Current Journal of Applied Science and Technology

35(1): 1-11, 2019; Article no.CJAST.48197
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Phytochemical Analysis of *Ocimum* Spp. - An Important Medicinal Plant

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Authors' contributions

This work was carried out in collaboration among all authors. Authors Kiran and PK designed the study, performed biochemical as well as statistical analysis, tabular calculation, graphical design, wrote the protocol and first draft of the manuscripts. Authors SK and AK managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2019/v35i130162
Editor(s):
(1) Dr. Tushar Ranjan, Department of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour (Bhagalpur)-813210, Bihar, India.
Reviewers:
(1) Ramaiah Maddi, Hindu College of Pharmacy, India.
(2) S. Parasuraman, AIMST University, Malaysia.
(3) Mustafa Sevindik, Akdeniz University, Turkey.
Complete Peer review History: http://www.sdiarticle3.com/review-history/48197

Original Research Article

Received 20 January 2019
Accepted 30 March 2019
Published 26 April 2019

ABSTRACT

The antioxidant activities of four genotypes of basil species were selected for biochemical studies, *viz.*, *Ocimum sanctum* L. (Rama Tulsi), *Ocimum tenuiflorum* L. (Krishna Tulsi), *Ocimum gratissimum* L. (Van Tulsi), and *Ocimum basilicum* L. (Babui Tulsi*)* for the antioxidant properties. *Ocimum* L. is considered useful in the treatment of respiratory system disorders, reduction of blood glucose level and is regarded as an 'adaptogen' or anti-stress agent. The phytochemical analysis of the plant leaves extract to the estimation of standard biochemical methods such as peroxidase, superoxide dismutase (SOD), polyphenol oxidase (PPO), catalase activity, antioxidant, flavonoids, total phenols, protein as well as total carbohydrates, starch and reducing and non-reducing sugar. The biochemical studies revealed that the starch (98 mg/gfw), the content was found to be higher in

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O. gratissimum. Similarly the other parameters such as total sugar (159 mg/gfw), non-reducing sugar (61mg/gfw), Peroxidase (142 U/mL), Catalase (188 U/mL), SOD (92.47 U/mL), PPO (462 U/mL) were found to be higher in *O. sanctum.* On the other hand, soluble protein (62 mg/gfw), total flavonoids (325 mg/gfw) and total flavonols (486 mg/gfw) were found to be higher in *O. tenuiflorum*. Total antioxidants (136.45 mg/gfw), reducing sugar (108 mg/gfw) and phenol (479 mg/gfw) were found to be higher in *O. basilicum.* The result shows that the best genotype among the four follows the trends *O. sanctum* > *O. basilicum* > *O. tenuiflorum* > *O. gratissimum.* On the basis of the result, the two genotypes can be recommended for medicinal purpose in the pharmaceutical application.

Keywords: Ocimum; antioxidant; phenols; flavonols.

1. INTRODUCTION

Ocimum is a large genus belonging to the Lamiaceae family, *Ocimum sanctum* L. commonly called Holy Basil or Queen of herb having economic and medicinal value. The genus *Ocimum* includes 35-150 species of annual and perennial herbs and shrubs native in Asia, Africa, South and Central America, but widely distributed around the world. *O. basilicum* (sweet basil) is native to Asia. The leaves contain essential oil, which contains eugenol, eugenal, carvacrol, methyl-chavicol, limatrol, and caryophyllene. The seeds contain oil composed of fatty acids and sitosterol. The roots contain sitosterol and three triterpenes A, B and C. Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum,* has been found to be largely responsible for the therapeutic potentials of Tulsi.

Sweet basil (*O. basilicum*) and Holy basil (*O. sanctum*) are the most widely grown basil species in the World either for the fresh market or for essential oil production [1]. *O. americanum, O. basilicum, O. sanctum,* and *O. tenuiflorum* are among the 178 high trade species with the estimated annual rate of 500-1000, 1000-2000, 2000-5000 and 2000-5000MT, respectively[2].

Different studies on therapeutic and biological potential of different species of Ocimum herb extract have exhibited numerous activities such as anticancer [3,4] rejuvenating properties, antiseptic effect, antiallergic [5] activation of antioxidative enzymes such as superoxide dismutase, catalase and glutathione peroxidase [6] adaptogenic and antistress properties [7], antiradiation activity [8], antioxidant activity [9] immunomodulatory [10], insect repellent [11], nematicidal [12], fungistatic [13], and antimicrobial [14] properties.

