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ORIGINAL ARTICLE

Characterization of Secondary Metabolites in Different Parts of *Ocimum gratissimum* L. by *in vitro* Antioxidant Activity and High-performance Liquid Chromatography–Diode-array Detector Analysis

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ABSTRACT

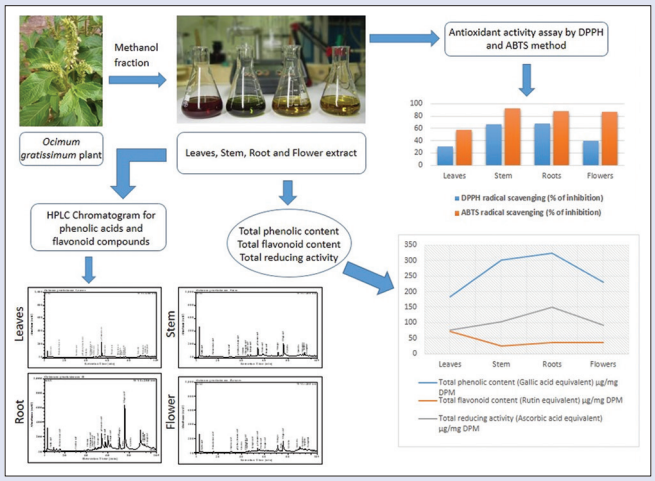
Background: *Ocimum gratissimum* L. (OG) has medicinal importance, especially in the treatment of epilepsy, fever, diarrhea, mental illness, etc., **Objectives:** The present study focused on evaluation of the total phenolic content, total flavonoid content, and reducing and antioxidizing activities from methanolic extract of different plant parts, namely leaves, stem, roots, and flowers of OG. **Materials and Methods:** The quantification of phenolic and polyphenolic compounds was assessed by high-performance liquid chromatography (HPLC). **Results:** The results revealed that the root extract showed the highest phenolic content (323.93 ± 2.062 µg/mg) and reducing property (150.57 ± 1.76 µg/mg), whereas the highest flavonoid content was found in leaves 72.09 ± 1.269 µg/mg. The highest radical scavenging activity (68.12% ± 0.527%) was observed for the root extract in 2, 2-diphenyl-1-picrylhydrazyl method. Similar significant results were observed by 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) method as well for all investigated extracts. HPLC–diode-array detector analysis was done to find the secondary metabolite composition of the methanol extracts which lead to the characterization of different flavonoids and phenolic acids in OG extracts. **Conclusion:** These results showed that this species can be a source of phenolic compounds and antioxidants, thereby suggesting it for consumption as a source of natural food antioxidant to help hinder against various detrimental effects of reactive species-induced ailments.

Key words: Antioxidants, flavonoids, high-performance liquid chromatography, *Ocimum gratissimum* L., phenolic compounds

SUMMARY

The present study aimed to investigate the antioxidant activities of different plant parts viz. leaves, stem, roots and flowers of Ocimum gratissimum. The antioxidant properties were evaluated by using DPPH and ABTS radical scavenging activity, reducing power and estimation of total phenolic and flavonoid content. The quantification of phenolic acids and flavonoids were carried out by the high performance liquid chromatography (HPLC). The result of this investigation showed that the methanol extracts of roots of the plant showed higher antioxidant activity as compared to the other parts. The HPLC analysis also showed the presence of phenolic acids and flavonoids in various amounts in these parts which can conclude them as potent natural antioxidant.

Abbreviations used: OG: *Ocimum gratissimum* L.; HPLC: High-performance liquid chromatography; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; ABTS: 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); HPLC-DAD: High-performance liquid chromatography–diode-array detector; ROS: Reactive oxygen species; BHT: Butylated hydroxytoluene; UV-VIS: Ultraviolet-visible; TPC: Total phenolic content; GAE: Gallic acid equivalent; DE: Dry extract of the concentrate; TFC: Total flavonoid content; RE: Rutin equivalent; AAE: Ascorbic acid equivalent; ANOVA: Univariate analysis of variance; Ac: Absorbance of control; At: Absorbance of test; USP: U. S: Pharmacopeia; ICH: International conference on Harmonization; IC: Inhibitory Concentration; DPM: Dry plant material; HIV: Human immunodeficiency infection; µg/mg: microgram per milligram; cm: centimeter; g: gram; mg: milligram; ml: milliliter; µg/ml: microgram per milliliter; nm: nanometer; µm: micrometer; µl: microliter; min: minutes; ml/min: milliliter per minute; mg/g: microgram per gram.



