrogen phosphate, 2, 2-azino-bis (3-ethyl benzothiazoline-6-sulfonic acids) diammonium salt (ABTS), potassium persulfate ($K_2S_2O_8$), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), methanol, tannic acid, hydrochloric acid, ferric chloride, potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid, potassium acetate (CH_3COOK), aluminium chloride ($alcl_3$), sodium carbonate, sodium hydroxide, nitric acid were purchased from E Merck India Ltd., Sigma chemicals, USA and Central Drug House (P) Ltd., New Delhi, India. All the chemicals and reagents used were of analytical grade.

2.3 Evaluation of Antioxidant Activity

Various assays have been used to determine the antioxidant potential of the leaves of *Ocimum basilicum* grown inside polyhouse and open field conditions. The methods used are ABTS free radical scavenging assay, DPPH free radical scavenging assay and FRAP assay were carried out in replication (n=3). The ascorbic acid (0.1mg/ml) used as a reference standard for the antioxidant assays.

2.3.1 DPPH Free Radical Scavenging Assay

The DPPH method was used for estimating inhibition concentration (IC_{50}) of the aqueous, ethanolic and hydroalcoholic extract according to Kedare *et al*. (2011)[14]. The methanolic solution of DPPH (2 ml of 0.1mmol) was added to the different aliquots(20-100 µl) of each extract (10 mg/ml), and then the final volume was made up to 3 ml in each test tube, and absorbance were observedafter 40 min., at 517 nm against a blank (methanol). The reference standard used was ascorbic acid[15-16]. The percentage FRSA (free radical scavenging activity) of DPPH radicals were calculated, and further IC_{50} value was determined as:

$FRSA\,(\%) = [(Ac - At)/Ac] \times 100$, Where, Ac = absorbance of control, At = absorbance of test.

The IC_{50} value calculated as:

Inhibitory Concentration (IC_{50}) value (mg/ml) = (Concentration of test (close to 50% FRSA) / FRSA close to 50 %) × 50

2.3.2 ABTS Free Radical Scavenging Assay

The total antioxidant activity was estimated as per the Re, R. *et al*. (1999)[17] method. The different concentrations (20-100 µl) of the extracts were added into test tubes and then makeup the volume up to 1 ml with distilled water. 1 ml of ABTS solution was added. Test tubes were shaken and kept in the dark for 5 min - 7 min. The absorbance of all the sample solutions was observed at 734 nm against methanol as blank. In the assay ABTS radical cation ($ABTS^{\bullet+}$) was produced when ABTS reacts with the potassium persulphate. $ABTS^{\bullet+}$ is a blue-green chromogen that shows absorbance maxima at 734 nm. The antioxidant activity observed according to the

extent of decolorisation. The antioxidants change the coloured radical cation ($ABTS^{\bullet+}$) to colourless ABTS, which is due to its hydrogen donating availability. The percentage FRSA and IC_{50} value were calculated similarly as mentioned above[15-16].

2.3.3 FRAP Free Radical Scavenging Assay

The reducing ability of medicinal plants was determined according to the method used by Maruthamuthu *et al.* (2016)[18] with modifications. Different concentrations of the extracts were added into test tubes and then made up the volume upto 1ml with distilled water. After that, 2.5 ml of phosphate buffer (0.2M, pH 6.6) was added to the above solution, followed by 2.5 ml of potassium ferricyanide (1 %). The resultant solution then incubated at 50 ^{0}C for 20 min, followed by the addition of the 2.5 ml of trichloroacetic acid (10 %). The mixture was then centrifuged for 10 min (3000 rpm). Further, taken 2.5 ml of the upper layer of the resultant solution and mixed with 2.5 ml of distilled water, followed by the addition of 0.5 ml of $FeCl_3$solution (0.1 %). Immediately after that measured the absorbance at 700 nm. The reference standard used was ascorbic acid[15-16]. The reducing ability was measured in terms of EC_{50} value (mg/ml).

