Research Article

Free Radical Scavenging Properties of *Ocimum basilicum Leaves*

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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. Intake of natural antioxidants preferentially from plant sources is most essential to avoid side effects. *Ocimum basilicum* L (*Lamiaceae*), a medicinally important plant commonly known as "Holy basil" found to contain many biological properties. The present study is aimed in evaluating the free radical scavenging potentials of *Ocimum basilicum* leaves extract. The leaves extract contains phytochemicals such as flavonoids, phenols, alkaloids, glycosides, saponins, tannins, phytosterols and triterpenoids. *Ocimum basilicum* is rich in minerals like copper, magnesium, calcium, zinc, sodium, and potassium. The antioxidant nature of the leaves extract was proved from DPPH, ABTS, NO, Superoxide radical scavenging assays, FRAP and TBARS assays. 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.

INTRODUCTION

Oxidative stress is the causative factor which is involved in the onset and progression of diabetes mellitus, cancer, neurodegenerative and cardiovascular disease. Free radicals are generated in human body during metabolic process and exposure with external environment resulting in oxidative stress induced damage on cells or tissues. Detonated production of free radicals cause oxidative stress, thus destroying internal redox balance and causing a variety of free radical mediated diseases[1].

Natural antioxidants of plant origin especially through foods are essential to eradicate a number of free radical mediated diseases[1]. 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 possess different biological effects. The oil of the plant contains eugenol, methyl eugenol, citral, and methyl chavicol [2]. *O. basilicum* leaves are used as antispasmodic, carminative, digestive, stomachic, and tonic [3,4].

O. basilicum produces triterpenoids, polyphenols, steroids, and phenylpropanoids some of which, such as basilol, ocimol, basilimoside, rosmarinic acid, hydroxycinnamic acids, oleanolic acid, and betulinic acid which possess various biological properties[5,6]. Ocimum sp., is shown to exert antibactericidal, antiinflammatory, antioxidative, antiulcer, antidiarrheal, chemopreventive and hypoglycemic properties [7-12]. 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 [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.

O. basilicum leaves

MATERIALS AND METHODS

Plant material- Identification and authentication

Matured *O. basilicum* leaves were selectively removed from the plant in and around areas of Vyasarpadi, Chennai, Tamil Nadu and identified by a plant taxonomist.

Preparation of ash

The *O.basilicum leaves* were shadow dried, finely powdered using electrical grinder. One hundred gram of properly powdered leaves were taken in a viterosil crucible and placed in an electrical muffle furnace overnight maintaining its temperature between 430-450˚ C because the loss of zinc may occur at >450˚ C and loss of potassium occurs if the temperature is too high (>480˚ C). The ash was then removed and dried in vacuum desiccators. The yield of ash in the powdered leaves was found to be 15.00g/100g.

Total Ash

2g of the ground air-dried material was accurately weighed, in a previously ignited and tared crucible

(usually of platinum or silica). The content was spread in an even layer and ignited by gradually increasing the heat to 450°C until it is white, indicating the absence of carbo following by cooling in a dessicator Ash value can be calculated by using formula:-

Ash value = Initial Weight – Final Weight × 100/ Initial Weight

Trace element analysis

2g of ash was digested with a triple acid (nitric acid, sulphuric acid and perchloric acid in the ratio of 11:6:3) for the complete removal of organic content. The digested sample was made up to 100 ml using deionized water and this sample is used for the assay of trace elements through atomic absorption spectroscopy using hollow cathode lamps. The determination of the trace element content of *O.basilicum* was carried out using an atomic absorption spectrometer (GBC-Avanta, Australia).

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 petroleum ether (60-80°C) for overnight. soxhalation was performed with 95% ethanol. Ethanol was 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.

Preliminary phytochemicals screening

Phytochemical screening of the leaves extract was performed according to standard established procedures.[14,15]

Free Radical Scavenging Assays

The *in vitro* anti radical scavenging potential of *O.basilicum leaves* extract (200-1000µg/ml) was determined using DPPH, ABTS, Nitric oxide scavenging and superoxide scavenging assays respectively [16-19] Ferric reducing antioxidant power (FRAP) assay was carried out according to the method of Benzie and Strain (1996)[20]. Thiobarbituric acid reactive substances (TBARS) assay. TBARS assay was conducted according to methods described.[21]

RESULTS

The leaves extract was found to contain of flavonoids, alkaloids, glycosides, phyosterol, phenols, tannins, proteins, saponins, sterols and triterpenes in the leaves extract. The ash content and mineral content is represented in table 1 and 2 respectively. *Ocimum basilicum* is rich in minerals like copper, magnesium, calcium, zinc, sodium, and potassium.

