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IN VITRO ANTI-OXIDANT ACTIVITIES OF AQUEOUS AND ETHANOL EXTRACT OF SEED OF *OCIMUM BASILICUM*

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ABSTRACT

Objective:

In the present study the aqueous and ethanol extract of *Ocimum basilicum* seed was studied for anti-oxidant activities.

Methods:

The antioxidant activities were investigated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) method, ABTS scavenging assay, hydrogen peroxide, superoxide anion radical scavenging assay and metal chelating ability on ferrous ions under *in vitro* condition.

Results:

The ethanolic extract of seed of *Ocimum basilicum* showed significant scavenging effect on DPPH (83.80±1.22) ABTS (74.85±0.824), Hydrogen peroxide (68.75 ±0.824), superoxide anion (78.37±0.808) and metal chelating (71.34 ±0.008) and the aqueous extract exhibited the inhibition for ABTS (63.78±0.824), hydrogen peroxide (54.69 ±0.824), superoxide anion (65.74±0.808) and metal chelating (65.74 ±0.008), DPPH (81.26 ±0.808) compared with standard drug.

Conclusion:

The study revealed the dose dependent increase in the scavenging activity of the extract and exhibited potent antioxidant activities. Therefore, it is suggested that the aqueous and ethanol extract of *Ocimum basilicum* is a source for natural antioxidant compounds and could have the potential use in the management against free radicals generated in response to oxidative stress.

Keywords: *Ocimum basilicum,* aqueous and ethanol extract, DPPH, ABTS, hydrogen peroxide, superoxide anion, metal chelating ability

1. INTRODUCTION

Antioxidants act as a major defense against radical-mediated toxicity by protecting from damages caused by free radicals **[1]**. Antioxidants can be defined as compounds that can delay and prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction **[2]**. The phenolic compounds in medicinal plants can play an important role in neutralizing free radicals, chelating transitional metals, quenching singlet and triplet oxygen **[3]**. Natural foods and food derived antioxidants such as phenolic phytochemicals and vitamins are known to function as chemo preventiveagents against oxidative damage **[4]**. Reactive oxygen species such as superoxide anion radicals and hydrogen peroxide can cause oxidative damage to macromolecules including DNA, lipids, proteins and small cellular molecules **[5-8]**. The antioxidant protection systems including enzymes (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymes protection (glutathione, vitamin C and E) play an important role in scavenging oxidants and preventing cell injury **[9, 10]**.

Medicinal plants have been used to treat human diseases in the world for centuries. Nowadays human beings are more interested in herbal medicines because of their low toxicity and good therapeutic performance **[11, 12]**. *Ocimum basilicum* commonly known as sweet basil belong to the family Lamiaceae **[13]**. *Ocimum basilicum* used for thousands of years for the treatment of stomachic, digestive and nervous disorders, diabetes and respiratory disorders, blood diseases, headache, dysentery, dizziness, piles, cough, paralysis, nervous temperament and numbness and also as taste improving and cardioprotective agent **[14-17]**. The essential oils are used to cure snake bites, acne and insect stings **[18]**. Basil tea cures vomiting, diarrhea, constipation and for mental fatigue **[19]**. The chemical constituents which have been isolated from the basil plants include flavonoids, terpenoids, tannins, saponin, glycosides and ascorbic acid **[20]**. The purpose of this work comprise the assessment of the antioxidant activity of aqueous and ethanol extracts of seed samples of *Ocimum basilicum* by different analytical methods such as using free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) method, scavenging ABTS radical cation, hydrogen peroxide, superoxide anion radical scavenging assay and metal chelating ability on ferrous ions under *in vitro* condition.

MATERIALS AND METHODS:

Collection of plant material:

The seed of *Ocimum basilicum were* collected in the month of February, 2020 from the Local market, Trichy, Tamil Nadu, India. The plant was authenticated by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirappalli. The voucher specimen dated on 04/02/2020.

Chemicals and Reagents:

Chemicals used in the study were ascorbic acid, gallic acid, tannic acid, catechol, butylated hydroxyl toluene, Folin-Ciocalteau reagent, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, potassium ferric cyanide, ferric chloride, sodium phosphate, ammonium molybdate, ferrous ammonium sulphate, Ethylene diamine tetra acetic acid (EDTA), Dimethyl Sulphoxide (DMSO), ammonium acetate, NADH, Nitro Blue Tetrazolium (NBT), Phenazine Methosulphate (PMS), hydrogen peroxide, sodium nitroprusside, sulphanilamide, Naphthyl ethylene diamine dihydrochloride (NEDD), glacial acetic acid, acetyl acetone, sulphuric acid, phosphoric acid, Trichloroacetic acid (TCA) and ferrozine. All the chemicals and solvents used were of analytical grade.