O. sanctum and *O. tenuiflorum* are acts as an adaptogen that helps the body and mind to encounter different physical, chemical emotional and infectious stresses, and restore physiological and psychological functions [15]. Such significant and health promising potential, in addition to its highly specific therapeutic actions, paved way for the broad range of Tulsa's traditional medical uses, and also contributes to its mythological importance and religious sanctity.

Primary metabolites directly involved in the growth and development of plants. Primary metabolites *viz*. chlorophyll, amino acid, nucleotides, and carbohydrates have a key role in the metabolic process such as photosynthesis, respiration and nutrient assimilation [16]. They are used as raw material and food additives. In this situation, the herbal medicinal plant can be used as a better opinion for the replacement of synthetic antioxidant agent. Antioxidant is the first aids of a defense system against free radicals include a number of an antioxidant enzyme such as superoxide dismutase, peroxidase, guaiacol peroxidase, Polyphenol oxidase and a non-enzymatic antioxidant such as glutathione, ABTS, Flavonoids, total phenol content, FRAP, DPPH, total tannin content, ascorbic acid,and dietary antioxidant etc. [17-19]. Therefore through the project work determine the biochemical test on antioxidant activity of four tulsi genotype namely *O. sanctum, O. tenuiflorum*, *O. gratissimum, and O. basilicum* has done the comparative studies of antioxidant of four genotypes of *Ocimum* genius.

2. MATERIALS AND METHODS

The estimation of biochemical studies on antioxidants of four *O. sanctum, O. tenuiflorum*, *O. gratissimum*, and *O. basilicum* genotypes was done by different methods. All analytical work was carried out at the Department of Biochemistry. The Tulsi plant was grown in climatic condition at 35/21°C with 75% relative humidity and collected the whole plant with wrapping the aluminium foils in 10 to 15 minute

from reaching the experimental laboratory. Mature plants of tulsi were collected from the herbal garden, Aromatic and Medicinal plant plot, Department of Horticulture, of Dr. RPCAU, Pusa. Leaves were cleaned from soil and washed with distilled water. Then grind to paste by mortar and pestle. The grind paste was collected in a centrifuged tube and centrifuged. The supernatant of samples were taken for the use of estimation of standard biochemical methods such as peroxidase [20], superoxide dismutase (SOD) [21], polyphenol oxidase (PPO) [22], catalase activity [23] and total carbohydrates [24] Soluble sugar estimated by the method of Trevelyan et al. [25], protein estimation by Lowry [26] method in 1951, total phenol with slight modification [27], total antioxidant [28], total flavonoids [28] and total flavonols [29].

2.1 Statistical Analysis

All the experiments were carried out in triplicates and data reported are the mean ± standard deviation. Calculation of linear correlation coefficient and correlation analysis were carried out using MS Office Excel 2007 (operating system window 7). The linear regression equation for a straight line is, Y = MX + C where, Y = absorbance of extract, M = slope of the calibration curve, X = concentration of extract, C = intercept. Using this regression equation, concentrations of extracts were calculated.

2.2 Catalase Assay

The method of Luck [23] was adopted to measure the catalase activity by1.0 g crushed fresh tulsi leaves mixed with 2.0 mL ice chilled assay phosphate buffer. After Centrifugation at 15,000 rpm for 15 min at 4°C, the 2 mL supernatant was collected to assay phosphate buffer. The assay mixture consists of 0.5mL extract and added 3.0 mL H_2O_2 phosphate buffer; absorbance is measured at 240 nm by UV-VIS Spectrophotometer. The catalase activity was expressed as Unit/mL or µ-mole/g/min. The catalase activity calculated by the following formula:

$$\text{Catalase activity} = \frac{\mu\text{M of sample}}{\text{Time in min.}} \times \text{Sample dilution}$$

2.3 Peroxidase Assay

The slightly modified method of Putter [20] was adapted to measure the peroxidase assay by 1.0 g freshly crushed leaves of Tulsi were mixed in 3.0 mL of chilled phosphate buffer at an ice box. After centrifugation at 18,000 rpm at 4°C for 15 min and the final volume of the supernatant was made up to 3.0 mL with phosphate buffer stored at the ice. For the assay mixture pipette out 3.0 ml buffer solution, 0.05 mL guaiacol solution, 0.10 mL extract, and 0.03 mL hydrogen peroxide solution in the cuvette and the absorbance is measured at 436 nm using a UV-VIS spectrophotometer. The Peroxidase activity was expressed as Unit/mL or µmole/g/min.