2.4 Evaluation of Antioxidant Constituents

2.4.1 Total Phenolic Contents

The total phenolic contents (TPC) of the extracts were estimated by using the Folin- ciocalteu method[19]. As per the method, 100 µl of the extracts were taken into the test tubes followed by the addition of the distilled water (3 ml). Then 0.5 ml of the folin-ciocalteu reagent was added. Mixed the resultant solution and added 2 ml of 20 per cent sodium carbonates solution just after 3 min. The solutions were mixed thoroughly and boil for at least 1 min in a water bath.The resultant solution turns to a blue colour solution by the complex formation, which formed due to the reaction of the sample with the phosphomol**y**bdic acid. The absorbance was measured at 650 nm. The total phenolic contents were measured in terms of catechol, and the values expressed as mg catechol equivalent/g (mg CE/g) on a dry weight basis[20,16].

2.4.2 Flavonoid Contents

The aluminium chloride method was used to determine the flavonoid contents (TFC) of the extracts[21]. As per the method, 100 µl of the extracts were taken into the test tubes followed by the subsequently addition of 80 per cent and 95 per cent ethanol. Then the aluminium chloride solution (100 µl) was added to each tube except the blank sample, followed by the addition of 100 µl potassium acetate solution. The solution was then thoroughly mixed in the vertex (1500 rpm) and incubated (30 min). The absorbance of the resultant solution was measured at 415 nm. The flavonoids contents were measured in terms of quercetin, and the values expressed as mg quercetin equivalent/g (mg QE/g) on a dry weight basis[16,22].

2.4.3 Tannin Contents

The tannin in plant extract was determined by folin-denis method[23]. As per the method, 100 µl of the extracts were mixed with 30 ml of water in a volumetric flask and 2.5 ml folin-denis reagent was added, followed by the addition of 35 per cent sodium carbonate solution. The resultant solution was mixed thoroughly and make-up the volume up to 50 ml with distilled water. Then placed the flask on the heating plate for 30 min (10 ^{0}C - 20 ^{0}C). The absorbance was observed at 700 nm. The tannin contents were measured in terms of tannic acid, and the values expressed as mg tannic acid equivalent/g (mg TAE /g) on a dry weight basis[16,22].

2.5 Chlorophyll Content

1 g finely chopped samples of leaves and roots are taken and ground to a fine pulp in 10 ml of 80 per cent acetone. Centrifuged for 5 min and filtered, then transfer the supernatant to a 100 mL volumetric flask. Repeat the same process until the residue turns colourless. Make up the volume in a conical flask to 100 mL with acetone and read the absorbance at 645, 663 and 652 nm against a blank(acetone)[23].

$$\text{mg chlorophyll a/g tissues} = 12.7\left(A663\right) - 2.69\left(A645\right) \times \frac{v}{1000 \times w}$$

$$\text{mg chlorophyll b/g tissues} = 22.9\left(A645\right) - 4.68\left(A663\right) \times \frac{v}{1000 \times w}$$

$$\text{mg chlorophyll total chlorophyll/g tissues} = 20.2\left(A645\right) - 8.02\left(A663\right) \times \frac{v}{1000 \times w}$$

2.6 Statistical Analysis

The results were expressed as Mean ± SD (Standard Deviation) (n=3). The results were interpreted by ANOVA (one-way analysis of variance) followed by Duncan's test and LSD at $P<0.05$ using SPSS 16.0 Software. The difference among the mean value was considered to be significant ($P<0.05$). Correlation between the antioxidant activity and antioxidant constituents like total phenolic, flavonoid and tannin contents were conducted on $p<0.05$ and $p<0.01$ probability levels.

3. RESULTS

3.1 Evaluation of Antioxidant Activity

The antioxidant activity was estimated in terms of IC_{50} (inhibition concentration 50); minimum IC_{50} value represents the maximum antioxidant activity**.** The results of the DPPH assay revealed that the IC_{50} value was varied from 0.090 to 0.462 mg/ml in the aqueous extract of the *Ocimum basilicum* grown in an open field condition from different plant growth stages, i.e. 15 days to 90 days of plant growth. The lowest IC_{50} value i.e. highest antioxidant activity, was found (0.090 mg/ml) in 90 days old plant followed by (0.115 mg/ml) in 75 days old plant. Whereas the IC_{50} value was ranged from 0.318 to 0.614 mg/ml in the aqueous extract of plant grown inside polyhouse condition. The highest antioxidant activity (IC_{50} value 0.318 mg/ml) was found in 90 days old plant followed by (0.406 mg/ml) in 75 days old plant.