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INTRODUCTION

Oxidation is an inevitable process in all living organisms. Free and nonfree radical species are simultaneously included in reactive oxygen species (ROS) production, thereby leading to hazardous and dreadful effects in the form of fatal diseases such as diabetes, atherosclerosis, ischemic cardiac disease, inflammation, suppression of immunity, diseases with neurodegeneration, cancer, and so on. These free radicals are produced as by-products during the formation of energy by the cell in the form of superoxide and hydroxyl radicals, etc., These ROS are beneficial in limited concentration but have detrimental effects on the cell when its concentration rises, thereby leading to oxidative stress. The living cell counteracts these mechanisms by producing antioxidants either naturally or in the form of food supplements. Several researches support the fact that flavonoids and phenolic compounds are potential antioxidants. The antioxidants scavenge the free radicals and compensate the damage caused by ROS.[1] In present days, medicinal plants are on highlights for being the natural sources of powerful antioxidants. Researchers are focusing on commercial development of these medicinal plants to enhance the health industries. Several evidences also claim that dietary phytometabolites such as flavonoids, phenols, and tannins have been used against various diseases and even in maintenance of health since ages.[2,3]

Ocimum gratissimum L. (OG), Lamiaceae, is a native plant to the African continent but is inhabited in India, Bangladesh, China, Thailand, Haiti, Egypt, and Indonesia as well. This plant is mentioned in several books of traditional medicines, especially Ayurveda and Unani medicinal practices. The prominent therapeutic potentialities of the plant have been customarily utilized in the treatment of epilepsy, fever, diarrhea, mental illness, upper respiratory tract infections, cough, and conjunctivitis.[4]

Meanwhile, researchers have searched several ways through which the medicinally important plant paves a pathway toward sustenance and solution of deadly diseases like cancer by cell reinforcement. Various studies have proved that the naturally occurring secondary metabolites in plants show a defensive mechanism against the genotoxicity which is caused during oxidative stress.[5] Evaluating the reducing activities and antioxidizing potentialities of OG and estimation of phenolic and polyphenolic compounds by high-performance liquid chromatography (HPLC) from its different plant parts, namely leaves, stem, roots, and flowers was the main aim of this investigation. The novelty of our study was to establish a comparative evaluation by estimating the phenolic compounds, flavonoid, and antioxidant activities of different parts of OG and quantifying the active phenolic and polyphenolic compounds present in the different parts through HPLC analysis and plant could be a potential source of natural antioxidants which corroborate its efficiency in traditional medicine and might be exploited on an industrial level as food additive and herbal drug formulations.

MATERIALS AND METHODS

Plant materials

The fresh plant material was collected from the locality of Hooghly and was sent to Central National Herbarium, Botanical Survey of India, Kolkata, for authentication. The voucher specimen (RU/BOT/JK/SG/001) was deposited in University Herbarium of Department of Botany, Ranchi University, Ranchi, for preservation. Each plant part was rinsed thoroughly with tap water. Then, samples were cut into small pieces and air-dried at room temperature.

Chemicals and equipments

To investigate the antioxidant activities, the phenolic and polyphenolic standards such as gallic acid, protocatechuic acid, catechin, rutin, gentisic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, salicylic acid, chlorogenic acid, p-hydroxy benzoic acid, ellagic acid, myricetin, quercetin, naringin, apigenin, and kaempferol along with 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1, 1-diphenyl–2–picrylhydrazyl (DPPH) were procured from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals such as Folin–Ciocalteus's phenol reagent, potassium ferricyanide, potassium per sulfate, aluminum chloride, ferric chloride, sodium carbonate, sodium dihydrogen phosphate, and trifluoroacetic acid and the HPLC grade solvents such as acetonitrile, methanol, and water were obtained from Merck (Germany). Reversed-phase HPLC analysis was achieved using Dionex Ultimate 3000 liquid chromatography coupled with a diode-array detector with 5 cm flow cell and gradient elution of mobile phase system, using Chromeleon system manager as data processor. The separation was achieved by a reversed-phase Acclaim C_{18} column (5 µ particle size, 250 mm × 4.6 mm).

Extraction of plant materials

The plant parts, namely leaves, stem, roots, and flowers were dried, pulverized, and were kept in a container which was airtight to analyze the phytochemicals. The plant material (1 g) was taken in 20 ml of 70% aqueous ethanol and agitation on magnetic stirrer for 2 h. The normal room temperature was maintained overnight. The extracts were filtered and undergone evaporation to dryness. The leftover dry extract (DE) was scrapped and weighed. The DE (25 mg) was dissolved in a minimum concentration of methanol and the volume was adjusted up to 25 ml. The extract was kept in a volumetric flask (25 ml) for further analysis.[6]