Preparation of Ethanol and Aqueous Extract:

The seed of *Ocimum basilicum* were washed in running water, and then shade dried for a week at 35-40^{o}C, after which it was grinded to a uniform powder of 40 mesh size. The ethanol and aqueous extracts were prepared separately by soaking 100 g each of the dried powder plant materials in 100 L of ethanol and aqueous using a soxhlet extractor continuously for 10 hr. The extracts were filtered through whatmann filter paper No. 42 (125mm) to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labeled sterile bottles and kept at -20^{o}C.

Antioxidant Activity

(DPPH Free Radical Scavenging Activity) Determination:

The antioxidant activity of the aqueous and ethanolic extract was examined on the basis of the scavenging effect on the stable DPPH free radical activity **[21]**. Ethanolic solution of DPPH (0.05mM) (300µl) was added to 40µl of aqueous and ethanolic extract with different concentrations (100-500 µg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 540nm.

Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate.

The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation.

Percent (%) inhibition of DPPH activity = [(A – B) / A] x 100

Where B and A are the absorbance values of the test and of the blank sample, respectively.

Scavenging of ABTS radical cation

To 1 ml of various concentrations of the extract or standard (Ascorbic acid), 1.0 ml of distilled DMSO and 3 ml of ABTS solution were added and incubated for 20 min. Absorbance of these solutions was measured spectrophotometrically at 734nm **[22]**.

% inhibition = [(Control- Test)/control]×100

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Various concentrations of the extract or standard ascorbic acid (20-100 µg/ml) in ethanol (1 ml) were added to 2ml of hydrogen peroxide solution in PBS. After 10 min the absorbance was measured at 230 nm **[23]**.

% inhibition = [(Control- Test)/control]×100

Superoxide anion radical Scavenging assay:

To various concentrations of the extracts (20-100 µg/ml), 1.0 ml of phosphate buffer (0.1 M, pH 7.2), 1.0 ml of NADH (2 mM), 1.0 ml of NBT (0.5 mM) and 0.1 ml of PMS (0.03 mM) were added. After 5 minutes incubation at room temperature, the absorbance was read at 562 nm against a reagent blank to determine the quantity of formazan generated. Gallic acid was used as the standards **[24]**.

% inhibition = [(Control- Test)/control]×100

Metal Chelating ability on ferrous ions:

The reaction mixture contained 1.0 ml of various concentrations of the extracts (20-100 μg/ml) and 0.05 ml of 2 mM $FeCl_3$. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. The reaction mixture was shaken vigorously and left standing at room temperature for 10 min and the absorbance of the reaction mixture was measured at 562 nm against a reagent blank. A lower absorbance of the reaction mixture indicated a higher ferrous ion chelating ability. The control contained all the reagents except sample. Ascorbic acid was used as standard for comparison **[25]**.

% Inhibition = [(Control- Test)/control]×100

RESULTS AND DISCUSSION

ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *OCIMUM BASILICUM* SEED SAMPLE BY DPPH ASSAY:

The result showed the potent antioxidant activities of aqueous and ethanolic extract of *Ocimum basilicum* seeds at high concentrations when compared with ascorbic acid **Table 1**. The aqueous extract presented 42.96 to 52.34% and ethanol extract expressed 62% to 83.8 % antioxidant activity at concentration (20-100 μg/ml) while ascorbic acid exhibited 70 to 84.60 % at the same concentration **(Figure 1)**.

Previous studies expressed the stronger antioxidant activity of water and ethanolic extract of basil **[26]**. The hexane extract of basil showed a maximal concentration – dependent antioxidant activity compared to the ethanol extract for the DPPH assay **[27]**.

ABTS RADICAL SCAVENGING ASSAY

ABTS assay is better to assess the antiradical capacity of both lipophilic and hydrophilic antioxidant because this method is based on the ability of antioxidant to reduce the ABTS radical cation. In the present work, the aqueous and ethanolic extracts of *Ocimum basilicum* seeds were evaluated for their ABTS radical cation scavenging activity. The ethanol extracts of *Ocimum basilicum* seeds showed maximum inhibition of 56.64 % to 74.85 % and aqueous extract expressed 48.34 % to 63.78 % of inhibition at a concentration of 20-100 μg/ml. Ascorbic acid was used as standard showed maximum inhibition of 70.57% to 83.88 % at concentration (20-100 μg/ml).

