Research Article

# *In vitro* Free Radical Scavenging and Antioxidant Effect of *Ocimum basilicum*

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**ABSTRACT**

Oxidative stress has been identified as the root cause of the development and progression of several diseases. Supplementation of exogenous antioxidants or boosting endogenous antioxidant defenses of the body is the most promising way of combating the detrimental effects of reactive oxygen species (ROS) induced oxidative damage. *Ocimum basilicum* L (*Lamiaceae*), a medicinally important plant commonly known as "Holy basil" found to contain many biological properties. The present study is aimed at evaluating the free radical scavenging potentials of *Ocimum basilicum* leaf extract. The antioxidant nature of the leaves extract was proved from Free radical scavenging was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS (2,2'-azinobis (3ethylbenzthiazoline-6-sulphonic acid), ferric reducing antioxidant power (FRAP), nitric oxide scavenging assay (NO), reducing power, hydroxy radical scavenging assay, superoxide radical scavenging (SOD), hydrogen peroxide radical assay, metal chelating activity as well as phosphomolypdenum assay. From the results obtained, *O.basilicum* leaves extract can be considered as a therapeutic agent for the treatment of free radical mediated diseases.

**Keywords:** *Ocimum basilicum*, Phytochemicals, Free radicals, Antioxidant property.

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## INTRODUCTION

Oxidative stress has been identified as a major causative factor in the development and progression of several life threatening diseases, including diabetes mellitus, cancer, neurodegenerative and cardiovascular disease. During metabolic process and exposure to external environment, a large amount of free radicals is generated in the human body which pose a major influence on biological macromolecules such as proteins, fatty acids and nucleic acids causing oxidative damage on cells or tissues or even resulting in gene mutation. Free radicals at high concentration in human body cause oxidative stress, thus destroying the internal redox balance and causing a variety of chronic diseases, even premature senility. In addition, supplementation with exogenous antioxidants or boosting of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress [1].

Natural antioxidants of plant origin, especially through foods are essential to eradicate a number of free radical mediated diseases[2]. In the series of various medicinal plants, *Ocimum basilicum.* Plays a significant role for its folkloric use in the treatment of various ailments. *Ocimum basilicum L* (Lamiaceae) also commonly known as "Holy basil" have possessed different biological effects. The oil of the plant contains eugenol, methyl eugenol, citral, and methyl chavicol[3]. *O. Basilicum* leaves are used as antispasmodic, carminative, digestive, stomachic, and tonic [4,5].

*O. basilicum* produces triterpenoids, polyphenols, steroids, and phenylpropanoids some of which, such as baseball, ocimol, basilimoside, rosmarinic acid, hydroxycinnamic acids, oleanolic acid, and betulinic acid which possess various biological properties[6,7]. Ocimum sp., is shown to exert antibactericidal, antiinflammatory, antioxidative, antiulcer, antidiarrheal, chemopreventive and hypoglycemic properties [8-13]. The observed effects of the plants might be due to its antioxidant power which in turn is attributed to the presence of flavonoids and polyphenols [14]. In the absence of systematic studies in the literature, the present study was aimed to investigate the antioxidant properties of *Ocimum basilicum* leaf extract.

## MATERIALS AND METHODS

### Plant material- Identification and authentication

Matured *O. basilicum* leaves were selectively removed from the plant in and around areas of Pudussery, Palakkad, Kerala and identified by a plant taxonomist. BSI/SRC/5/23/2022/Tech/630.

### Preparation of *O.basilicum* leaves extract

#### *Delipidation and extraction*

*O. basilicum* leaves were washed, dried in a hot air oven at 40°C and subsequently ground in to powder in an electrical grinder. Delipidation was performed with methanol, ethyl

acetate for overnight. soxhalation was performed with 95% methanol, ethyl acetate evaporated in a rotary evaporator at 40-50°C under reduced pressure. The yield of the leaves extract was around 13.5 % of dry weight.