2.4 Superoxide Dismutase (SOD) Assay

The assay of superoxide dismutase by Kakkar, *et al.* [21] was done by 1.0 g of freshly isolated Tulsi leaves was crushed in mortar-pestle with 3.0 ml of potassium phosphate buffer at the ice. Centrifuged the crushed sample at 5000 rpm on 4^0C for 10 min and collect supernatant were used for more analysis. The assay mixture contained 1.2 mL of sodium pyrophosphate buffers, 0.1 mL of PMS, 0.3 mL of NBT, 0.2 mL of extract. The reaction was initiated by the addition of 0.2mL of NADH. The mixture was incubated at 30°C for 1 min and arrested by the addition of 1.0 mL of glacial acetic acid. The reaction mixture was then shaken with 4.0 mL of n-butanol and allowed to stand for 10 minutes. The intensity of the chromogen in the butanol layer was measured at 560 nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in 60 seconds. The activity of SOD calculates by the following methods:

$$\%\ \text{inhibition of NBT reduction by SOD} = \frac{\text{control OD} - \text{treatment OD}}{Control\ OD} \times 100$$

2.5 Polyphenol Oxidase (PPO) Assay

The enzyme extract was prepared by homogenizing 1.0 g freshly isolated leaves in 4.0 mL of the extraction medium containing Tris-HCl, sorbitol and NaCl. The homogenate was centrifuged at 2000 g for 10 minutes at 4^0C and the final volume of the supernatant was made up to 5.0 mL with extraction medium. Phosphate buffer (2.5 mL) and 0.3 mL of catechol solution were added in the cuvette and the spectrophotometer was set at 495 nm. The enzyme extract (0.2 mL) was added and the change in absorbance was recorded for every 0 minutes up to 5 minutes in a spectrophotometer.

The activity of PPO (Unit/mL) one unit of PPO is defined as the amount of enzyme that transforms 1.0 mole of dinitrophenol to 1.0 mole of Quinone per minute under the assay condition. The method to be slight modification by Esterbauer et al. [22] was adopted. The activity can be calculated from the slope of Absorbance vs time X dilution factor of the enzyme.

2.6 Total Phenolic Content

Total phenolic content is determined by 1.0 g of freshly isolated leaves of Tulsi were crushed in mortar-pestle in 2 mL of 50% ethanol and allow to stand for 15 min. Centrifuge it at 5000 rpm for 15 min and the supernatant was used for assay.1.0 mL of sample and 1.0 mL of Folin-Ciocalteu (2N) reagent were added to a 25 mL volumetric flask. The contents were mixed and allowed to stand for 5 min at room temperature. Then 10 mL of a 7.5% sodium carbonate solution was added and allowed to stand at 30°C for the 30-minute measure at 750 nm. Total phenol content was standardized against 0.1 mg/mLof Gallic acid and expressed as (GAE)mg/100 g fresh weight of leaves. Total phenol method with the slight modification of Singleton and Rossi [27].

2.7 Estimation of Total Flavonoids

For the estimation of flavonoids by Cameron *et al.*[28] method through 1.0 g of freshly isolated leaves of *Ocimum* was homogenized in 2.0 mL of methanol: H_2O (2:1) and 2.0 mL of methanol: H_2O (1:1). The homogenate was filtered and make up the volume to 2.0 mL with Methanol: H_2O (2:1). To 1.0 mL of filtrates, added 4 mL of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) and kept in a boiling water bath for 15 min. The absorbance was read at 340 nm. The flavanoids were standardized against mg/mLof catechine and expressed as mg (catechine) s/100 g fresh weight of leaves.

