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Research article

EVALUATION OF ANTIOXIDANT ACTIVITY OF *OCIMUM TENUIFLORUM*, AN IMPORTANT MEDICINAL HERB.

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ABSTRACT: Objective - The present study was carried out to evaluate the antioxidant activities and total phenolics assay of *Ocimum tenuiflorum*. **Methods -** The methanolic and ethanolic leaf extracts of this plant have been analyzed for their free radical-scavenging activity in different *in vitro* systems, e.g. DPPH radical scavenging activity, total phenolic content, FRAP radical scavenging activity etc. The free radical scavenging activities were compared with standard antioxidants like ascorbic acid. **Results-** The highest phenolic content was estimated with 3.66g/100gm. *Ocimum tenuiflorum* extract through Ethanol: water (70:30) solvent type shows high inhibition of DPPH activity. The highest total antioxidant activity of the extracts was found to be 211.5 Fe (II)/g for extracts through Ethanol: water (70:30) solvent type. **Conclusions -**The present study reveals that *O. tenuiflorum* would exert several beneficial effects by virtue of their antioxidant activity and could be used in drug formulation.

Key words- *Ocimum tenuiflorum*, DPPH, Total phenolic content, FRAP radical scavenging activity.

INTRODUCTION

Tulsi, the “Queen of Herbs", is the most sacred herb of India. *Ocimum* belongs to the Lamiaceae family, which has close to 252 genera and 6700 species [1].There are three important varieties of Tulsi namely, Rama Tulsi (*Ocimum sanctum*), Krishna Tulsi (*Ocimum tenuiflorum*) and Vana Tulsi (*Ocimum gratissimum*). Leaves have petioles and are ovate up to 5cm long, usually slightly toothily. The flowers are purplish in elongate racemes in close chorals. The two main morph types cultivated in India and Nepal are green-leaved and purple-leaved. Tulsi is cultivated for religious and medicinal purposes and for religious and medicinal purposes, and for its essential oil.

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins and carbohydrates for energy. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts and immune system decline and brain dysfunction [2]. Luckily, free radical formation is controlled naturally by various beneficial compounds known as “antioxidants”. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being.

MATERIALS AND METHODS

Collection of plant material

Wild variety of *Ocimum tenuiflorum* collected by uprooting method form Regional Plant Resource Centre, Bhubaneswar, Odisha, India and maintained in the green house. The species identification was examined by comparing its morphological features and microscopic examination of its anatomy.

Preparation of leaf extract by methanol & ethanol

The *Ocimum tenuiflorum* leaves were cut in to small pieces for extraction crude extract. Four solvent systems were used for the (extraction A): methanol: water (70:30), (extraction B) methanol: water (50:50), (extraction C) ethanol: water (70: 30) and (extraction D) ethanol: water (50:50). 200 g of the fresh leaves were macerated in about 1200 ml four different solvents for 72 hours. The extracts were filtered, respectively, first through cotton wool, then Whatman filter paper no. 42 (125 mm). The solvents were completely removed by rotary evaporator and further removal of water was carried out by freeze drying. The dry extracts were weighed, respectively, stored in clean sample bottles.

Determination of total phenolic content:

The total phenolics content (TPC) of the plant extracts was determined spectrophotometrically using Folin-Ciocalteau's reagent. 50 µL of the samples in triplicate was added into the test tubes followed by 1.5 mL of 2N Folin-Ciocalteau reagent (diluted 10 times) and 1.2 mL of 20% sodium carbonate [3]. The contents of the tubes were mixed thoroughly and stored at dark for 30 min. Phenols react with phosphomolibdic acid of Folin-Ciocalteau's reagent in alkaline medium and produce blue colored complex, that could be measured at 765 nm and expressed as mg Gallic acid per gm of plant material with Gallic acid as the standard.