***Free Radical Scavenging Assays***

The *in vitro* anti radical scavenging potential of *O.basilicum leaves* extract (100-500µg/ml) was determined using DPPH[15], ABTS[16], FRAP[17], Nitric oxide[18], Reducing power[19], hydroxy radical[20] superoxide scavenging[21], hydrogen peroxide[22], metal chelating activity as well as phosphomolypdenum assay[23,24].

**Statistical analysis**

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

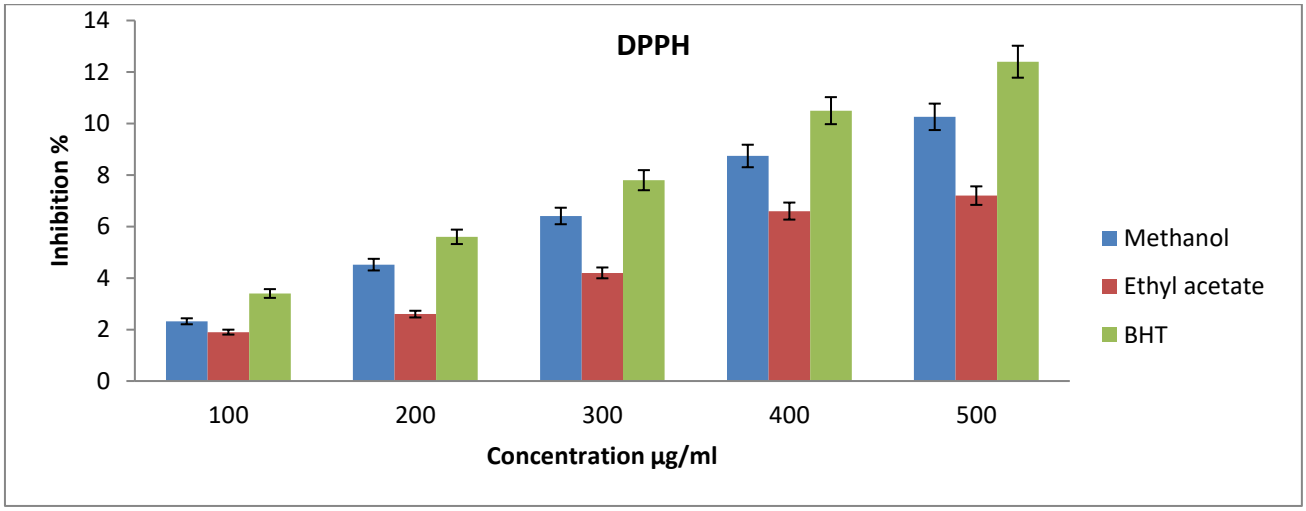


**Figure 1:** DPPH effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

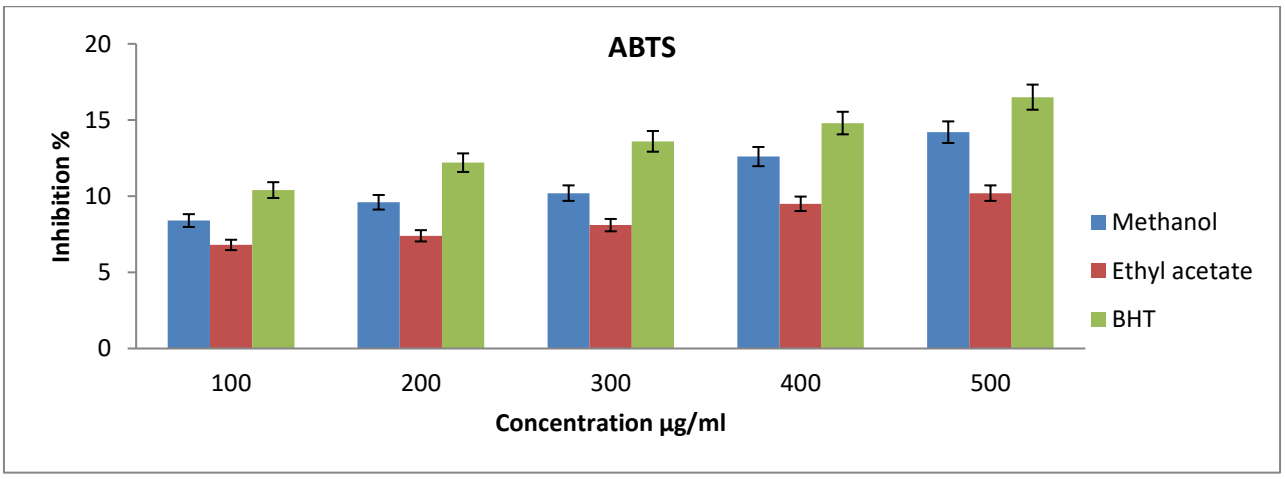


**Figure 2:** ABTS effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

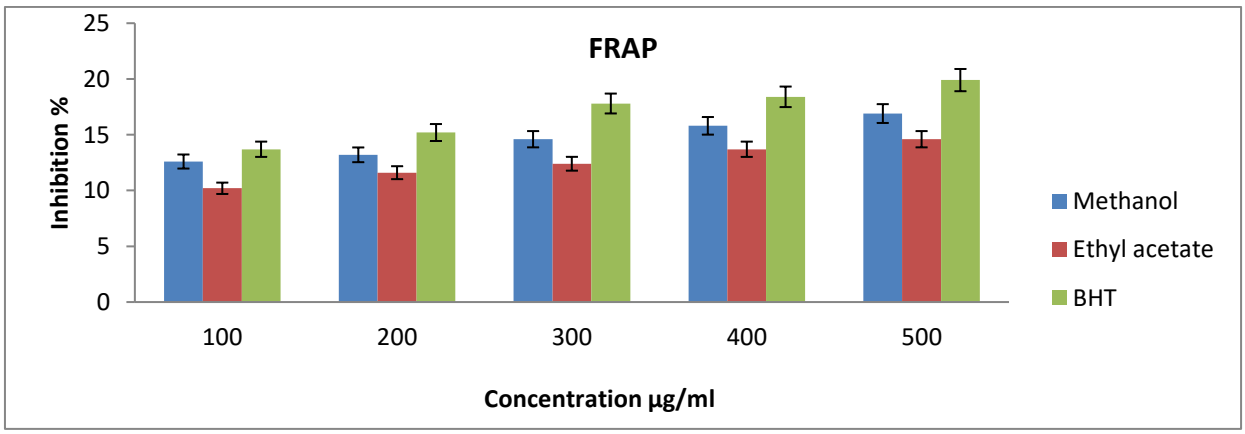


