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Antioxidant Properties of *Ocimum basilicum* Leaves Extract: An *in vitro* study

Sriram Prasath G[1*], Santhana Bharathi P[1], Subramanian S[2]

[1]*PG and Research, Department of Biochemistry, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai, India*

[2]*Department of Biochemistry, University of Madras, Guindy campus, Chennai, India*

***Corresponding author:** Prasath S, Department of Biochemistry, Dwaraka Doss Goverdhan Doss Vaishnav College Arumbakkam, Chennai, India. E-mail: gsriramprasath@gmail.com*

ABSTRACT

Free radicals are considered as the chief causative factor in the progression of various diseases such as diabetes mellitus and cancer. In order to scavenge superfluous free radicals and maintain the human body, the consumption of dietary antioxidants is essential. Synthetic antioxidants have toxic effects to some extents. Therefore, the intake of natural antioxidants preferentially from plant sources is essential. Ocimum basilicum L (Lamiaceae), a medicinally important plant commonly known as "Holy basil" have been reported to possess a wide range of pharmacological properties. In the present study, an attempt has been made to analyze the antioxidant properties of Ocimum basilicum leaves extract. Phytochemical analysis of the leaves extract revealed the presence of phenols, alkaloids, flavonoids, glycosides, saponins, tannins, phytosterols and triterpenoids. The total phenolic and flavonoid content were found to be 284.72 ± 1.44 mg Gallic acid equivalent and 43.65 ± 0.21 mg quercetin equivalent respectively. The carbohydrate and protein content was found to be 8.15 ± 0.27 mg /g and 4.2 ± 0.32 mg /g of the leaves extract respectively. The leaves extract was found to be antioxidant in nature which is evident from DPPH, ABTS, NO, Superoxide radical scavenging assays. At a concentration of 1000µg/ml, the extract significantly scavenged 84% of DPPH radicals (IC50=586.3 µg/ml) and 79% ABTS radicals (IC50=727.9 µg/ml). O. basilicum leaves extract exhibited a maximum of 81% superoxide scavenging activity (IC50= 604.2 µg/ml) at a concentration of 1000 µg. The extract exhibited a maximum of 83% NO scavenging potential (IC50= 652.60 µg/ml). Thus it can be concluded that Ocimum basilicum leaves extract can be used for the treatment of free radical mediated diseases such as diabetes mellitus and cancer.

Keywords: *Ocimum basilicum;* Phytochemicals; Free radicals; Anti-oxidant property

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 with external environment, a large amount of free radicals are generated in 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 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].

Plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS induced oxidative damage. Synthetic antioxidants have side effects after prolonged use. Hence, natural antioxidants of plant origin especially through foods are essential to eradicate a number of free radical mediated diseases. Although phytotherapy continues, most of the traditional medicinal plants have not received scientific or medical scrutiny. One such medicinal plant, which lacks scientific evidence for its folklore use is *Ocimum basilicum.*

Ocimum basilicum L (Lamiaceae) also commonly known as “Holy basil” has been reported to possess different pharmacological effects. Essential oil of the plant is composed of interesting terpenoids and phenylpropanoids such as eugenol, methyl eugenol, citral, and methyl chavicol [2].The leaves of *O. basilicum* are traditionally used as antispasmodic, carminative, digestive, stomachic, and tonic [3,4]. The merit of the traditional use of *O. basilicum* has been supported by some former studies from the genus Ocimum, providing several biologically active constituents present in the extracts and essential oils of the plants. Since, the complexity and chemical diversity of the compounds present in medicinal herbs play an important role in the discovery and development of new compounds, detecting the naturally occurring constituents in plants seems to be of value to achieve a better understanding of the relation between chemical constituents and biological properties of a medicinal plant.

Figure 1: *O. basilicum* leaves.