Determination of DPPH radical scavenging activity:

Scavenging activity of *Ocimum tenuiflorum* against DPPH radicals was assessed. Briefly, 0.1 mM DPPH-methanol solution was mixed with 1 ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30 min at 25º C in dark, the decrease in the absorbance at 517nm was measured [4]. Control contained methanol instead of antioxidant solution while blanks contained methanol instead of DPPH solution in the experiment. Ascorbic acid is as positive controls. The inhibition of DPPH radicals by the samples was calculated according to the following equation:

$$\text{DPPH scavenging activity}(\%) = \left[\frac{1-(\text{absorbance of the sample-absorbance of blank})}{\text{absorbance of the control}}\right] \times 100$$

Determination of FRAP radical scavenging activity:

A modified method of Benzie and Strain [5] was adopted for the ferric reducing antioxidant power (FRAP) assay. It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue colour which can be read at 593µnm. 1.5 mL of freshly prepared FRAP solution, containing 25µmL of 300 mM acetate buffer pH 3.6, 2.5µmL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40µmMµHCl, and 2.5µmL of 20 mM ferric chloride {$FeCl_3 \cdot 6H_2O$} solution, was mixed with 1µmL of the extracts, and the absorbance was read at 593 nm. The standard curve was linear between 100 and 500μM $FeSO_4 \cdot 7H_2O$. Results are expressed in μM Fe (II)/g dry plant material and compared with that of ascorbic acid.

Statistical analysis

The entire assays were done in triplicate for all the treated plants extracted under different solvents. The data obtained was subjected to statistical.

RESULTS

Determination of total phenolic content in different solvent:

Phenolics content of the different extracts of *Ocimum tenuiflorum* was determined. Total phenolic content was obtained from the regression equation of the calibration curve of gallic acid (Fig-1) and expressed as gallic acid equivalent (GAE). The phenolic content of the leaves extracts are as follows: 4.33, 2.10, 3.16 and 1.88 g/100g of plant material for extracts A, B, C, and D respectively.

Determination of DPPH radical scavenging activity content in the different solvents:

In the present study several free radical scavenging activities of *Ocimum tenuiflorum* extracts of leaf and stem were evaluated. DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts, thus it was considered important to screen the *Ocimum tenuiflorum* extracts for antioxidant activity against DPPH radical. In this study, *Ocimum tenuiflorum* extract through Ethanol: water (70:30) solvent type shows high inhibition of DPPH activity shows that 73% in leaf extract (Fig-2). None of the *Ocimum tenuiflorum* extracts were as effective DPPH scavengers as the positive control (99.8 %).

Determination of FRAP radical scavenging activity content in different solvents:

The positive control ascorbic acid had a value of 287μM The total antioxidant activity of the extracts was 197.3, 162.3, 211.5 and 178 Fe(II)/g for Extracts A, B, C and D respectively (Fig- 3). The reducing ability of the leaves extracts are in the order: Extract D > C > B > A.

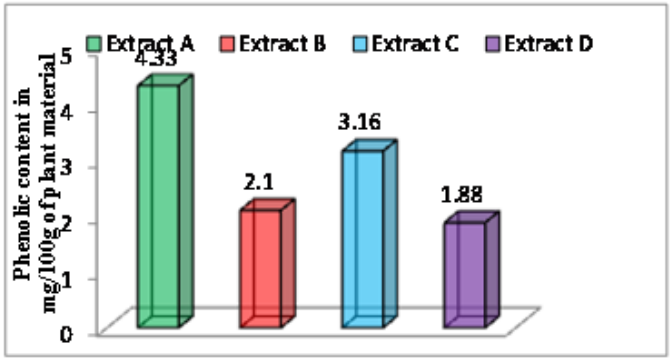

Fig- 1: Phenolic content of leaf extracts in *O.tenuiflorum* from different types of solvent.

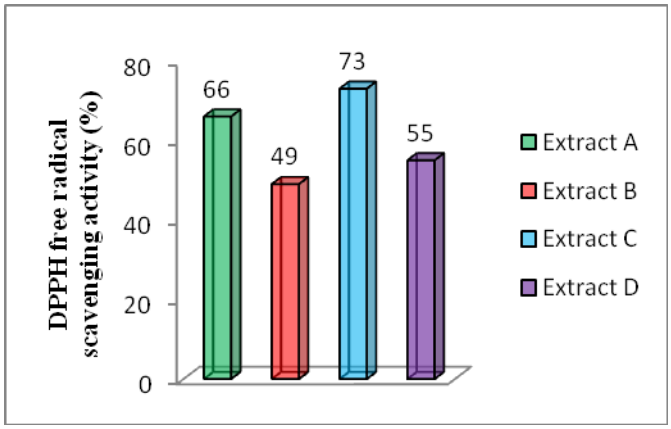

Fig-2: DPPH free radical scavenging activity of different solvents of *O. tenuiflorum* leaf extracts.