**Figure 3:** FRAP effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

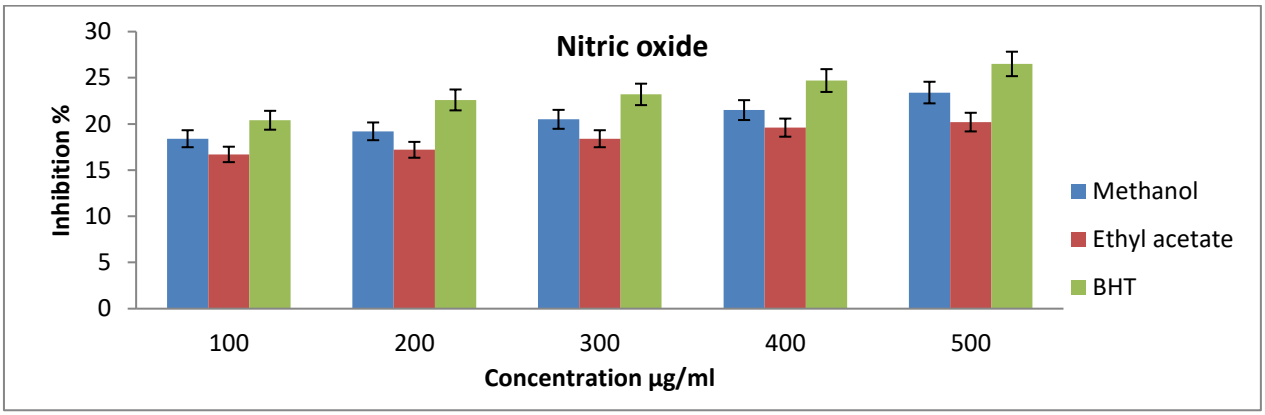


**Figure 4:** Nitric oxide effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

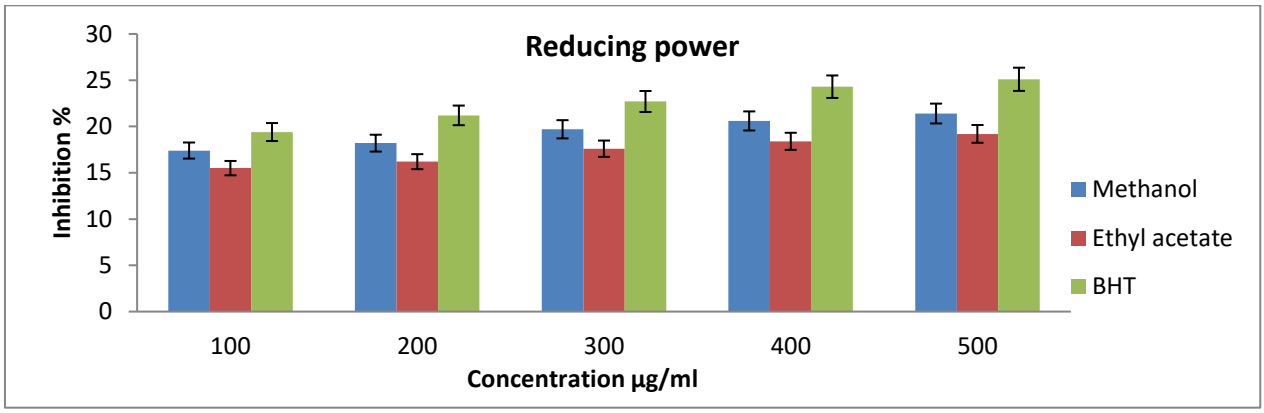


**Figure 5:** Reducing power effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

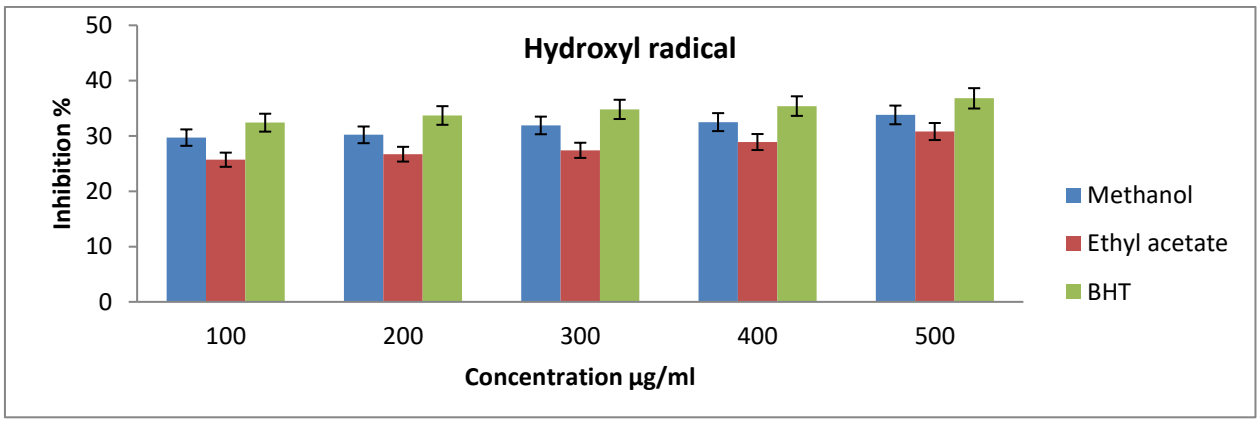


**Figure 6:** Hydroxyl radical effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

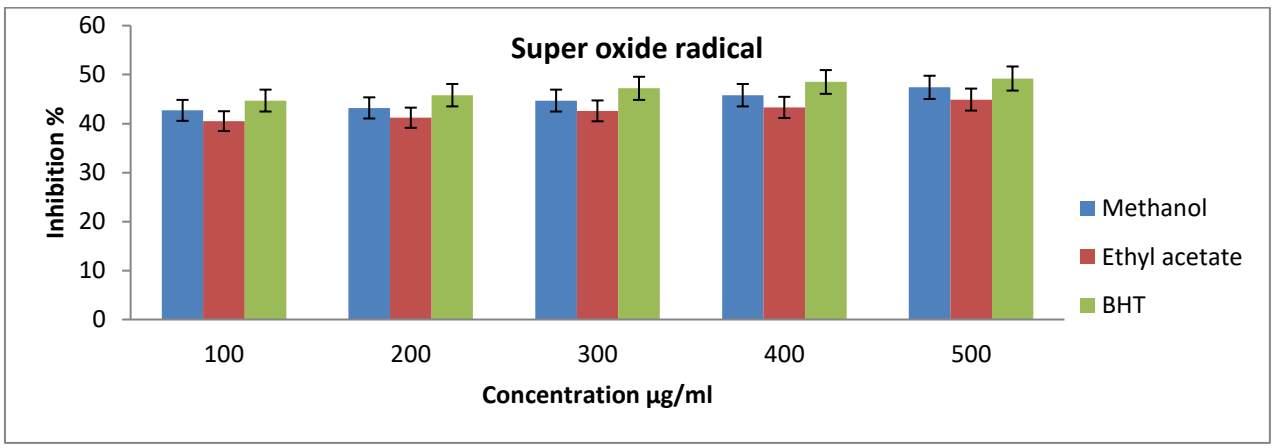


**Figure 7:** Superoxide radical effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

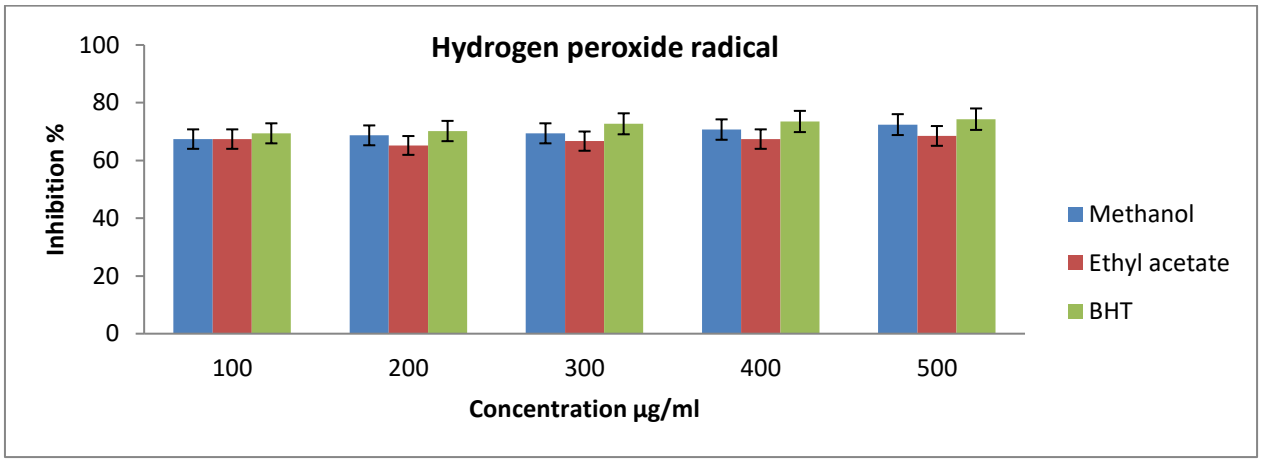


**Figure 8:** Hydrogen peroxide effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

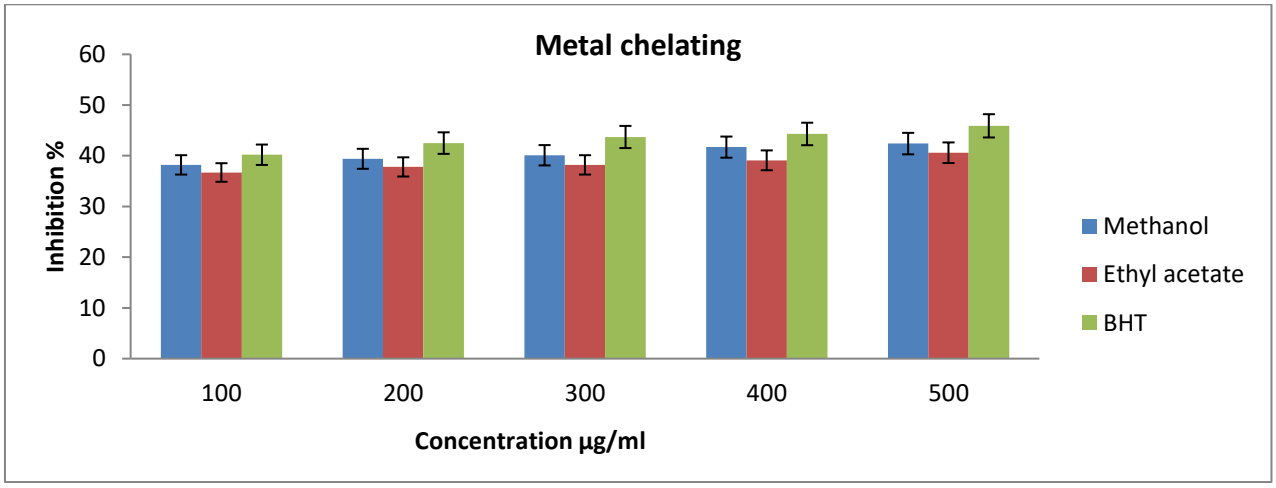