2.8 Total Flavonols

The expression of total flavonols by Kumaran and Karunakaran [29] method with slight modification, in 1.0 g of freshly isolated *Ocimum* leaves was crushed in mortar-pestle in 2.0 mLof dist. H_2O. After centrifugation, the final volume of the supernatant was made up to 4 mLwith dist. H_2O. To 2.0 ml of extract of leaves, 2.0 ml of 2% $AlCl_3$- ethanol and 3.0 mL of 5% sodium acetate solution were added. The absorbance was read at 440 nm after 2.5hr at 20°C. The total Flavonols was standardized against 1 mg/mL Quercetin and expressed as mg/100g fresh weight of leaves.

2.9 Estimation of Total Antioxidant Activity (Phosphomolybdic Acid Method)

The antioxidant activity calculates by Kumaran and Karunakaran [29] method, estimation of antioxidant in 1.0 g of freshly cursed *Ocimum* leaves homogenate in 2.0 mL of distilled water. The homogenate was centrifuged at 5000 rpm for 15 minutes and the final volume of the supernatant was diluted up to 3.0 ml. An aliquot of 0.4 mL of the sample solution was combined in a vial with 4.0 mL of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The vials were capped and incubated into the water bath at 90°C for 90 minutes. Cooled the sample at room temperature and the absorbance was measured at 695 nm against a blank. The antioxidant activity was standardized against mg/mL of ascorbic acid and expressed as mg (Ascorbic Acid)/100 g fresh weight of leaves. The percentage of DPPH (2, 2-diphenyl-1-picrylhydrazyl) decolouration was calculated as follows:

DPPH scavenging effect % = (1-As/Ac) × 100

Where Ac is the absorbance of the control and As is the absorbance of the test sample.

2.10 Extraction of Soluble Protein

The estimation of protein was done by Lowry [26] method. The soluble protein determines in1.0 g of fresh leaves of Tulsi was crushed in 10mL of 80% ethyl alcohol and centrifuged at 5000 rpm for 10 minutes. The residue was washed with 3 mL of 80% ethyl alcohol and centrifuged at 5000 rpm for 10 minutes. The residue so obtained was air dried and extracted in 6mL of 0.1M Phosphate buffer (pH 6.7). After centrifugation at 5000 rpm for 10 minutes, the residue was discarded. To the supernatant was added 2 mL of 15 per cent trichloroacetic acid (TCA) and the mixture was left for 20 minutes at 0°C. The precipitate so obtained was separated from TCA Soluble components by centrifuging at 5000 rpm for 10 minutes. The precipitate was taken and dissolved in 4.0 mL of 0.3N NaOH. After centrifugation at 5000 rpm for 10 minutes, the supernatant was used for protein estimation.

5 ml of reagent 'C' was added to 0.1 mL of the aliquot prepared in 0.3N NaOH and was allowed to stand for 10 minutes at room temperature. After this period, 0.5 mL of reagent 'D' was added to the mixture followed by stirring. After 30 minutes absorbance was measured at 670 nm on spectro-colourimeter 103. The total quantity of protein present in the material was calculated using a standard curve prepared by taking 50-300 µg of bovine serum albumin (BSA) from a stock solution (1 mg/mL) of BSA in water.

2.11 Extraction of Carbohydrates

The extraction of carbohydrates by McCready et al. [24] method. The fresh leaf of *ocmium,* 1.0 gm was homogenized in 80:20 ratio of ethanol: water centrifuged at 5000 rpm for 10 minutes in REMI table centrifuge. The residue was ground twice with 80 per cent ethanol and centrifuged again at 3000 rpm for 10 minutes to extract the sugar. The alcohol extract was pooled after centrifugation for use in sugar estimation while the residue was used for determination of starch.

2.11.1 Determination of starch

The starch estimated by the method of Trevelyan et al. [25]. Sugar-free residues were added 5 mLof water and 6 mL of 52 per cent perchloric acid. The mixture was allowed to stand at 0°C for 20 minutes with occasional stirring. After centrifugation at 5000 rpm for 5 minutes, the residue was again treated with water and perchloric acid in a similar manner and centrifuged. The supernatants containing dissolved starch were pooled and diluted to 50 mL with water.

Anthrone reagent test tubes for use were placed in an ice bath. To these tubes were added 0.2 mL of starch extract, 0.8 mL of water and 4 mL of ice-cold anthrone reagent and the contents mixed well. Blanks were run simultaneously in test tubes. Where in the starch extract was replaced by water? The tubes were placed in boiling water for 10 minutes. The intensity of colour developed was read at 620 nm on a UV-spectrophotometer.