Previous studies reported the presence of natural antioxidants and phenolic compounds in white and red holy basil leaves of *Ocimum sanctum* and also reported the higher values in ABTS scavenging assay than DPPH and FRAP assays **[28]**. The leaves extract of *Ocimum basilicum* was found to be antioxidant in nature and at a concentration of 1000 μg/ml, the extract significantly scavenged 84% of DPPH radicals and 79% of ABTS radicals **[29] (Table 2)**.

Scavenging of hydrogen peroxide

Hydrogen peroxide forms OH in the presence of metal ions and oxygen facilitates this reaction. The radical scavenging capacity may be attributed to phenolic compounds in ethanol extract with the ability to accept electrons, which can combine with free radical moiety to decrease hydroxyl radical **[30]**. The maximum hydrogen peroxide scavenging activity was found in ethanol extracts of *Ocimum basilicum* seeds with the maximum inhibition of 57.14 % to 68.75 % and aqueous extract exhibited 42.34 % to 54.69 % scavenging activity at a concentration 20-100 µg/ml. Ascorbic acid was used as standard showed maximum inhibition 69.27% to 81.86% at concentration (20-100 µg/ml).

Umar *et al* (2012) studied the antioxidant activity of basil by different methods like DPPH free radical scavenging, hydrogen peroxide scavenging, reducing power, ferric thiocyanate method and metal chelating activities **[31] (Table 3)**.

Metal chelating activity

The transition metal ions Fe^{2+} possess the ability to move electrons and also allow the formation and propagation of many radical reactions **[32].** The Ferrozine can quantitatively form complexes with Fe^{2+}. The absorbance of Fe^{2+} ferrozine complex was decreased in a dose dependent manner, i.e. the activity was increased by increasing the concentration of aqueous and ethanol extract of seed of *Ocimum basilicum* from 20 to 100 µg/ml. The chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion. **[33].** The result proved that the ethanolic extract possessed Fe^{2+} chelating activity and may play a protective role against oxidative damage induced by metal catalyzed decomposition reactions. The ethanolic extract of *Ocimum basilicum* showed very strong chelating activity as compared to the aqueous extract due to high concentration of flavonoids compounds that can chelate metal ions. The maximum metal chelating activity was found in ethanol extracts of *Ocimum basilicum* seeds with the maximum metal chelation activity of 56.32 % to 71.34 % than aqueous extract of 49.33 % to 65.74 % .Standard ascorbic acid was showed maximum inhibition of 62.68% to 88.59 % at concentration of 20-100 µg/ml.

Earlier studies reported that the methanolic extract of leaves of *Ocimum basilicum* showed a significantly higher chelating percentage (82.84%) at concentration 100 µg/ml that aqueous extract 72.33% **[34]** **(Table 4)**.

Superoxide anion radical scavenging activity

Superoxide anion derived from dissolved oxygen, riboflavin and methionine reduce the yellow dye NBT and to produce the blue formazan. Antioxidants are able to inhibit the blue NBT formation **[35]** and decrease of absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture. The maximum superoxide anion radical scavenging activity was found in ethanol extracts of *Ocimum basilicum* seeds with the maximum scavenging activity of 54.15 % to 78.37 % and for aqueous extract 49.45 % to 66.84 % .The standard ascorbic acid showed maximum inhibition of 65.86% to 84.74 % at a concentration of 20-100 µg/ml.

The previous literature reported that the superoxide scavenging activity of *O. Basilicum* leaves extract showed 81% superoxide scavenging activity (IC50= 604.2 µg/ml) and Nitric oxide scavenging activity was 83% with IC50 value of 652.60 µg/ml **[36] (Table 5)**.