**Figure 9:** Metal chelating effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

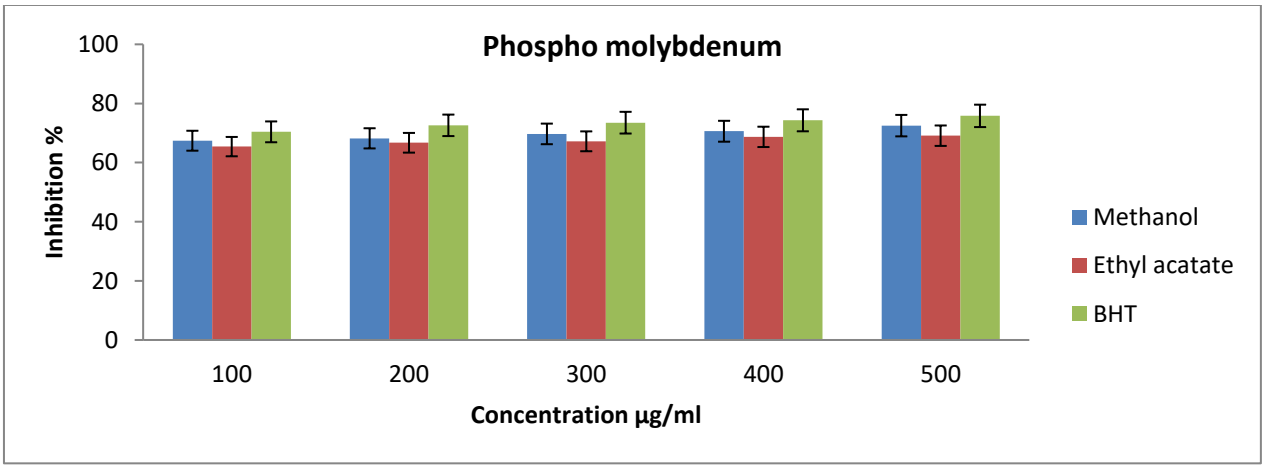


**Figure 10:** Phospho molybdenum effect of Methanol and ethyl acetate leaves extract of *Ocimum basilicum*

## RESULTS

Figure 1 and 2 shows the effect of *O.basilicum* leaves extract methanol, ethyl acetate on the DPPH and ABTS radicals present in the reaction mixtures. The extract at a concentration of 100 -500µg/ml, significantly scavenged of DPPH radicals with an $IC_{50}$ value of 10.26,7.2µg/ml and ABTS radicals having $IC_{50}$ values of 14.2, 10.2µg/ml. BHT values 12.4, 16.5µg/ml.

Figure 3 and 4 shows the effect of the FRAP power of the *O.basilicum* increases extract methanol, ethyl acetate with the increasing concentration was 16.9,14.6µg/ml and the $IC_{50}$ value of BHT was 19.9µg/ml. The