A calibration curve was prepared by taking 10 ml to 50 µg of glucose from a stock solution (25 mL/100 mL) and the colour developed, as in the extract of the sample mentioned above. The value of starch content was calculated by multiplying the amount of glucose so obtained by a factor of 0.9 taking into account the amount of water taken up during starch hydrolysis.

2.11.2 Estimation of total soluble sugar

The total sugar estimated by the method of Trevelyan et al. [25]. Taken a 400 mg fresh sample of *tulsi* leaves was extracted with 80% ethanol in a mortar pestle and then the sample was centrifugation at 3000 rpm for 10 min and the supernatant was collected. Extraction was repeated 4 times with 80% ethanol and the supernatant was collected in a volumetric flask and final volume of the extract was made to 25 mL with 80% ethanol. The extract of 0.3 mL was pipetted out in a separate test tube and test tube placed in a boiling water bath for 3 minutes to evaporate the ethanol. 1 mL of Millipore water was added in each test tube and mixed well and pipette out 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mL of glucose solution (100 µg/mL) in another set of the test tube for preparation of standard curve and made the volume to 1 mL to distilled water. Blank was prepared by adding 1 ml of distilled water and 4mLof anthrone reagent. 4mLof 0.2% anthrone reagent (200 mg anthrone in 100 mL H_2SO_4) was added in each test tube and placed in ice-cold water. The intensity of colour read at 600nm on a spectrophotometer. Compared to the absorbance with the glucose standard curve and the amount of total soluble sugar content were calculated.

3. RESULTS AND DISCUSSION

Four species of tulsi such as *O. basilicum, O. sanctum, O. gratissimum, O. tenuiflorum* were taken for the biochemical studies such as total sugar, reducing sugar, non-reducing sugar, soluble protein, starch, phenol, total flavanoids, total flavonols, total antioxidants and enzymatic studies such as superoxide dismutase (SOD), Polyphenol oxidase (PPO), peroxidase, catalase. A study by Hu, et al. [30] used an assay system for free radicals to confirm the antioxidant action of tulsi extract. Tulsi contains more than 120 substances which are responsible for its different types of health and nutritional benefits. In the present study, photochemical analysis and Antioxidant activity in four different genotypes of Ocimum have been undertaken.

3.1 Biochemical Studies

3.1.1 Starch

Starch is biodegradable and renewable in nature. They are increasingly being considered as an

eco-friendly alternative to the use of synthetic additives in many other products, including plastics, detergents, pharmaceutical tablets, pesticides, cosmetics and even oil-drilling fluids [31]. The highest amount of starch was observed in *O. gratissimum* (98 mg/gfw) and the minimum amount was observed in *O. tenuiflorum* (79 mg/gfw).

Quantitative estimation of sugar shows that the content of sugar (Fig. 1) is more in *O. sanctum* (159 mg/gfw) and the minimum amount was observed in *O. tenuiflorum* (109 mg/gfw). In earlier studies also, more or less similar findings have been reported [16]. Plant sugar can be used as artificial sweeteners and they can even help diabetics by supporting the body in its rebuilding [31]. Total soluble sugar has been studied in different plant species reported in leaves of *M. indica* [32] and *C. obtusifolia* L. [33].

3.1.2 Reducing sugar and non-reducing sugar

Reducing sugar was found to be higher in *O. basilicum* (108 mg/gfw) and the lower amount in *O. gratissimum*(72 mg/gfw). The content of non-reducing sugar was found to be higher in *O. sanctum (*61 mg/gfw) as compare to *O. basilicum* (33 mg/gfw).

3.1.3 Total soluble protein

Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in the future [34]. Total levels of protein (Fig. 1) were found to be higher in *O. tenuiflorum* (62 mg/gfw) and the lower amount in *O. sanctum* (40 mg/gfw). In earlier studies also, more or less similar findings have been reported [16,32].

3.2 Enzymatic Antioxidants

3.2.1 Polyphenol oxidase (PPO)

Phenoloxidases are copper-containing proteins that catalyze the aerobic oxidation of phenolic substrates (Fig. 2) to quinines, which are auto-oxidized to dark brown pigments known as melanins [22]. These can be estimated spectrophotometrically at 495nm. The highest amount Polyphenol oxidase (PPO) activity was found in *O. sanctum* (462 U/ml) and the lowest activity was found in *O. gratissimum* (224 U/mL). In earlier studies also, more or less similar findings have been reported [16].