The results of the ABTS assay revealed that the IC_{50} value was ranged from 0.064 to 0.344 mg/ml in the plant grown in an open field condition from 15 days to 90 days. The highest antioxidant activity (IC_{50} value 0.064 mg/ml) was found in 90 days old plant followed by (0.085 mg/ml) in 75 days old plants. Whereas the IC_{50} value was ranged from 0.085 to 0.401 mg/ml in the aqueous extract of plant grown inside polyhouse

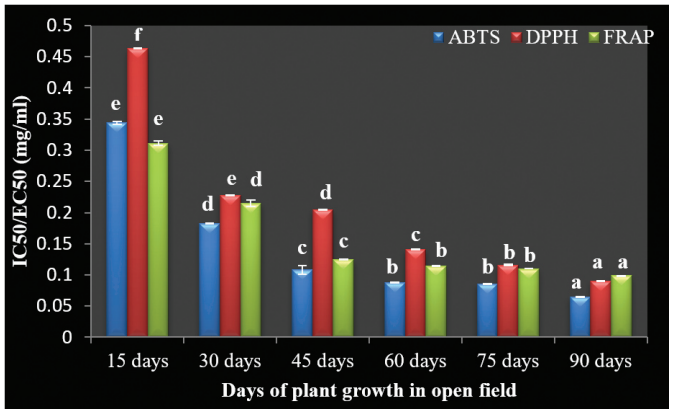


Figure2. Antioxidant activity of aqueous extracts of leaves of *Ocimum basilicum* grown in an open field, the IC_{50}/EC_{50} value with different alphabets (a-e) are significantly different (P<0.05) according to Duncan's and LSD test.

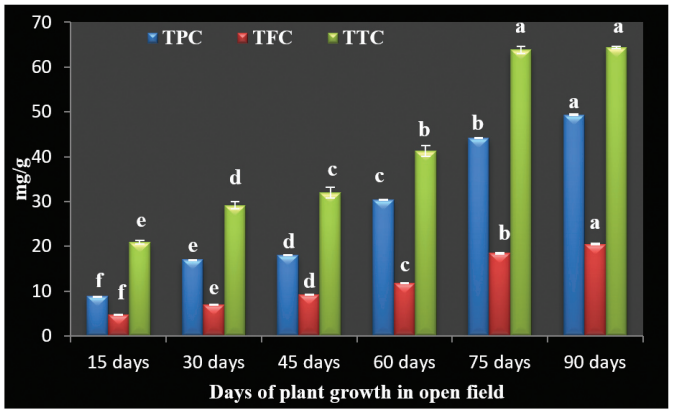


Figure 4. Total phenolic, flavonoid and tannin contents of aqueous extract of leaves of *Ocimum basilicum* grown in an open field condition, the values with different alphabets (a-e) are significantly different (P <0.05) according to Duncan's and LSD test.

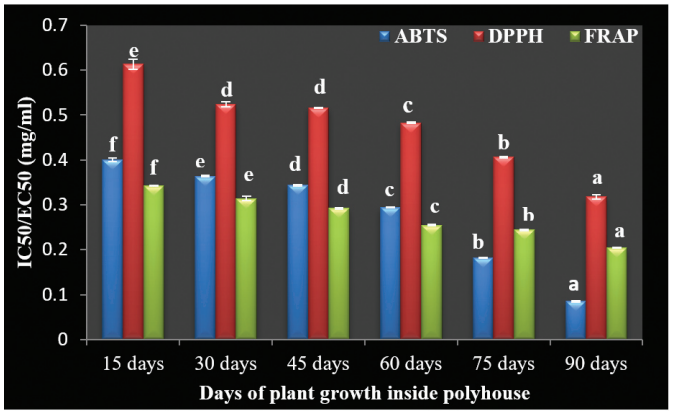


Figure3. Antioxidant activity of aqueous extracts of leaves of *Ocimum basilicum* grown inside polyhouse condition, the IC_{50}/EC_{50} value with different alphabets (a-e) are significantly different (P <0.05) according to Duncan's and LSD test.