scavenging of nitric oxide by *O.basilicum* was increased concentration of 23.4, 20.2 µg/ml of *O.basilicum* 50% of nitric oxide generated by incubation was scavenged. The $IC_{50}$ value of BHT was 26.5µg/ml.

Figure 5 shows the effect the reducing power *O.basilicum* methanol, ethyl acetate was increased in quantity of sample. The *O.basilicum* could reduce the most $Fe^{3+}$ ions, which had a lesser reductive activity than the standard of BHT. The $IC_{50}$ value of *O.basilicum* and BHT was 21.4, 19.2µg/ml and 25.1µg/ml respectively.

The results for hydroxyl scavenging assay are shown in Fig.6. The concentrations for inhibition were found to be 33.8, 30.8 and 36.8µg/ml for the *O.basilicum* and BHT, respectively.

Figure 7 and 8 shows the effect of the superoxide scavenging activity of *O.basilicum* leaves methanol, ethyl acetate extract showed superoxide scavenging activity ($IC_{50}$= 40.4,44.9 µg/ml), *O.basilicum* showed concentration dependent activity and the $H_2O_2$ scavenging effect at a concentration was 72.42, 68.5µg/ml. This was comparable to the scavenging effect on the concentration of BHT was 74.3, 49.2µg/ml.

Figure 9 and 10 shows the effect of the metal chelating activity and phosphomolybdenum reduction of *O.basilicum* methanol, ethyl acetate to the quantity of the sample. The $IC_{50}$ value of *O.basilicum* was 42.4,40.6µg/ml and 45.9 µg/ml as a standard BHT significantly the phosphomolybdenum 72.5 and 69.1µg/ml and 75.8 µg/ml for the *O.basilicum,* BHT.

## DISCUSSION

Phytochemicals, are produced in plants to protect themselves from the environmental stress and infections. Phytochemicals play a preventive role in the treatment of diabetes and cancer [25-27]. Primary metabolites produced in plants are maintained plant cells, while secondary metabolites are responsible for normal growth, development and defense of plants [28]. These compounds are mostly nitrogen-containing alkaloids or nitrogen-deficient terpenoids and phenolics[29]. Flavonoids and phenolic acids are biosynthetically derived from the acetate and shikimate pathways (from phenylalanine or tyrosine) [30,31]. It has been reported that *Ocimum basilicum* L. Contains various compounds such as the flavonoid, alkaloid, phenol and essential oil contains flavonoid compounds with the greatest potential as an antioxidant [32].