Estimation of total phenolic content

The Folin–Ciocalteus's methodology was followed to evaluate the total phenolic content (TPC) in various concentrations (100, 200, 400, 800, and 1000 µg/ml) from different plant parts. With 0.5 ml of tenfold diluted Folin–Ciocalteu's reagent, 4.5 ml of distilled water and 0.5 ml of the extract were added and mixed. The mixture was incubated for an hour by maintaining the room temperature and then absorbance was taken at 765 nm. The experiment was performed in triplicates and was communicated as gallic acid equivalent (GAE) in µg/mg DE of the plant materials.[6]

Estimation of total flavonoid content

To 2 ml of extracts in different concentrations (100, 200, 400, 800, and 1000 µg/ml), 2 ml of 2% $AlCl_3$ in ethanol was added followed by incubation in the dark by maintaining the room temperature for an hour. Then, absorbance was measured at 420 nm. The total flavonoid content was calculated and expressed as rutin equivalent (RE) in µg/mg of DE.[6]

Estimation of reducing power

The reducing power was evaluated following the protocol given by Datta *et al*. 2019. The reducing activity was evaluated and expressed as ascorbic acid equivalent (AAE) in µg/mg of DE.[6]

Estimation of antioxidant activities

The antioxidizing potentialities were determined from plant extracts of different parts using DPPH. Besides, ABTS radical cation was also used for analyzing the free radical scavenging property.[6] The potentiality of DPPH and ABTS radical scavenger was deliberated, using the following equation:

DPPH/ABTS scavenge (%) = ([Ac– At]/Ac) × 100

Where Ac signifies the absorbance in the absence of sample extract, i.e., DPPH/ABTS solution and At signifies the absorbance with sample extracts in different concentrations.

Estimation of phenolic acids and polyphenolic compounds by high-performance liquid chromatography

The HPLC analysis for estimation of phenolic acids and polyphenolic compounds was performed following the protocol by Datta *et al.* 2019 which was validated by standard USP and ICH guidelines. The methanol extract of OG (20 mg) was mixed in 20 ml mobile phase solvent (methanol: 0.5% acetic acid in water: 1:9) and before injecting into the HPLC system, the filtration of the test solution was done through 0.45 µm membrane filter. Methanol (solvent A) was being used as a mobile solvent phase and 0.5% aqueous acetic acid solution (solvent B). The column temperature was maintained at 25°C thermostatically and the injection volume was kept at 20 µl. The gradient elusion was prepared in different percentages, namely solvent A (10%) and solvent B (90%) with a flow rate in the first 27 min being 1 ml/min to 0.7 ml/min. The solvent A changed from 10% to 40% and flow rate was reduced to 0.7 ml/min in 50 min and for 40% solvent A and 60% solvent B, the flow rate was 0.7 ml/min at first for 2 min and afterward flow rate was decreased from 0.7 to 0.3 ml/min in 65 min, and from 40% to 44% solvent A, the flow rate was 0.3–0.7 ml/min in 70 min. From 44% solvent A, the flow rate was 0.7–1 ml/min for 10 min duration then swapped from 44% to 58% with a flow rate of 1 ml/min for 5 min followed by 58%–70% solvent A in 98 min with a consistent flow rate of 1 ml/min. Before the injection of another sample, the flexible stage was resumed to the initial condition (solvent A: solvent B: 10:90) at 101 min and was allowed to run for the next 4 min. The total running time for the complete estimation of each sample was 105 min. The retention time and spiking with standards under comparable conditions were the recognition point for compound identification. The measurement of integrated peak area was carried out to quantify the phenolic acids and flavonoids. The quantification of phenolic acids and polyphenolic compounds was calculated by using the calibration curve and plotting peak area against respective standard sample concentration.

Statistical analysis

All the experiments were carried out using triplicate samples and the results were presented as mean ± standard error by using the Statistical Package for the Social Sciences (SPSS variant 7.5) (SPSS Inc, International Business Machines (IBM), USA). Tukey's test at 95% confidence level and statistical significance was followed, thereby accepting at $P < 0.05$ level. The linear regression graphs were used to calculate the IC_{50} values.

RESULTS AND DISCUSSION

The TPC, flavonoid content, reducing activity, and antioxidizing activity from different parts of the plant of OG are exhibited in Table 1.

Total phenolic content

The TPC was communicated as GAE which was expressed in µg/mg dry plant material (DPM), as shown in Table 1 and Figure 1. The highest phenolic content was noticed in root extract (323.93 ± 2.062 µg/mg GAE). The lowest phenolic content was recorded in leaf extract (182.40 ± 1.26 µg/mg GAE). The phenolic compounds are the most vital secondary metabolites in the plant which are responsible for the antioxidizing activity by inactivation of lipid free radicals and prevention of hydroperoxide conversion to oxyradicals.[7,8] This is the reason that the evaluation of TPC serves as a promising parameter to estimate the antioxidant potential. HPLC can detect the different components present in different amounts in the extract. In previous researches, the phenolic contents of leaves were reported to have the highest amount.[9] However, the present study exhibits a striking difference in the results.