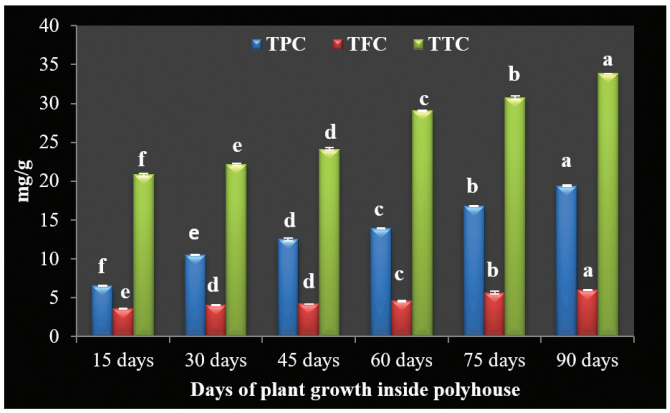


Figure 5. Total phenolic, flavonoid and tannin contents of aqueous extract of leaves of *Ocimum basilicum* grown inside polyhouse condition, the values with different alphabets (a-e) are significantly different (P <0.05) according to Duncan's and LSD test.

condition. Maximum antioxidant activity (IC_{50} value 0.085 mg/ml) was found in 90 days old plants followed by (0.182 mg/ml) in 75 days old plants.

The antioxidant activity was estimated in terms of EC_{50} (effective concentration 50), minimum EC_{50} value represents the maximum antioxidant activity**.** The results of the FRAP assay revealed that the EC_{50} valuewas varied from 0.099 to 0.310 mg/ml in a plantgrown in an open field conditionfrom different plant growth stages, i.e. from 15 days to 90 days. The maximum antioxidant activity (EC_{50} value 0.099 mg/ml) was exhibited by 90 days old plant followed by (0.110 mg/ml) in 75 days old plants. Whereas theEC_{50} value was ranged from 0.204 to 0.343 mg/ml in the aqueous extract of plant grown inside polyhouse condition. Maximum antioxidant activity (IC_{50} value 0.204 mg/ml) was recorded in 90 days old plants followed by (0.247 mg/ml) in 75 days old plants. Figure 2 and Fig. 3 represented the antioxidant activity of aqueous extracts of leaves of *Ocimum basilicum* grown in an open field and inside polyhouse conditions, respectively.

3.2 Evaluation of Antioxidant constituents

The total phenolic contents varied from 08.79 to 49.32 and 06.54 to 19.42 mg CE/g d.w. in aqueous extracts of leaves of *Ocimum basilicum* grown in open field condition and inside polyhouse condition, respectively,from 15 days to 90 days of plant growth. The results revealed that the maximum phenolic contents (49.32 mg/g) found in aqueous extract of 90 days old plants leaves grown in open field condition followed by the 75 days old plants (44.143mg/g). Similarly, the aqueous extract of plant grown inside polyhouse condition represented that the highest phenolic contents (19.42 mg/g) found in 90 days old plant leaves followed by the 75 days old plants (16.79 mg/g). The total phenolic content expressed as mg catechol equivalent per g of sample on a dry weight basis.

The flavonoid contents varied from 04.77 to 20.49 and 03.59 to 06.01 mg QE/g d.w. in aqueous extracts of leaves of *Ocimum basilicum* grown in open field condition and inside polyhouse condition, respectively, from 15 days to 90 days of plant growth. The results revealed that the maximum flavonoid contents (20.49 mg/g) found in aqueous extract of 90 days old

Table 1. Pearson's correlation analysis of antioxidant constituents with antioxidant activities of Ocimum basilicum grown in an open field and inside polyhouse conditions

Antioxidant parameters	Correlation coefficient (r)					
	IC_{50} ABTS assay	IC_{50} DPPH assay	EC_{50} PFRAP assay	TPC	FC	TC
IC_{50} ABTS assay	1	0.928**	0.940**	-0.782**	-0.743**	-0.767**
IC_{50} DPPH assay		1	0.965**	-0.856**	-0.859**	-0.827**
EC_{50} FRAP assay			1	-0.859**	-0.855**	-0.841**
TPC				1	0.981**	0.991**
FC					1	0.975**
TC						1