Figure 1 and 2 shows the effect of *O.basilicum* leaves extract on the DPPH and ABTS radicals present in the reaction mixtures. The extract at a concentration of 1000µg/ml, significantly scavenged 84 % of DPPH radicals with a IC_{50} value of 586.3µg/ml and 79 % ABTS radicals having IC_{50} value of 727.9µg/ml.

Table 1: Ash content of *Ocimum basilicum* leaves

Constituent	Content (%)
Total Ash	12 ± 0.20
Water soluble Ash	4.40 ± 0.08
Acid soluble ash	4.55 ± 0.10

Table 2: Mineral Composition of *Ocimum basilicum* Leaves

Element	Concentration (ppm)
Calcium (Ca)	36.60
Iron (Fe)	12.5
Magnesium (Mg)	10
Sodium (Na)	103
Potassium (K)	900
Zinc (Zn)	0.85
Copper (Cu)	0.48

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

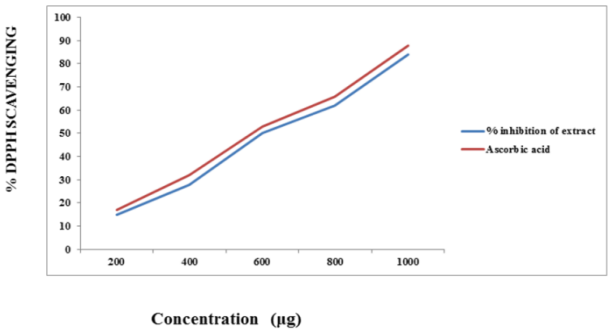
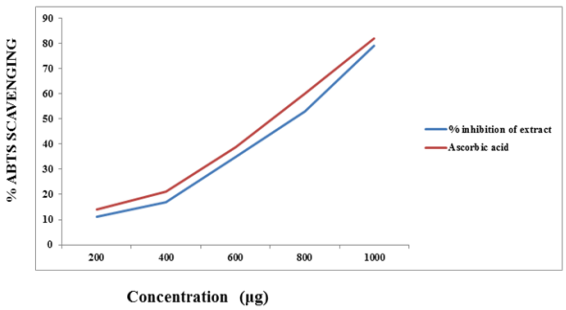


Figure 2: ABTS radical scavenging potential of *Ocimum basilicum leaves* extract



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 baslicum leaves* extract

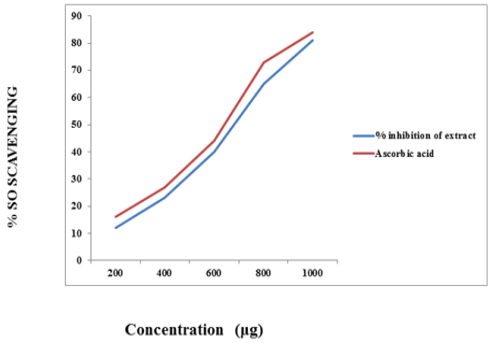


Figure 4 :Nitric oxide radical scavenging potential of *Ocimum basilicum leaves* extract

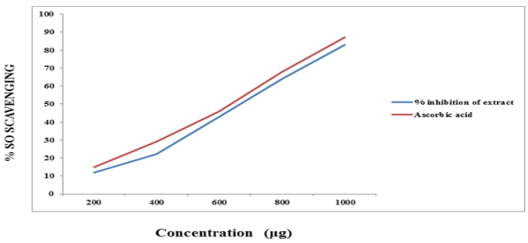
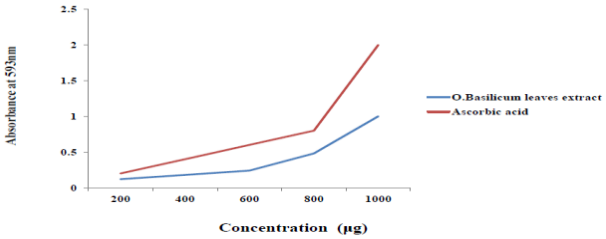


Figure 5: Ferric Reducing Antioxidant Power (FRAP) Assay



Antioxidant activities of the ethanolic extracts of the *O.basilicum* as determined by the FRAP assay. In the FRAP assay the absorbance of *O.basilicum* was found to be 0.12, 0.18, 0.24, 0.48, 1.00 at 200-1000 µg/ml respectively (Figure 5). The percentage inhibition using the TBARS assay ranged from 24.00 to 80.00% for the leaves extract.