Total flavonoid content

Flavonoids are also an extensive group of natural metabolites and are prominent for their health-promoting benefits through cell signaling.[10,11] In many studies, it has been proven to have several pharmacological

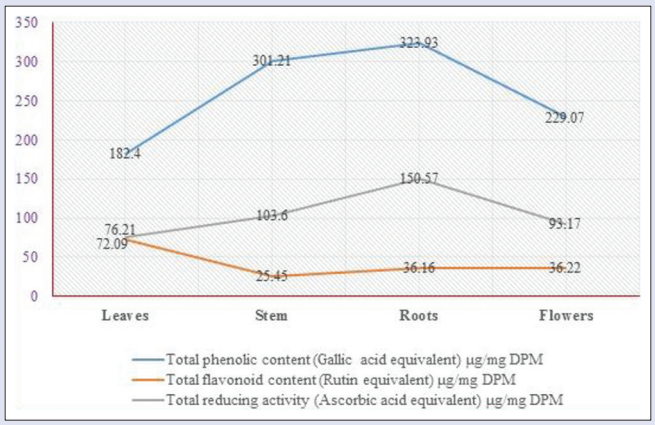

Figure 1: Graphical representation showing total phenolic content, total flavonoid content, and total reducing activity from different plant parts, namely leaves, stem, roots, and flowers of *Ocimum gratissimum* L.

Table 1: Antioxidant activities of different plant parts of *Ocimum gratissimum* L.

Plant part extract	Mean±SEM				
	Total phenolic content (gallic acid equivalent), µg/mg DPM	Total flavonoid content (rutin equivalent), µg/mg DPM	Total reducing activity (ascorbic acid equivalent), µg/mg DPM	Antioxidant activity	
				DPPH radical scavenging (percentage of inhibition)	ABTS radical scavenging (percentage of inhibition)
Leaves	182.40±1.26^{d}	72.09±1.269^{a}	76.21±1.004^{d}	30.38±0.732^{d}	57.40±0.079^{d}
Stem	301.21±0.824^{b}	25.45±0.286^{d}	103.60±2.52^{b}	67.09±0.732^{b}	93.01±0.079^{a}
Roots	323.93±2.062^{a}	36.16±0.127^{c}	150.57±1.76^{a}	68.12±0.527^{a}	87.61±0.073^{b}
Flowers	229.07±4.54^{c}	36.22±0.063^{b}	93.17±1.76^{c}	39.71±0.053^{c}	87.36±0.073^{c}

Each value in the table was obtained by calculating the average of three experiments and data are presented as mean±SEM. Statistical analysis were carried out by Tukey's test at 95% confidence level and statistical significance were accepted at the $P<0.05$ level. The superscript letters $^{a, b, c}$ and ^{d} denotes the significant differences within same parameters of different extract of the plant. SEM: Standard error of the mean; DPM: Dry plant material; DPPH: 2, 2- diphenyl-1-picrylhydrazyl; ABTS: 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

and biological activities which also include anti-inflammatory, anti-carcinogenic, anti-thrombotic, anti-allergic, hepatoprotective, and even free radical scavenging activities.[11] The highest amount of flavonoid was recorded in leaves (72.09 ± 1.269 µg/mg RE) and the lowest in stem (25.45 ± 0.286 µg/mg RE) which is represented in Table 1 and Figure 1. The result showed a good congruence with the earlier studies where leaf extracts were reported to have the highest flavonoid content.[10,12,13]

Total reducing power

The reducing activity is expressed in µg AAE/mg of DPM, as shown in Table 1 and Figure 1. The highest reducing activity was recorded in root extract (150.57 ± 1.76 µg/mg AAE) and the lowest was recorded in leaf extract (76.21 ± 1.004 µg/mg AAE). The reducing potential of the extracts actually measures its ability to donate electrons.[10] The promising results show that the root extract of the plant has ability to donate electrons and hence can also have the ability to scavenge free radical.

Antioxidizing activity

Free radical scavenging of DPPH and ABTS is communicated in percentage of inhibition (%) of DE of plant materials, as shown in Table 1 and Figure 2. DPPH radical scavenging was recorded in root extract (68.12% ± 0.527%) immediately followed by stem extract (67.09% ± 0.732%). In ABTS radical scavenging, the stem extract exhibited highest inhibition (93.01 ± 0.079%) and lowest inhibition showed by the leaves extract (57.40 ± 0.079%).