** Indicates correlation significance at P < 0.01 (2-tailed). TPC- total phenolic content, FC-flavonoid contens, and TC-tannin contents.

plants leaves grown in open fieldcondition followed by the 75 days old plants (18.43 mg/g). Similarly, the aqueous extract of plant grown inside polyhouse condition represented that the highest flavonoid contents (06.01 mg/g) found in 90 days old plant leaves followed by the 75 days old plants (5.65 mg/g).The total flavonoid content expressed as mg quercetin equivalent per g of sample on a dry weight basis.

The tannin contents varied from 20.85 to 64.42 and 20.82 to 33.88 mg TAE/g d.w. in aqueous extracts of leaves of *Ocimum basilicum* grown in open field condition and inside polyhouse condition, respectively, from 15 days to 90 days of plant growth. The results revealed that the maximum tannin contents (64.42 mg/g) found in aqueous extract of 90 days old plants leaves grown in open field condition followed by the 75 days old plants (63.88 mg/g). Similarly, the aqueous extract of plant grown inside polyhouse condition represented that the highest tannin contents (33.88 mg/g) found in 90 days old plant leaves followed by the 75 days old plants (30.75 mg/g). The total tannin content expressed as mg tannic acid equivalent per g of sample on a dry weight basis. Figure 4 and Fig. 5 represented the total phenolic, flavonoid and tannin contents of aqueous extracts of leaves of *Ocimum basilicum* grown in an open field and inside polyhouse conditions, respectively.

3.3 Chlorophyll Content

The total chlorophyll content was varied from 0.255 to 0.926 mg/100 g and 0.548 to 1.470 mg/100 g f.w. in the leaves of *Ocimum basilicum* grown in an open field condition and inside polyhouse condition, respectively, from 15 days to 90 days of plant growth. The highest chlorophyll content (0.926 mg/100 g) was found in 15 days old plant, followed by (0.841 mg/100 g) in 30 days old plants grown in open field condition. The concentration of chlorophyll was decreased with the maturity of the plants. The maximum chlorophyll content (1.470 mg/100 g) was found in 90 days old plant followed by (0.938 mg/100 g) in 75 days old plants grown inside polyhouse condition. The concentration of chlorophyll was increased with the maturity of plants grown inside polyhouse conditions.

3.4 Correlation Analysis

The Pearson'scorrelation analysis of antioxidant constituents such as total phenolic, flavonoids and tannin contents with the antioxidant potential of leaves extract of *Ocimum basilicum* grown in an open field and inside polyhouse conditions are represented in Table 1; the values of correlation coefficient showed that the antioxidant constituents exhibited a significant negative correlation with the IC_{50}/EC_{50} value of different antioxidant methods such as the ABTS, DPPH and FRAP assay. The results also revealed that the IC_{50} value of ABTS assay had a significantly positive correlation with the IC_{50} and EC_{50} value of the DPPH and FRAP assay, respectively. (Table 1).

4. DISCUSSION

Antioxidants are the compounds that suppress or inhibited the oxidation of free radicals by oxidising themselves. These compounds break the chain reaction of free radicals without becoming a free radical itself. They often have known as reducing agent[2]. The present study assessed the antioxidant potential and antioxidant constituents of the aqueous leaves extract of *Ocimum basilicum* Linn. grown in an open field and inside polyhouse conditions with the maturity of the plant in every 15 day's interval. The antioxidant activity determined by the ABTS, DPPH free radical scavenging assay and FRAP assay. The results revealed that the leaves extract of plant grown in an open field conditions possesses a significantly higher antioxidant activity than the plant grown inside polyhouse conditions. Further, the 90 days old plant showed maximum antioxidant potential among other plant growth stages in both open field and inside polyhouse conditions. Prasath S *et al.*(2019)[5] reported the antioxidant properties of ethanolic leaves extract of *Ocimum basilicum*. The study revealed that the ethanolic leaves extract significantly scavenged DPPH and ABTS radicals with IC_{50} values 586.3 µg/ml and 727.9 µg/ml, respectively. The study also reported that the extract exhibited superoxide and NO scavenging activity with IC_{50} values 604.2 µg/ml and 652.60 µg/ml. The total phenolic and flavonoid contents exhibited in the ethanolic extract of leaves were 284.72 mg and 43.6 mg, respectively. The previous study, therefore, concluded that the free radical activity of leaves extract is evident from the *in-vitro* antioxidant methods, and the activity is probably due to the phytochemical contents of the leaves.

