Research Article

Antioxidant and free radical scavenging activity of *Ocimum basilicum* – An *in vitro* study

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ABSTRACT

Aim: Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. They play an important role in inhibition and radical scavenging, thus providing protection against diseases. A great number of medicinal plants contain chemical compounds which possess antioxidant activities. Natural antioxidants are mainly phenolic compounds that may exist in all parts of the plants. Antioxidant potential of the ethanolic extract of *Ocimum basilicum* was studied using different *in vitro* free radical scavenging models such as 2, 2 Diphenyl-1-picryl hydrazyl radical (DPPH) and hydrogen peroxide. The DPPH results have been compared with the standard ascorbic acid. The extract showed good dose-dependent free radical scavenging property in both the models used in this study. **Materials and Methods:** The free radical scavenging activity of the ethanolic extract of *O. basilicum* was determined using DPPH using ultraviolet-spectrometry at 517 nm. The DPPH solution was prepared in 95% methanol. **Conclusion:** *In vitro,* antioxidant effects of *O. basilicum* were tested using DPPH and catalase method. The extract *O. basilicum* expressed the strongest antioxidant activity. The extracts of *O. basilicum* leaves showed good free radical scavenging activity. The broad range of antioxidant activity of this extract indicates the potential of the plant as a source of natural antioxidants with potential application to reduce oxidative stress and consequent health benefits. The plant may thus be exploited in the pharmaceutical and food industries. The study, therefore, not only reveals the spices as accessible reservoirs of natural antioxidants to be utilizes nutritionally and pharmaceutically but also very importantly, provides good scientific justification for increased domestication of these plants.

KEY WORDS: 2, 2 Diphenyl-1-picrylhydrazyl radical, Antioxidant activity, Free Radical, Scavenging, Hydrogen peroxide, *Ocimum basilicum*

INTRODUCTION

Reactive oxygen species (ROS) exert oxidative damaging effects by reacting with nearly every molecule found in living cells including protein, lipid, amino acids, and DNA if excess ROS are not eliminated by the antioxidant system. They play important roles in aging and the pathogenesis of age-related disorders such as cancer, hypertension, atherogenesis, Alzheimer's disease, and Parkinson's disease. Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables.[1] They are also available as dietary supplements. Well-known antioxidants include enzymes and other substances, such as Vitamin C, Vitamin E, and beta carotene, which are capable of counteracting the damaging effects of oxidation. Different parts such as seeds, leaves, and bark of stem and root are known to contain substantial amounts of phytoconstituents such as phenolics, flavonoids, and tannins having the ability to inhibit the free radicals. The significance of antioxidants in preventive medicine is well known. Increasing interest has been devoted to searching for new, effective natural antioxidants due to their beneficial health effects. Antioxidants reduce the oxidative stress in cells and are, therefore, useful in the treatment of many human diseases such as cancer, cardiovascular, and inflammatory diseases.[2] Many plant species have similar antioxidant potentials as that of synthetic antioxidant without potential side effect and are used as an alternative in the food processing industry and in preventive medicine.

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The plant Tulsi or Holy Basil (Botanical name *Ocimum sanctum*) belongs to family Lamiaceae. It is a tropical plant which grows as weed and also cultivated. Tulsi is worshipped by Hindus and is an important symbol of Hindu religion. It is a very common sight to find Tulsi Vrindavan (A special structure where Tulsi is grown) in houses of Hindus. The Tulsi shrub is an erect plant which grows to a height of 50–60 cm tall. It has hairy stems, opposite, ovate leaves, and purple flowers. Leaves have a strong scent. It is light to digest and dries tissue secretions. It can penetrate deep tissues and has anthelmintic properties. Due to these properties, it normalizes kapha and vata. Leaves, flowers, seeds, and roots of Tulsi are used in ayurvedic preparations. Some of the main chemical constituents of Tulsi are: Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene (about 8%), β-elemene (c.11.0%), and germacrene D (about 2%).[3]