The methodology followed to evaluate the antioxidant activity is dependent on the reaction condition and substrate, therefore, all the methods do not exhibit similar value, but a similar pattern is noticed.[14] The antioxidant activity was determined by using DPPH and ABTS. The results showed a very nominal difference that is why can be considered almost similar. Due to the presence of double bond in the C-ring and arrangement of hydroxyl group, flavonoids possess higher antioxidant activity. An eminent free radical scavenging activity is exhibited either due to the presence of ortho group (quercetin), hydroxyl group in meta position (kaempferol), or compounds having a double bond in the C-ring structure, between C2 and C3 hydroxyl group. It has also been previously reported that the molecular weight of phenolic compound is directly proportional to the free radical scavenging activity, depending on the number of aromatic rings and hydroxyl groups being substituted.[6] The present study showed that in both DPPH and ABTS assays, the root and the stem extract showed almost similar results and hence both can be considered to have good antioxidizing activity.

Identification and quantification of phenolic acids and polyphenolic compounds in *Ocimum gratissimum* by high-performance liquid chromatography

In the analysis, the phenolic acids such as gallic acid, gentisic acid, chlorogenic acid, protocatechuic acid, caffeic acid, p-hydroxyl benzoic acid, vanillic acid, p-coumaric acid, syringic acid, sinapic acid, ferulic acid, ellagic acid, and salicylic acid and the flavonoids such as catechin, naringin, rutin, myricetin, quercetin, naringenin, apigenin, and kaempferol were detected which were being conveyed as µg/mg DPM and are represented in Table 2. All the compounds under investigation have responded at 280 nm and separated successfully. Besides, the identified compounds which were recorded by the absorption spectra [Figures 3-6] are also comparable for all plant extracts as well as standard substances.[6] Caffeic acid and salicylic acid were reported only in leaf and root extracts. p-hydroxy

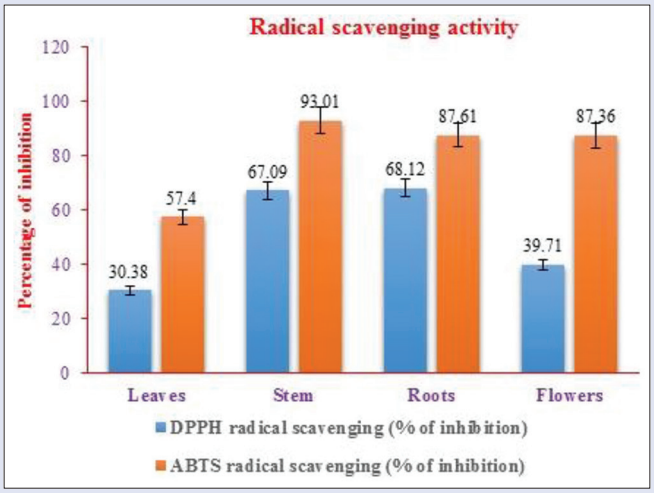

Figure 2: Graphical representation showing comparative antioxidant activity by 2, 2- diphenyl-1-picrylhydrazyl and 2, 2′- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) method

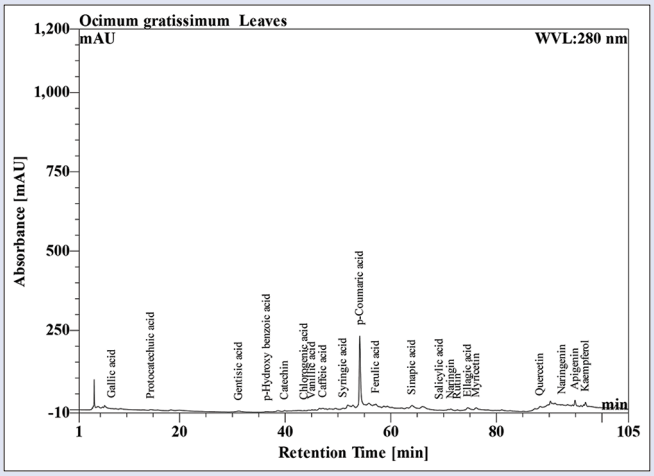

Figure 3: High-performance liquid chromatography chromatogram of leaf extract of *Ocimum gratissimum* L.

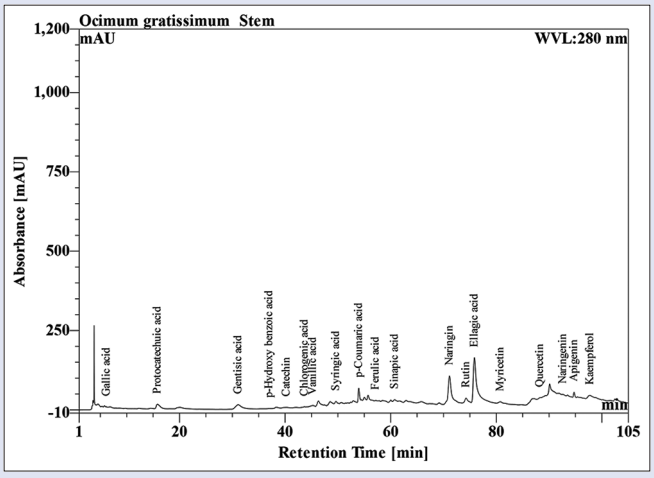

Figure 4: High-performance liquid chromatography chromatogram of stem extract of *Ocimum gratissimum* L.