Concerning the prior phytochemical reports from O. basilicum, it mainly produces triterpenoids, polyphenols, steroids, and phenylpropanoids some of which, such as basilol, ocimol, basilimoside, rosmarinic acid, hydroxycinnamic acids, oleanolic acid, and betulinic acid, have been proved for having prominent biological properties [5,6]. Ocimum sp., possess bactericidal, anti-inflammatory, antioxidative, antiulcer, antidiarrheal, chemopreventive and hypoglycemic activity [7-12]. The profound medical effects of this herb may be attributed to its antioxidant power due to flavonoids and polyphenols content [13]. In the absence of systematic studies in the literature, the present study was aimed to investigate the antioxidant properties of *Ocimum basilicum* leaves extract.

MATERIAL AND METHODS

Plant material- Identification and authentication

Fresh, green and matured *O. basilicum* leaves were collected from Vyasarpadi, Chennai, Tamil Nadu and identified by a plant taxonomist.

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, which was stored in an airtight brown container at 5°C until further use. The powdered leaves were delipidated with petroleum ether (60-80°C) for overnight. It was then filtered, and soxhalation was performed with 95% ethanol. Ethanol was evaporated in a rotary evaporator at 40-50°C under reduced pressure. The yield was around 13.5% of dry weight.

Preliminary phytochemicals screening

The ethanolic extract of *O. basilicum* leaves were subjected to phytochemical screening for the qualitative analysis of various [14,15].

Quantitative analysis of total polyphenol, flavonoid, carbohydrate and protein content

Total polyphenol content in the ethanolic extract of *O. basilicum leaves* was determined according to the Folin-Ciocalteu colorimetric method [16,17]. Total flavonoid content in the ethanolic extract of *O. basilicum leaves* was determined according to the method of Quettier- Deleu et al., [18] with minor modifications. The carbohydrate content was measured by 3, 5-dinitrosalicylic acid method [19] and the estimation of proteins was carried out by the Lowry (1951) method [20].

In vitro antioxidant assays

The free radical scavenging capacity of the ethanolic extract of *O. basilicum leaves* extract (200-1000 µg/ml) was determined using DPPH, ABTS, Nitric oxide scavenging and superoxide scavenging assays respectively [21-24].

RESULTS

Qualitative analysis of the leaves extract showed the presence of flavonoids, alkaloids, glycosides, phyosterol, phenols, tannins, proteins, saponins, sterols and triterpenes in the leaves extract which is presented in Table 1.

Table 1: Phytochemical analysis of *Ocimum basilicum leaves* extract.

Phytoconstituents	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Phytosterol	+
Diterpenes	-
Triterpenoids	+
Glycosides	+
Anthraquinones	-
Phenols	+

Total phenol content and flavonoid content (Table 2) was found to be 284.72 ± 1.44 mg Gallic acid equivalents, 43.65 ± 0.21 mg quercetin equivalent respectively. The carbohydrate and protein content was found to be 8.15 ± 0.27 mg /g and 4.2 ± 0.32mg /g of the leaves extract respectively.

Table 2: Quantitative analysis of flavonoid, phenolic, carbohydrates and protein content of *Ocimum basilicum* leaves extract

Constituent	Content
Phenolic content	284.72 ± 1.44 mg Gallic acid equivalent
Flavonoid content	43.65 ± 0.21 mg quercetin equivalent.
Carbohydrate content	8.15± 0.27 mg /g of the leaves extract
Protein content	4.2 ± 0.32 mg /g of the leaves extract

Figures 1 and 2 shows the dose dependent effect of *O. basilicum* leaves extract on the percentage inhibition of DPPH and ABTS radicals present in the reaction mixtures. At a concentration of 1000 µg/ml, the extract significantly scavenged 84% of DPPH radicals (IC_{50}=586.3 µg/ml) and 79% ABTS radicals (IC_{50}=727.9 µg/ml).