3.2.2 Peroxidase

Peroxidase reduces HO^- to water while oxidizing a variety of substrates. Thus, peroxidases are oxidoreductases which use HO^- as an electron acceptor for catalyzing different oxidative reactions [35-37]. The highest amount of Peroxidase activity was found in *O. sanctum* (142U/mL) and the lowest activity was found in *O. gratissimum* (88.76 U/mL).

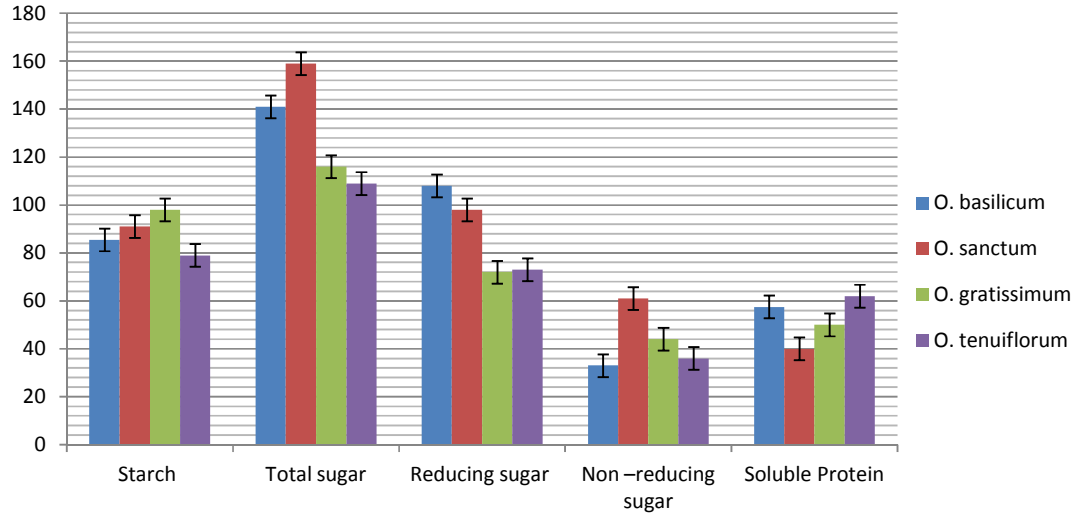

Fig. 1. Starch, total sugar, reducing sugar, non-reducing sugar (mg/gfw) and soluble protein (mg/gfw) content in *O. basilicum, O. sanctum, O. gratissimum, O. teniuiflorum*