Table 2: Quantitative estimation of phenolic acids and flavonoids of different plant parts of *Ocimum gratissimum* by high-performance liquid chromatography

Phenolic acids/flavonoids	Total amount present (µg/mg dry extract) (Mean±SEM)			
	Leaves	Stem	Roots	Flowers
Gallic acid	0.004±0.001^{d}	0.323±0.001^{b}	0.645±0.001^{a}	0.206±0.001^{c}
Protocatechuic acid	0.102±0.001^{d}	0.838±0.001^{b}	1.464±0.001^{a}	0.505±0.001^{c}
Gentisic acid	0.118±0.001^{d}	0.352±0.001^{a}	0.628±0.001^{a}	0.345±0.001^{c}
p-hydroxy benzoic acid	0.053±0.001^{a}	0.043±0.001^{b}	ND	0.013±0.001^{c}
Catechin	0.149±0.001^{d}	0.494±0.001^{b}	0.726±0.001^{a}	0.484±0.001^{c}
Chlorogenic acid	0.019±0.001^{c}	0.342±0.001^{a}	0.055±0.001^{b}	0.055±0.001^{b}
Vanillic acid	0.031±0.001^{d}	1.155±0.001^{a}	0.058±0.001^{c}	0.185±0.001^{b}
Caffeic acid	0.184±0.001^{a}	ND	0.044±0.001^{b}	ND
Syringic acid	0.033±0.001^{d}	0.556±0.001^{a}	0.236±0.001^{c}	0.259±0.001^{b}
p-coumaric acid	1.997±0.001^{b}	1.366±0.001^{c}	5.080±0.001^{a}	0.463±0.001^{d}
Ferulic acid	0.482±0.001^{c}	0.785±0.001^{b}	2.712±0.001^{a}	0.020±0.001^{d}
Sinapic acid	0.112±0.001^{b}	0.001±0.001^{d}	0.827±0.001^{a}	0.051±0.001^{c}
Salicylic acid	0.036±0.001^{b}	ND	0.376±0.001^{a}	ND
Naringin	0.164±0.001^{d}	3.413±0.001^{c}	7.242±0.001^{a}	5.393±0.001^{b}
Rutin	0.075±0.001^{c}	ND	1.280±0.001^{a}	0.468±0.001^{b}
Ellagic acid	0.567±0.001^{d}	15.295±0.001^{b}	36.288±0.001^{a}	11.400±0.001^{c}
Myricetin	0.415±0.001^{a}	0.331±0.001^{b}	0.308±0.001^{c}	0.107±0.001^{d}
Quercetin	0.399±0.001^{a}	0.067±0.001^{b}	ND	0.025±0.001^{c}
Naringenin	0.020±0.001^{d}	2.454±0.001^{a}	0.865±0.001^{b}	0.045±0.001^{c}
Apigenin	0.370±0.001^{c}	2.139±0.001^{a}	0.515±0.001^{b}	0.153±0.001^{d}
Kaempferol	0.172±0.001^{a}	0.040±0.001^{d}	0.160±0.001^{b}	0.079±0.001^{c}

Each value was obtained by calculating the average of triplicated experiments and data are presented as mean±SEM. Statistical analysis were carried out by Tukey's test at 95% confidence level and statistical significance were accepted at the $P<0.05$ level. The superscript letter $^{a, b, c}$ and ^{d} denotes the significance of various parameters. Letter 'a' is most significant followed by b, c and d consecutively. ND: Not detected; SEM: Standard error of the mean

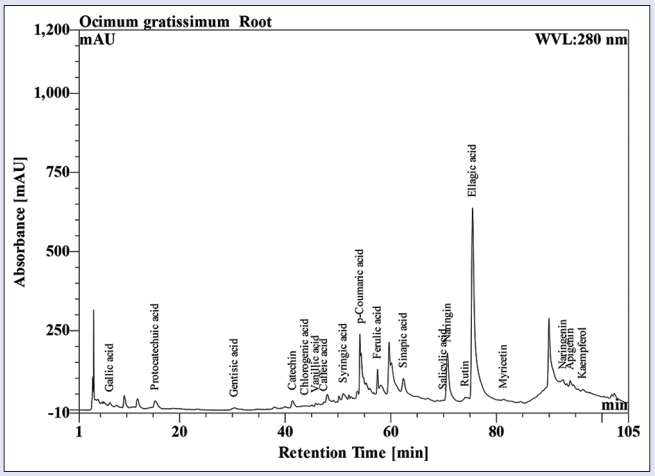

Figure 5: High-performance liquid chromatography chromatogram of root extract of *Ocimum gratissimum* L.