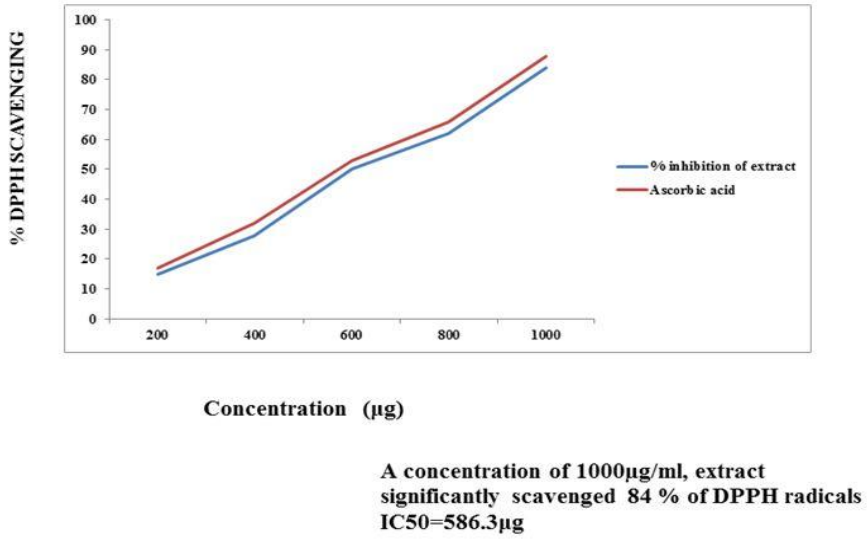

Figure 1: DPPH radical scavenging potential of *Ocimum bascillum* leaves extract.

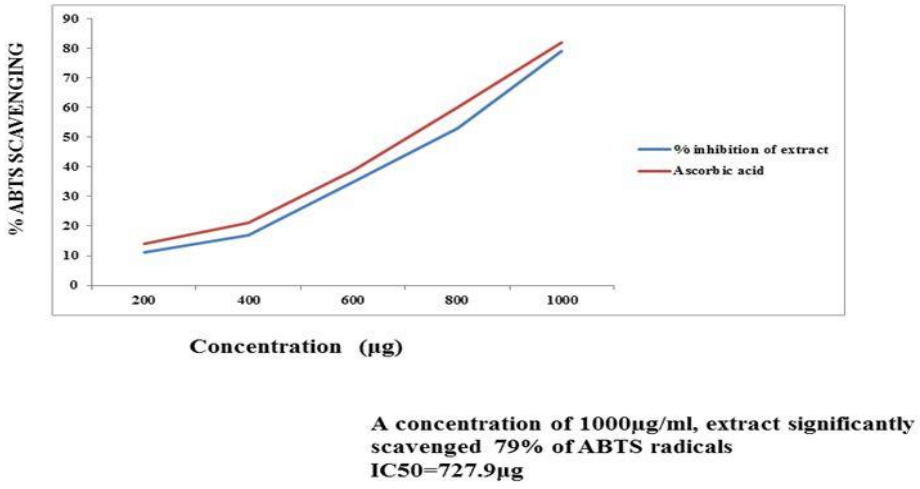

Figure 2: ABTS radicals scavenging potential of *Ocimum basilicum* leaves extract.

The superoxide scavenging activity of *O. basilicum* leaves extract is graphically represented as Figure 3. *O. basilicum* leaves extract showed 81% superoxide scavenging activity (IC_{50}= 604.2 µg/ml) and Nitric oxide scavenging activity (Figure 4) was 83% with IC_{50} value of 652.60 µg/ml.