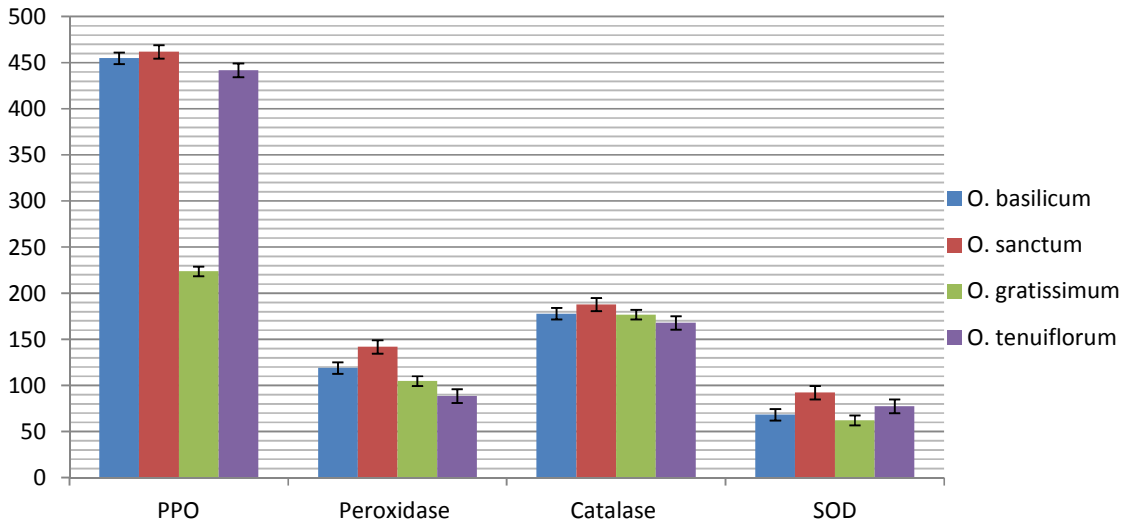

Fig. 2. The figure depicted the amount of PPO, peroxidase, catalase and SOD enzymatic antioxidant activity (µmole/min/gfw) *of O. basilicum, O. sanctum, O. gratissimum, O. teniuiflorum*

3.2.3 Catalase

Catalase is an antioxidant enzyme which plays an important role in the body defence mechanism against the harmful effects of the reactive oxygen species (ROS) and free radicals in biological systems [35-37]. Tulsi showed a significant catalase activity (Fig. 2) with an increase in time with the highest activity in *O. sanctum* (188 U/mL) and the lowest activity was observed in *O. tenuiflorum*(168U/mL).

3.2.4 Superoxide dismutase

Superoxide Dismutase (EC1.15.1.1) catalyzes the reduction of superoxide anions to hydrogen peroxide. It plays a critical role in the defence of cells against the toxic effects of oxygen radicals. SOD has suppressed apoptosis in cultured rat ovarian follicles, neural apoptosis in neural cell lines, and transgenic mice by preventing the conversion of NO to peroxynitrite, an inducer of apoptosis [38]. The highest amount of Superoxide dismutase (SOD) activity was found in *O. sanctum* (92.47 U/mL) and the lowest activity was found in *O. gratissimum* (62.36 U/mL). In earlier studies also, more or less similar findings have been reported [16].

3.3 Total Phenolic Content

Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as the number and location of hydroxyl groups. In many *in vitro* studies, phenolic compounds demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids [39]. Plant phenol may interfere with all stages of the cancer process, potentially resulting in a reduction of cancer risk [10]. Phenolic compounds have been studied in different plant species like *Arachis hypogaea* and *Simmondsia chinensis* [40]; *Chlorophytum borivilianum* [41], *Balanites aegyptica L.* [42], *Terminalia catappa* [43]. In the present investigation, the content of total phenolics in extracts of Tulsi was found to be highest in *O. tenuiflorum* (667 mg/gfw) while lowest in *O.gratissimum* (468 mg/gfw). In earlier studies also, more or less similar findings have been reported [44].

3.3.1 Total flavonoids

Flavonoids also exist as valuable antioxidants in plant foods. There is abundant evidence from *in vitro* biochemical studies that flavonoids defend cells from lipid peroxidation, and from human intervention studies that dietary supplements decrease the incidence of cancer and atherosclerosis [39]. The total Flavonoids content was found to be highest in *O. tenuiflorum* (325 mg/gfw) and the lowest was found in *O. gratissimum* (310 mg/gfw).

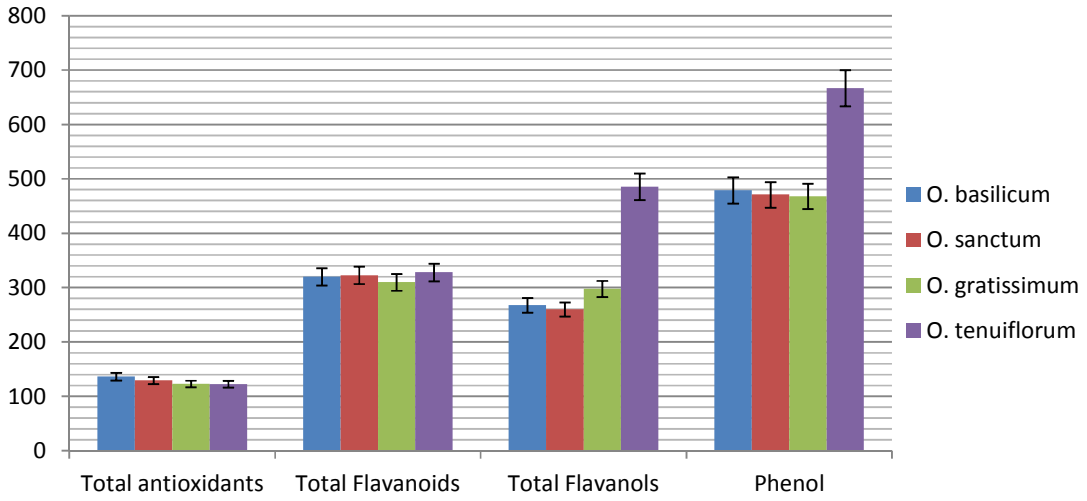

Fig. 3. Total antioxidants, total flavonoids, total flavonols and phenol content (mg/gfw) of *O. basilicum, O. sanctum, O. gratissimum* and *O. teniuiflorum*