DISCUSSION

Total ash includes physiological ash, which is derived from the plant tissue. Non-physiological ash is derived from environmental contaminations such as sand and soil. *O.basilicum* show a variation in contents of compounds according to differences in growing conditions, such as soil type, climate which may change the ash content depending upon presence of various contaminants thus becoming an important parameter of quality assessment.

Ocimum basilicum is rich in minerals like copper, magnesium, calcium, zinc, sodium, and potassium which do play a pivotal role in insulin metabolism. It has been reported that the percentages of minor mineral elements content were (Iron from 0.98 to 4.35 mg/100g), (Zinc from 10.44 to 17.72 mg/100g), (Copper from 0.45 to 3.75 mg/100g) [22] (Mlitan, et al., 2014). A high concentrations of potassium, (483mg/100g); calcium, (460mg/100g); moderate amount of sodium, (159mg/100g); appreciable concentrations of phosphorus, (35.9mg/100g); and (10.5mg/100g) in *Ocimum basilicum* has been reported. Ocimum basilicum also contains has higher concentration of crude protein [23] . The results of the present study are in line with earlier reports.

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 [24-26]. Primary metabolites produced in plants are maintains plant cells, while secondary metabolites are responsible for normal growth, development and defense of plants [27]. These compounds are mostly nitrogen-containing alkaloids or nitrogen-deficient terpenoids and phenolics [28]. Flavonoids and phenolic acids are biosynthetically derived from the acetate and shikimate pathways (from phenylalanine or tyrosine)[29-30]. 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 [31].

In vitro antioxidant assays are used to measure and confer antioxidant activity to plants; however, each of these has its own limitations regarding applicability. In these assays, plants are generally assessed for their function as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators, after which they are classified as primary (chain-breaking) and secondary (preventive) antioxidants. Primary antioxidants act by donating a hydrogen atom, while secondary antioxidants function via binding of metal ions capable of catalyzing oxidative processes and scavenging oxygen, absorbing UV radiation, inhibiting enzymes or decomposing hydroperoxide.

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 [32]. 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 antioxidants present in the plants. 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 1000µg/ml, the extract significantly scavenged 84 % of DPPH radicals (IC_{50}=586.3µg/ml) and 79 % ABTS radicals (IC_{50}=727.9µg/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. 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.
In the present study, the absorbance of *O. basilicum* leaves extract clearly increased which is due to the

formation of the Fe2+- TPTZ complex with increasing concentration as seen. The leaves extract showed a significant antioxidant activity. The high percentage inhibition observed in the TBARS assay indicate the ability of the leaves extract to inhibit linoleic acid peroxidation. The antioxidant activity of the plant extract may be due to the presence of phenolic compounds with redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and as well as potential metal chelators[33]

CONCLUSION

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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REFERENCES