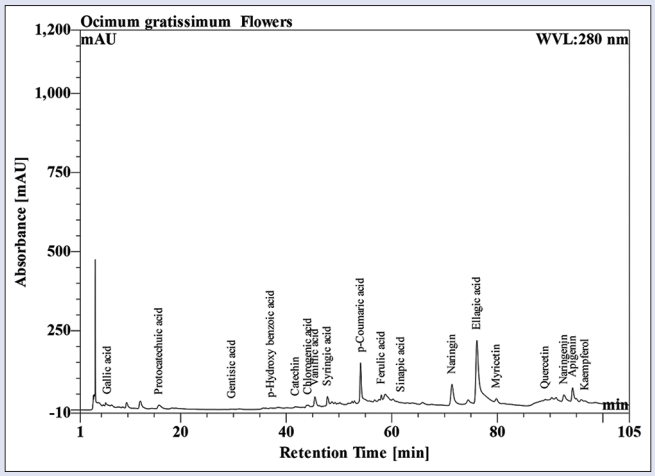

Figure 6: High-performance liquid chromatography chromatogram of flower extract of *Ocimum gratissimum* L.

benzoic acid and quercetin were absent only in root extract. Rutin was found to be absent in only stem extract. The leaf extract of the plant contains highest amount of p-hydroxy benzoic acid (0.053 ± 0.001 µg/mg DPM), caffeic acid (0.184 ± 0.001 µg/mg DPM), myricetin (0.415 ± 0.001 µg/mg DPM), quercetin (0.399 ± 0.001 µg/mg DPM), and kaempferol (0.172 ± 0.001 µg/mg DPM). The stem extract was found to show highest amount of gentisic acid (0.352 ± 0.001 µg/mg DPM), chlorogenic acid (0.342 ± 0.001 µg/mg DPM), vanillic acid (1.155 ± 0.001 µg/mg DPM), syringic acid (0.556 ± 0.001 µg/mg DPM), and naringenin (2.454 ± 0.001 µg/mg DPM). The highest amount of gallic acid was recorded in the root extract of the plant (0.645 ± 0.001 µg/mg DPM), protocatechuic acid (1.464 ± 0.001 µg/mg DPM), gentisic acid (0.628 ± 0.001 µg/mg DPM), catechin (0.726 ± 0.001 µg/mg DPM), p-coumaric acid (5.080 ± 0.001 µg/mg DPM), ferulic acid (2.712 ± 0.001 µg/mg DPM), sinapic acid (0.827 ± 0.001 µg/mg DPM), salicylic acid (0.376 ± 0.001 µg/mg DPM), naringin (7.242 ± 0.001 µg/mg DPM), rutin (1.280 ± 0.001 µg/mg DPM), and ellagic acid (36.288 ± 0.001 µg/mg DPM).

The HPLC analysis detected the presence of gallic acid which is generally found in plants either in the form of esters or in the free states which goes about as a pioneering anticancerous agent. Some normal vegetables too have some amount of gallic acids which is comparable to the amount of gallic acid in methanol extract present in all parts of the plant, such as chili pepper (3.33 mg/g), lemon (2.03 mg/g), spinach (1.82 mg/g), onion bulb (1.55 mg/g), cabbage (0.49 mg/g), and so on.[6,15] The presence of protocatechuic acid in the plant can be concluded to be helpful in treating different

diseases such as neurodegenerative and hepatic disorders[16] The occurrence of p-hydroxybenzoic acid in the plant also justifies its usage againt liver ailments such as cirrhosis of the liver and liver malignancy[6,17] The usage consumption of this plant containing chlorogenic acid may be useful in decreasing the glucose levels, thereby serving as an antidiabetic agent.[18] The plant extracts were found to contain catechin which demonstrates that this plant may be added to cell reinforcement and restorative properties.[19] The occurrence of vanillic acid in the plant which was investigated may get related to having hepatoprotective activities.[20] The presence of caffeic acid helps increasing the production of collagen, premature aging, and antimicrobial and antioxidant activity.[21] The noticeable amount of syringic acid in the plant extract is remarkable for its antimalignancy, hostile to proliferative, narcotic, decongestant, and hepatoprotective activities.[22] p-coumaric acid has been measured in OG which demonstrates its defensive capacity to reduce the formation of carcinogenic nitrosamines in the stomach.[23] The ferulic acid which is one of the significant phenolics is exceptionally found in this plant promptly brings down the cholesterol level in serum and builds sperm reasonability[24] The presence of sinapic acid was also recognized in the plant which has antimicrobial and anticancerous.[25] Rutin is a polyphenolic compound with glycosidic linkage having natural impacts having antidiabetic effects[26] and anticancerous properties.[27] A sufficient amount of rutin in this plant puts forward their potentiality as helpful operators and justifies the legends' application. An immense amount of ellagic acid was recognized in the plant under examination and utilization of it would be valuable for antimutagenic, antimicrobial, and cell reinforcement properties and inhibitors of human immunodeficiency infection.[25] The plant parts also contain a good measure of myricetin and can be used as an antidiabetic agent.[28] An extensive level of quercetin recognized in the plant under examination can be utilized for anticancerous,[29] antihistamine, and also anti-inflammatory activities.[30] The presence of apigenin might be helpful against cardiovascular diseases, neurological disorders, and mutagenesis.[31] The prominent presence of kaempferol can be utilized to prevent the lipid proteins of low-density being oxidized, thereby indicating a potential defensive role in atherosclerosis, cardiovascular maladies, malignancy, and so on.[32]