Tulsi has anti-inflammatory properties as it reduces vata. Hence, its external application on swollen parts helps to diminish swelling and pain.[4] Tulsi helps in many skin disorders. It is effective in skin rashes, insect bites, and itching. Leaves of this plant are effectively used in ringworm infections and leukoderma. Tulsi leaves act as a nervine tonic and help to sharpen memory.[5] Paste and Juice of Tulsi leaves help to reduce acne, pimples, and scars. Crushed leaves of Tulsi are very effective in fever, cough, bronchitis, and other diseases of lungs. It helps in expectoration of excess mucous secretion. Tulsi acts as a cardiac tonic and purifies the blood.[6] The *O. sanctum* L. has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic, and diaphoretic actions.[7]

Uses of *Ocimum basilicum*

Basil (*O. basilicum* L.) is aromatic herbs that are used extensively to add a distinctive aroma and flavor to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics. Conventionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunction. The antioxidant activities of basil and thyme have been investigated using various model systems and assays. The antioxidant activity of the ethanol extract of basil (*O. basilicum* L.) was investigated by electrochemical measurements. In the study of essential oils produced from various cultivars of *O. basilicum* L., Linalool (21.1–33.8% of total quantified volatile compounds), estragole (35.9–56.2%), eugenol (1.12–4.36%), and 1,8-cineole (3.40–4.37%) were also determined as major constituents. Major aroma compounds found in volatile extracts of basil exhibited varying amounts of antioxidative activity. In particular, eugenol, thymol, carvacrol, and 4-allylphenol were found in basil and thyme, exhibited potent antioxidant activity, comparable to the known antioxidants, BHT, and a-tocopherol. Considering the abundance of these aroma chemicals in natural plants, the total activity may be comparable, or more, than those of known antioxidants. Furthermore, ingestion of these aroma compounds may help to prevent *in vivo* oxidative damage, such as lipid peroxidation, which is associated with cancer, premature aging, atherosclerosis, and diabetes.

MATERIALS AND METHODS

Measurement of the Antioxidant Activity

2, 2 Diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging test: The free radical scavenging activity of the ethanolic extract of *O. basilicum* was determined using DPPH using ultraviolet-spectrometry at 517 nm. The DPPH solution was prepared in 95% methanol.[8,9] The extract was mixed with 95% methanol to prepare the stock solution (10 mg/100 ml). From the stock solution 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml were taken in five test tubes and diluted with the same solvent to get a final concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml, respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing 1 ml of the test extract and after 30 min, the absorbance was taken at 517 nm using spectrophotometer. A similar procedure was repeated with distilled water instead of extract which serves as a control. Ascorbic acid was used as a standard. 95% methanol was used as blank. All the tests were performed in triplicate to avoid test error. Percentage scavenging of the DPPH free radical was measured using the following equation.

$$\%\ \text{of DPPH radical scavenging} = \frac{\left(\begin{array}{l}\text{Absorbance of control}\\ -\ \text{Absorbance of test sample}\end{array}\right)}{(\text{Absorbance of control})} \times 100$$

Hydrogen Peroxide Scavenging Activity

The ability of plant extracts to scavenge hydrogen peroxide is determined by taking 0.5 ml of hydrogen peroxide (1 ml of 30% of hydrogen peroxide was made up to 45 ml with distilled water), 1 ml of sodium phosphate buffer pH 7.4, 0.01 m, w/v (mixing 30 ml of solution A-156 mg of sodium dihydrogen phosphate was dissolved in 100 ml of distilled water; and with 70 ml of solution B-178 mg of disodium hydrogen phosphate was dissolved in 100 ml of distilled water), and 0.4 ml water. 0.1 ml of the sample was added to initiate the reaction. 2 ml dichromate acetic acid

Table 1: 2, 2 Diphenyl-1-picrylhydrazyl radical radicals scavenging activity of ascorbic acid and ethanolic extract of *Ocimum basilicum*

Concentration of the extract (µg)	Scavenging activity of ascorbic acid (%)	Scavenging activity of the extract (%)
20	40.32	18.36
40	55.36	21.34
60	78.15	40.78
80	90.18	76.91
100	98.10	81.25