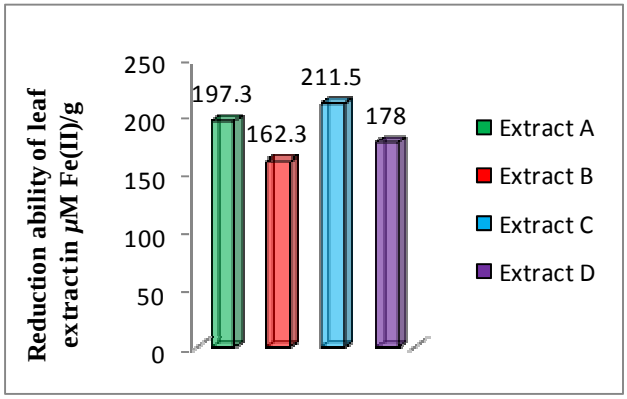

Fig-3: Reduction ability of leaf extracts from different solvent in µM Fe (II)/g.

DISCUSSION

Determination of total phenolic content in different solvent:

Total phenolic content was obtained from the regression equation of the calibration curve of gallic acid. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties. According to our study, the high phenolic content in of *O. tenuiflorum* can explain its high free radical scavenging activity. The phenolic content of the leaves extracts are as follows: 4.33, 2.10, 3.16 and 1.88 g/100g of plant material for Extracts A, B, C, and D, respectively. Phenolic content of *Ocimum gratissimum* was reported [6].

Determination of DPPH radical scavenging activity content in the different solvents:

In the present study several free radical scavenging activities of *Ocimum tenuiflorum* extracts of leaf and stem were evaluated. DPPH assay method is based on the reduction of methanol is solution of the colored free radical, DPPH, by a free-radical Scavenger. DPPH, a protonated radical, has characteristic absorbance maxima at 517 nm, which decreases with the scavenging of the proton radical. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants [7,8].The stable free radical DPPH has been widely used to test the free radical-scavenging ability of various dietary antioxidants [9].Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy. As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. It was repoted that *O. gratissium* extract was the most potent scavenger (81.1%) while *O. americanum, O .minimum, O. citriodorum, O. kilimandscharicum, O. grandiflorum, O. lamiifolium* and *O. selloi* had significantly lower scavenger activity as 77.4 %, 70.1 %, 60. 6 % 56.2 %, 51.3 %, 46.2 % and 42.4 % respectively [9]. In this study, *Ocimum tenuiflorum* extract through Ethanol: water (70:30) solvent type shows high inhibition of DPPH activity shows that 73% in leaf extract (Fig-2). None of the *Ocimum tenuiflorum* extracts were as effective DPPH scavengers as the positive control (99.8 %). The results of DPPH-free radical scavenging assay suggest that the extracts are capable of scavenging free radicals via electron (or) hydrogen donating mechanisms and thus should be potent enough to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices. The scavenging effect of the leaves extracts and standards on the DPPH radical decreased in the following order: Ascorbic acid > Extract D > Extract A > Extract B > Extract C.

Determination of FRAP radical scavenging activity content in different solvents:

FRAP (Ferric reducing antioxidant power) is one of the most rapid test and very useful for routine analysis. The antioxidant activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2, 4, 6-tri-(2-pyridyl)-s-triazine) and $FeCl_3 \cdot 6H_2O$. It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III) TPTZ) complex to ferrous tripyridyltriazine (Fe (II) TPTZ) at a low pH. (Fe (II) TPTZ) has an intensive blue color which can be read at 593 nm. The reducing ability of the extracts was in the range of 175–281 μM Fe (II)/g. The ethanol: water extract of the dried leaves had a comparable reducing power as the standard ascorbic acid used. The reducing ability of the leaves extracts are in the order: Extract D > C > B > A.

CONCLUSION

The extracts of *Ocimum tenuiflorum* leaves showed good free radical scavenging activity. The ethanol: water extract of the fresh leaves showed the best results. The broad range of antioxidant activity of this extract indicates the potential of the plant as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress and consequent health benefits. The methanol extract of the fresh leaves also had the highest amounts of polyphenols. It can thus be concluded that the leaves of *Ocimum tenuiflorum* possess more antioxidant activity and it also depends upon which types of solvent is used for extraction. Though the ethanol: water extract of the dried leaves showed the highest antioxidant activity, the methanol extract possesses higher amounts of plant phenol which are also known to be responsible for the antioxidant activity of most of the plants. The plant may thus be exploited in the pharmaceutical and food industries.

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