The antioxidant property of plant confers their free radical scavenging potential their bioactive components and to understand the mechanism of action of their phytoconstituents[33]. In the present study, *O. basilicum* leaves scavenge DPPH and ABTS radicals in a concentration dependent manner. The amount of DPPH which is reduced may be estimated by observing a decrease in absorbance at 517 nm. ABTS assay involves reduction of the color intensity of ethanolic solution containing pre-formed radical monocation of ABTS which is generated by oxidation of ABTS with potassium persulfate due to the radical scavenging activity of anti-oxidants present in the plants [34]. The change in intensity of the color is directly proportional to the antioxidant efficiency of the compound in plant extract. *O. basilicum* leaves extract at a concentration of 1000 µg/ml, the extract significantly scavenged of DPPH radicals ($IC_{50}$=10.26,7.2 µg/ml) and ABTS radicals ($IC_{50}$=14.2,10.2 µg/ml)

Antioxidants can be explained as reductants, and inactivators of oxidants [35]. Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity.. In this study, we used a FRAP assay because it is quick and simple to perform, and the reaction is reproducible and linearly related to the molar concentration of the antioxidant and FRAP assay was used by several authors for the assessment of antioxidant activity of various food product samples[36, 37]. The reducing power of the *Ocimum basilicum* increases with the increasing concentration was 16.9,14.6 µg /ml.

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is associated with several diseases[38]. *Ocimum basilicum* inhibited nitrite formation in a concentration dependent manner (100-500µg/ml). This may be due to the presence of antioxidant principles in the *Ocimum basilicum* which complete with oxygen to react with nitric oxide. The scavenging of nitric oxide 23.4, 20.2 µg/ml of *Ocimum basilicum* of nitric oxide generated by incubation was scavenged.

The reducing power of the *Ocimum basilicum* was evaluated by the transformation of $Fe^{3+}$ to $Fe^{2+}$ through electron transfer ability, which serves as a significant indicator of its antioxidant activity. Reductions are also reported to react with certain precursors of peroxide, thus preventing peroxide formation[39]. The presence of antioxidant substances in the compound samples causes the reduction of the $Fe^{3+}$ ferric cyanide complex to the ferrous form. Therefore, $Fe^{2+}$can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The $IC_{50}$ value of *Ocimum basilicum* was 21.4,19.2µg/ml respectively.

Hydroxyl radical scavenging capacity of *Ocimum basilicum* is directly related to its antioxidant activity[40]. This method involves in vitro generation of hydroxyl radicals using $Fe^{3+}$ /ascorbate/EDTA/ $H_2O_2$ system using Fenton reaction. The concentrations for inhibition were found to be 33.8, 30.8µg/ml for the *Ocimum basilicum* respectively.

Superoxide radicals generated *in vitro* by the system was determined by NBT photo reduction method. The decrease of absorbance at 560 nm with the plant extract indicates the consumption of superoxide anion in the reaction mixture[41]. *O. basilicum* leaves extract exhibited a maximum of superoxide scavenging activity ($IC_{50}$= 47.4, 44.9 µg/ml).

Hydrogen peroxide is a weak oxidizing agent that inhibits the oxidation of essential thiol (-SH) groups directed by few enzymes. It can probably react with $Fe^{2+}$ and possible $Cu^{2+}$ ions to form hydroxyl radicals[42]. From the results, *Ocimum basilicum* showed concentration dependent activity and the

$H_2O_2$ scavenging effect at a concentration was 72.4, 68.5µg/ml.

Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury[43,44]. The chelating ability of ferrous ions by the *Ocimum basilicum* was estimated by the method. Ferrozine can quantitively form complexes with $Fe^{2+}$. In the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. The metal chelating activity of *Ocimum basilicum* is present 42.4µg/ml and 40.6µg/ml.

The phosphomolybdenum method is based on the reduction of $M_0$ (VI) to $M_0$ (V) by the antioxidant compounds and the formation of green phosphate/$M_0$ (V) complex with the maximal absorption at 695 nm [45]. The $IC_{50}$ value of *Ocimum basilicum* was 72.5µg/ml and 69.1µg/ml .

## CONCLUSIONS

*O.basilicum* leaves is rich in biologically active ingredients and minerals of known pharmacological actions. Free radical potential of the *O.basilium* leaves extract is evident from in vitro antioxidant assays. The antioxidant property might be due to the presence of flavonoids and phenols present in the leaves. Thus, *Ocimum basilicum* leaves can be used for the treatment of free radical mediated diseases such as diabetes mellitus and cancer.

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