Table 2: Hydrogen peroxide scavenging activity of the ethanolic extract hawthorn berries

Concentration of the extract(µg)	Scavenging activity of the extract %
20	26.75
40	40.28
60	60.46
80	69.39
100	78.35

reagent (Dichromate acetic acid – 5% potassium dichromate with glacial acetic acid in ratio 1:3) was added after 15, 30, 45, and 60 s to arrest reaction to the control tubes. The tubes were then heated for 10 min allowed to cool and the green color developed was read at 240 nm using a spectrophotometer. Extract (20–100 µg/ml) in distilled water is added to hydrogen peroxide and the absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.[10,11] The percentage of hydrogen peroxide scavenging is calculated as follows [Tables 1 and 2].

$$\% \text{ scavenged } (H_2O_2) = (A0–A1/A0) \times 100.$$

Where; A0 is the absorbance of control and A1 is the absorbance of test.

RESULTS AND DISCUSSION

DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. In this analysis, the scavenging behavior of the ethanolic extract was similar to that of ascorbic acid. The DPPH radical scavenging activity of ascorbic acid and ethanolic extracts increased in a dose-dependent manner. At a concentration of 100ug/ml both ethanolic extract and standard ascorbic acid showed 81.25% and 98.10% antioxidant activity by DPPH radicals scavenging assay.

Hydrogen peroxide (H_2O_2) is a byproduct of respiration and is made in all living cells. Hydrogen peroxide is harmful and must be removed as soon as it is produced in the cell. Cells make the enzyme catalase to remove hydrogen peroxide. Different plant materials show very different amounts of catalase activity and the most metabolically active tissues show the greatest activity. Hydrogen peroxide scavenging depends on the phenolic content of the extract which can donate electrons to H_2O_2 thus neutralizing it into water. The ethanolic extract of the Hawthorn berries was capable of scavenging H_2O_2 in a dose-dependent manner. Basil leaf (*O. basilicum* L.) contains various compounds such as flavonoid, alkaloid, phenol, and essential oil, so it needs to be fractionated to find out the flavonoid compound with the greatest potential as an antioxidant. This research was aimed to know the chemical compound, antioxidant potential of the ethanolic extract and ethyl acetate fraction from basil leaf. The basil leaf was extracted by maceration using ethanol 70%. The crude extract was fractionated with ethyl acetate.

The ethanolic extract and ethyl acetate fraction were screened of phytochemical content including identification of flavonoids, alkaloids, and polyphenolics. Phytochemicals are produced in plants to protect themselves from environmental stress and infections. Phytochemicals play a preventive role in the treatment of diabetes and cancer. Primary metabolites produced in plants are maintains plant cells, while secondary metabolites are responsible for normal growth, development, and defense of plants. These compounds are mostly nitrogen-containing alkaloids or nitrogen-deficient terpenoids and phenolics. Flavonoids and phenolic acids are biosynthetically derived from the acetate and shikimate pathways (from phenylalanine or tyrosine). It has been reported that *O. basilicum* L. contains various compounds such as flavonoid, alkaloid, and phenol and essential oil contains flavonoid compound with the greatest potential as an antioxidant.[12]

O. basilicum contains a significant amount of protein which is on par with the earlier report.

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic antioxidant to prevent the free radicals implicated diseases which can have serious effects on the cardiovascular system, either through lipid peroxidation or vasoconstriction.

CONCLUSION

In vitro, antioxidant effects of *O. basilicum* were tested using DPPH and CATALASE method. The extract *O. basilicum* expressed the strongest antioxidant activity. The extracts of *O. basilicum* leaves showed good free radical scavenging activity. The broad range of antioxidant activity of this extract indicates the potential of the plant as a source of natural antioxidants with potential application to reduce oxidative stress and consequent health benefits. The plant may thus be exploited in the pharmaceutical and food industries.

The study, therefore, not only reveals the spices as accessible reservoirs of natural antioxidants to be utilized nutritionally and pharmaceutically but also very importantly, provides good scientific justification for increased domestication of these plants.

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