1. Kasote DM, Hegde MV, Katyare SS Mitochondrial dysfunction in psychiatric and neurological diseases: cause(s), consequence(s), and implications of antioxidant therapy. Biofactors., 39(4), 2013, 392-406.
2. Bunrathep S, Palanuvej C, Ruangrungsi N. Chemical compositions and antioxidative activities of essential oils from four ocimum species endemic to Thailand. J Health Res, 21, 2007, 201–203.
3. Rahimi R, Shams-Ardekani MR, Abdollahi M, A review of the efficacy of traditional Iranian medicine for inflammatory bowel disease. World J Gastroenterol., 16(36), 2010, 4504-14.
4. Velázquez C, Calzada F, Torres J, González F, Ceballos G, Antisecretory activity of plants used to treat gastrointestinal disorders in Mexico. J Ethnopharmacol. 103(1), 2006, 66-70.
5. Siddiqui BS, Aslam H, Ali ST, Begum S, Khatoon N. Two new triterpenoids and a steroidal glycoside from the aerial parts of Ocimum basilicum. Chem Pharm Bull (Tokyo), 55(4), 2007, 516-9.
6. Strazzer P, Guzzo F, Levi M. Correlated accumulation of anthocyanins and rosmarinic acid in mechanically stressed red cell suspensions of basil (Ocimum basilicum). J Plant Physiol, 168(3), 2011, 288-93.
7. Chattopadhyay RR, A comparative evaluation of some blood sugar lowering agents of plant origin. Journal of Ethnopharmacology, 67, 1999, 367–372.
8. Klem MA, Nair MG, Sraassburg GM, and Dewitt DL, Antioxidant and cyclooxygenase inhibitory phenolic compounds from Ocimum sanctum Linn. Phytomedicine 7, 2000, 7–13.
9. Koga T, Hirota N, and Takumi K, Bactericidal activities of essential oils of Basil and Sage against a range of bacteria and the effect of these essential oils on Vibrio parahaemolyticus. Microbiological Research, 154, 1999, 267–273.
10. Maity TK, Mandal SC, Saha BP, and Pal M, Effect of Ocimum sanctum roots extract on swimming performance in mice. Phytotherapy Research, 14, 2000, 120–121.
11. Offiah VN and Chikwendu UA, Antidiarrheal effects of Ocimum gratissimum leaf extract in experimental animals. Journal of Ethnopharmacology 68, 1999, 327–330.
12. Uma Devi P, Ganasoundri A, Vindra B, and Srinivasan KK, Radiation protection by the Ocimum flavonoids orientin and vicenin: mechanism of action. Radiation Research, 154, 2000, 455–460.
13. Kaushik G, Satya S, Khandwal RK, and Naik SN, Commonly consumed Indian food materials in the management of diabetes mellitus. Diabetes and Metabolic Syndrome: Clinical Research and Reviews 5, 2008, 31–37.
14. Harborne JB, Methods of extraction and isolation. In: Phytochemical Methods. pp. 60-66, Chapman and Hall, London, 1998.
15. Kokate CK. Practical Pharmacognosy. pp. 107–111, Vallabh Prakashan, New Delhi, India. 2005.
16. Brand-Williams W, Cuvelier ME, Berset C. Use of a free-radical method to evaluate antioxidant activity. Food Sci Technol-Leb. 28, 1995, 25–30.
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26 (9-10), 1999, 1231-1237.
18. Marcocci L, Maguire JJ, Droylefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb. Biochem. Biophys. Res. Commun. 201, 199, 748-755.
19. Fontana M., Mosca L, and Rosei M A,. Interaction of enkephalines with oxyradicals. Biochemical Pharmacology, 61, 2001, 1253-1257.
20. Benzie IF1, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 239(1), 1996 Jul 15, 70-6.
21. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. J. Food Sci., 58, 1993. 1407-1410.
22. Mlitan AM, Sasi MS, Alkherraz AM. Proximate and Minor Mineral Content in Some Selected Basil Leaves of Ocimum gratissimum L, in Libya. International Journal of Chemical Engineering and Applications, 5(6), 2014, 502-505
23. Shuaib, OR., Adeniran, OI, Musah, M, Yerima H, Sani H, Amusat, Comparative Nutritional And Anti-Nutritional Analysis Of Ocimum grattissimum and Ocimum basilicum. Academia Arena, 7(7), 2015, 77-81.
24. Malireddy S, Kotha SR, Secor JD, Gurney TO, Abbott JL, Maulik G, Maddipati KR, Parinandi NL. Phytochemical antioxidants modulate mammalian cellular epigenome: implications in health and disease. Antioxid Redox Signal 17(2), 2012, 327-339.
25. Doughari JH, Dr.Venketeshwer Rao (Ed.). Phytochemicals: Extraction Methods, Basic Structures and Mode of action as Potential Chemotherapeutic Agents, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, 2012, 978-953.
26. Mamta Saxena, Jyoti Saxena, Rajeev Nema, Dharmendra Singh, and Abhishek Gupta, Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry, 1, 2013, 2278- 4136.
27. Kliebenstein DJ, Osbourn A. Making new molecules - evolution of pathways for novel metabolites in plants. Curr Opin Plant Biol. 15, 2012, 415–23.
28. Patra B, Schluttenhofer C, Wu Y, Pattanaik S, Yuan L. Transcriptional regulation of secondary metabolite biosynthesis in plants. Biochim Biophys Acta. 1829, 2013, 1236–47.
29. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther, 96, 2002, 67-202.
30. Dewick PM. The Shikimate Pathway: Aromatic Amino Acids and Phenylpropanoids, in Medicinal Natural Products: A Biosynthetic Approach, 3rd Edition. Chichester, UK: John Wiley & Sons, Ltd; 2009.
31. Shuaib OR, Adeniran OI, Musah M, Yerima H, Sani H, Amusat K. Comparative Nutritional And Anti-Nutritional Analysis Of *Ocimum grattissimum* and Ocimum basilicum. Academia Arena, 7(7), 2015, 77-81,
32. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. Analyst. 127, 2002, 183–98.
33. Pietta PG, Flavonoids as antioxidants. J. Nat. Prod., 63, 2000, 1035-1042.

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