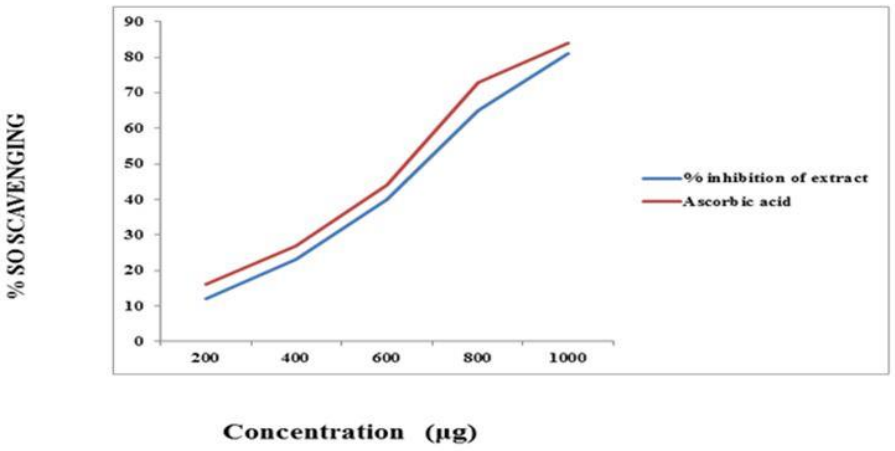

Figure 3: Superoxide radical scavenging potential of *Ocimum basilicum* leaves extract.

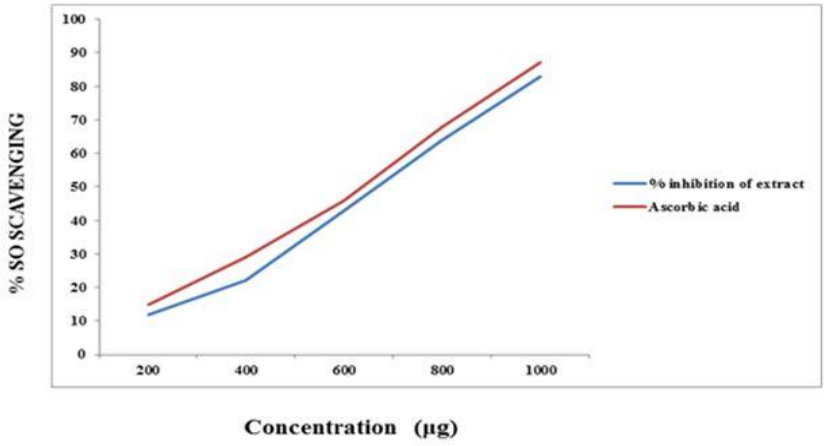

Figure 4: Nitric oxide radical scavenging potential of *Ocimum basilicum* leaves extract.

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 maintains 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 flavonoid, alkaloid, phenol and essential oil contains flavonoid compound with the greatest potential as an antioxidant [32]. *Ocimum basilicum* contains significant amount of protein which is on par with earlier report [33].

The antioxidant property of plant confer their free radical scavenging potential their bio active components and to understand the mechanism of action of their phytoconstituents [34]. In the present study, *O. basilicum* leaves scavenge DPPH and ABTS radicals in a concentration dependent manner. Bioactive compounds (Free radical quenchers) of the plants may react with DPPH which is a purple colored stable free radical and convert it into a colorless α-α-diphenyl-β-picryl hydrazine. 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 [22]. 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 84% of DPPH radicals (IC_{50}=586.3 µg/ml) and 79% ABTS radicals (IC_{50}=727.9 µg/ml)

Superoxide radicals generated *in vitro* by the system was determined by NBT photoreduction method. The decrease of absorbance at 560 nm with the plant extract indicates the consumption of superoxide anion in the reaction mixture [35]. Superoxide radical is converted by SOD to hydrogen peroxide, which produces reactive hydroxyl radicals. *O. basilicum* leaves extract exhibited a maximum of 81% superoxide scavenging activity (IC_{50}= 604.2 µg/ml). Nitric oxide It plays an important role in N-methyl-D-aspartate (NMDA) receptor activation and the induction of significant oxidative stress. NO induced oxidative stress causes lipid peroxidation and neuronal cell death by DNA damage. In the present study nitric oxide scavenging activity was 83% with IC_{50}value of 652.60 µg/ml.

CONCLUSION

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

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