3.3.2 Total flavonols

Flavonols are a class of flavonoids that have the 3-hydroxy flavone backbone. Flavonols are present in a wide variety of fruits and vegetables. Flavonoids are extensively degraded to various phenolic acids, some of which still possess a radical-scavenging ability. The total Flavonols content was found to be highest in *O. tenuiflorum* (486 mg/gfw) and the lowest was found in *O. sanctum* (249.9 mg/gfw).

3.3.3 Total antioxidant

The antioxidant capacity of the fractions was measured spectrophotometrically through Phospho-molybdenum method, which was based on the reduction of Mo (VI) to Mo (V) by the sample analyses and the subsequent formation of green phosphate/Mo (V) compounds with maximum absorption at 695 nm [28,36]. The highest amount of total Antioxidant activity was found in *O.basilicum* (136.45 mg/gfw) and the lowest activity was found in *O.tenuiflorum* (122.65 mg/gfw). The phenolic compounds could be the main cause of antioxidant power of *O. bascilicum* plant, in accordance with the previous findings that many phenolic compounds in plants are good sources of natural antioxidants [3,45].

There are thousands of herbal plants in the world but the *O. sanctum* is considered to be the queen of herbs due to its greater medicinal values. Tulsi contains various phytochemicals, having strong antioxidant, antibacterial [46], antiviral, adaptogenic, and immune-enhancing properties that promote general health and support the body's natural defence against stress and diseases. Some of the main chemical constituents of *tulsi* are oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, Linalool, β-caryophyllene (about 8%), β-element (c.11.0%), and germacrene D (about 2%). Traditionally crude extracts of various parts of plants have been used for their antidiabetic, antioxidant, antistress, antihyperlipidemic and antibacterial properties [47-48]. Future research on sacred basil should be emphasized for control of various diseases especially it should be explored as a significant remedy regarding neuropsychological disorders for the welfare and service of mankind.

4. CONCLUSION

The leaves of the plant have enough bioactive properties as shown in the different parameter. The data may signify the investigations of different bioactive compounds from the plant tulsi species and the requisite level of activity (pharmacological) would be considered for further scrutiny to develop the potential drug molecule. Free radicals are the cause of several major disorders [17-19]. So, evaluation of antioxidant activity in Ocimum genotypes could result in the discovery of natural antioxidants with pharmacological and food value. The importance of phenol compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known. However, only limited work has been done on species. Therefore, the present work

aimed to analyze the antioxidant potential and phytochemical studies of these species of tulsi. Free radicals cause biological damage through oxidative stress and such processes lead to many disorders like neurodegenerative disorders, and cancer. The graph shows that the starch content (98 mg/gfw) was found to be higher in *O. gratissimum.* Similarly the other parameters such as total reducing sugar (159 mg/gfw), phenol (479 U/mL) and total antioxidants (136 mg/gfw) are higher in *O. basilicum* while total sugar (159 mg/gfw), non-reducing sugar (61 mg/gfw), PPO (462 U/mL), peroxidase (142 U/mL), catalase (188 U/mL) and SOD (92.47 U/mL) are higher in *O. sanctum.* On the other hand, soluble protein (62 mg/gfw), total flavonoids (325 mg/gfw) and total flavonols (486 mg/gfw) were found to be higher in *O. tenuiflorum*. However, the data obtained on enzymatic antioxidant activity in comparison to enzymatic levels in medicinal plants need an extensive investigation. The results concluded that the biochemical studies of *O. sanctum* are good potential in the degradation of free radicals. Therefore, it is suggested that tulsi could be a potential source of natural antioxidant. Farmers could be recommended to cultivate this genotype of tulsi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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