Table 1: ***In vitro*** **DPPH scavenging assay of extracts of** ***Ocimum basilicum*** **seeds (Values are mean ± SEM of three replicates)**

S.NO	CONCENTRATION	DPPH scavenging activity		
		AQUEOUS	ETHANOLIC	ASCORBIC ACID
1	20 (µg/ml)	42.96±0.843	62.0±1.25	70.00±1.31
2	40 (µg/ml)	44.53±0.838	75.00±1.50	79.80±1.35
3	60 (µg/ml)	46.87±0.835	78.00±1.35	83.00±1.40
4	80 (µg/ml)	48.43±0.832	81.4±1.46	82.80±1.48
5	100 (µg/ml)	52.34±0.832	83.8±1.22	84.60±1.52

Table 2: ***In vitro*** **ABTS scavenging activity of the extracts of** ***Ocimum basilicum*** **seeds (Values are mean ± SEM of three replicates)**

S. No.	CONCENTRATION	Antioxidant activity by ABTS assay		
		AQUEOUS	ETHANOLIC	ASCORBIC ACID
1	20 (µg/ml)	48.34±0.839	59.64±0.832	70.57±0.808
2	40 (µg/ml)	52.68±0.838	63.45±0.832	74.67±0.808
3	60 (µg/ml)	59.37±0.832	65.15±0.824	79.28±0.832
4	80 (µg/ml)	61.24±0.832	69.95±0.832	81.98±0.824
5	100 (µg/ml)	63.78±0.832	74.85±0.824	83.88±0.824

Table 3: ***In vitro*** **Hydrogen peroxide scavenging activity of extracts of** ***Ocimum basilicum*** **seeds (Values are mean ± SEM of three replicates)**

S. No.	CONCENTRATION	Hydrogen peroxide scavenging assay		
		AQUEOUS	ETHANOLIC	ASCORBIC ACID
1	20 (µg/ml)	42.34±0.841	57.14±0.832	69.27±0.824
2	40 (µg/ml)	48.73±0.839	58.54±0.824	74.77±0.824
3	60 (µg/ml)	50.14±0.832	61.54±0.832	76.18±0.824
4	80 (µg/ml)	52.33±0.832	66.95±0.824	79.98±0.824
5	100 (µg/ml)	54.69±0.824	68.75±0.824	81.86±0.824

Table 4: *In vitro* metal chelating activity of the extracts of *Ocimum basilicum* seeds (Values are mean ± SEM of three replicates)

S. No.	CONCENTRATION	metal chelating activity		
		AQUEOUS	ETHANOLIC	ASCORBIC ACID
1	20 (µg/ml)	49.33±0.834	56.32±0.822	62.68±0.824
2	40 (µg/ml)	52.84±0.832	58.46±0.824	73.75±0.824
3	60 (µg/ml)	58.47±0.824	61.60±0.824	78.64±0.818
4	80 (µg/ml)	62.46±0.824	65.27±0.824	83.44±0.824
5	100 (µg/ml)	65.74±0.825	71.34±0.008	88.59±0.808

Table 5: *In vitro* superoxide anion radical scavenging activity of extracts of *Ocimum basilicum* seeds (Values are mean ± SEM of three replicates)

S. No.	CONCENTRATION	Superoxide anion radical scavenging activity		
		AQUEOUS	ETHANOLIC	GALLIC ACID
1	20 (µg/ml)	48.45±0.824	54.15±0.824	65.86±0.826
2	40 (µg/ml)	53.64±0.824	62.36±0.824	67.66±0.824
3	60 (µg/ml)	56.82±0.824	68.56±0.824	71.37±0.832
4	80 (µg/ml)	63.17±0.824	76.57±0.808	82.28±0.828
5	100 (µg/ml)	65.74±0.824	78.37±0.808	84.78±0.824

CONCLUSIONS

Ocimum basilicum seeds extract possessed abundant phenolic and flavonoids contents and exhibited excellent antioxidant activities. The ethanolic extract of seed of *Ocimum basilicum* shows significant inhibitory effect on DPPH (83.80±1.22) ABTS (74.85±0.824), Hydrogen peroxide (68.75 ±0.824), superoxide anion (78.37±0.808) and metal chelating (71.34 ±0.008) and the aqueous extract showed the inhibition for ABTS (63.78±0.824), hydrogen peroxide (54.69 ±0.824), superoxide anion (65.74±0.808) and metal chelating (65.74 ±0.008), DPPH (81.26 ±0.808) compared with standard drug. Thus, *Ocimum basilicum* seed can be used for the treatment of free radical mediated diseases such as diabetes and cancer and also further investigations on the isolation and characterization of bioactive compounds from the seed extracts would help to ascertain its potency.

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AUTHOR CONTRIBUTION

All authors contribute equally to this manuscript.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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