The medicinal plants possess various secondary metabolites, which attributes the antioxidant properties and helpful for the prevention of chronic diseases[3-4,24]. The secondary metabolites of the plants, typically the phenolic compounds, show their antioxidant potential mainly due to their hydrogen donating and metal-chelating properties. The phenolic compounds such as flavonoid, anthocyanin and tannin have possessed a significant antioxidant potential for different antioxidant asaays[25], and showed a protective response against several reactive oxygen species such as hydroxyl radical, superoxide anion, peroxyl radical, peroxynitrite and hypochlorous acid[26]. The present study revealed that the leaves extract exhibited appreciable quantities of total phenolic, flavonoids and tannin contents. The results showed that the leaves extract of plant grown in an open field conditions exhibited significantly maximum antioxidant constituents compared to the plant grown inside polyhouse conditions. Further, the 90 days old plant showed significantly higher antioxidant constituents among others in both open field and inside polyhouse conditions. Surveswaran *et al*. (2010)[27] reported the variation in phytochemicals constituents within the plant parts and suggested that the variation can be attributed to the endogenous physiological changes and specific metabolic process of the plant[25]. The results of the present study showed a satisfactory agreement with the previously reported[28-30]. Chlorophyll is a pigment that provides a green colour to plants. The study observed that among the leaves sample, the leaves grown in open field condition showed higher chlorophyll contents than the leaves grown inside polyhouse conditions. The data of plants grown in an open field conditions showed that the concentration of chlorophyll decreased with the maturity of the plant. Whereas the plants grown inside polyhouse conditions showed that the concentration of chlorophyll increased with the maturity of the plant. The Pearson's correlation analysis showed a negative correlation of antioxidant constituents *viz.*total phenolic, flavonoids and tannin contents with the IC_{50}/EC_{50} values by different antioxidant assay. Therefore, the data reflects that the higher the contents of antioxidant constituents, the lower the IC_{50}/EC_{50} value, and the higher will be the antioxidant potential. The data suggested that the phenolic, tannin and flavonoid constituents are the significant contributors to the antioxidant activity of the plant extracts. The findings also revealed that the IC_{50} value of the ABTS assay of all the samples had a significant positive correlation with their IC_{50} value of DPPH assay and an EC_{50} value of the FRAP method. The present study data was accordant with the previous work, which reported the correlation of antioxidant constituents with the antioxidant potential[4,31]. Hence, It can be anticipated that the phenolic components of the plants are the major compounds responsible for the antioxidant activities of leaves extract by different antioxidant assay.

5. CONCLUSION

The study concluded that the aqueous leaves extract of *Ocimum basilicum* grown in open field conditions exhibited significantly higher antioxidant activity and constituents than the plant grown inside polyhouse conditions. Further, the 90 days old plant showed significantly maximum antioxidant activity and constituents followed by the 75 days old plant and least antioxidant activity and constituents exhibited by the15 days old plantin both open field and inside polyhouse conditions. The study also observed that the leaves grown in open field condition showed higher chlorophyll contents than the leaves grown inside polyhouse conditions. The antioxidant constituents *viz.* total phenolic, flavonoids and tannin contents of leaves exhibited significant correlation with its antioxidant activity. Therefore, the study reflects that the phenolic, tannin and flavonoid constituents are the significant contributors to the antioxidant activity. Consequently, the findings provide substantial evidence that the *Ocimum basilicum* leaves can be valued as sources of effective natural antioxidants and can be utilised as an accessible source of natural antioxidants with consequent health benefits.

6. ACKNOWLEDGEMENT

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He was involved in the field work related to the experiment and also in analysis of samples.