CONCLUSION

According to the above findings, it can be concluded that the plant OG can be exploited as a natural resource of antioxidants. The antioxidant and nutraceutical potentialities of this plant can promote its selection in such a group of plants which are used as nutritional supplements in food industry as well as in development of antioxidant-based drugs by pharmaceutical industries.

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Conflicts of interest

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REFERENCES

1. Omodamiro OD, Ekeleme CM. Comparative study of *in vitro* antioxidant and antimicrobial activities of *Piper guineense, Curcuma longa, Gongronemalati folium, Allium sativum, Ocimum gratissimum*. World J Med Med Sci 2013;1:51-69.
2. Torel J, Cillard J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radicals. Phytochem 1996;25:383-5.
3. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, *et al*. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod 1998;61:71-6.
4. Usunobun U, Uwadiae E. *In vitro* medicinal studies on *Ocimum gratissimum* leaves. ARC J Pharma Sci 2016;2:1-5.
5. Seal T, Chaudhuri K, Pillai B, Chakrabarti S, Mondal T, Auddy B. Evaluation of antioxidant activities, toxicity studies and the DNA damage protective effect of various solvent extracts of *Litsea cubeba* fruits. Heliyon 2020;6:1-9.
6. Datta S, Sinha BK, Bhattacharjee S, Seal T. Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants. Heliyon 2019;5:1-37.
7. Sahreen S, Khan MR, Khan RA. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. Food Chem 2010;122:1205-11.
8. Florence OJ, Adeolu AA, Anthony JA. Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus*. Rec Nat Prod 2011;5:29-42.
9. Chiu YW, Lo HJ, Huang HY, Chao PY, Hwang JM, Huang PY, *et al*. The antioxidant and cytoprotective activity of *Ocimum gratissimum* extracts against hydrogen peroxide-induced toxicity in human HepG2 cells. J Food Drug Anal 2013;2:252-60.
10. Oriakhi K, Oikeh EI, Ezeugwu N, Anoliefo O, Aguebor O, Omoregie ES. *In vitro* antioxidant activities of extracts of *Vernonia amygdalina* and *Ocimum gratissimum* leaves. J Pharm Bioresour 2014;11:58-65.
11. Ayodele OD, Oyegbade O, Oseni SR. Phytochemical analysis and antioxidant activities of dry and fresh leaves of *Petivera alliacea* and *Ocimum gratissimum*. Int J Sci 2015;24:1-3.
12. Prasad MP, Jayalakshmi K, Rindhe GG. Antibacterial activity of *Ocimum species* and their phytochemical and antioxidant potential. Int J Microbiol Res 2012;4:302-7.
13. Venuprasad MP, Kandikattu HK, Razack S, Khanum F. Phytochemical analysis of *Ocimum gratissimum* by LC-ESI-MS/MS and its antioxidant and anxiolytioc effects. S Afr J Bot 2014;92:151-8.
14. Abaza L, Youssef BN, Manai H, Haddada FM, Methenni K, Zarrouk M. Che´toui olive leaf extracts: Influence of the solvent type on phenolics and antioxidant activities. Grasas Aceites 2011;62:96-104.
15. Romaric GB, Fatoumata AL, Oumou HK, Mamounata D, Imael HN, Mamoudou HD. Phenolic compounds and antioxidant activities in some fruits and vegetables from Burkina Faso. Afr J Biotechnol 2011;10:13543-7.
16. Kakkar S, Bais S. A review on protocatechuic Acid and its pharmacological potential. ISRN Pharmacol 2014;26:1-9.
17. Tverdal A, Skurtveit S. Coffee intake and mortality from liver cirrhosis. Ann Epidemiol 2003;13:419-23.
18. Uma DB, Ho CW, Wan WM, Aida WM. Optimization of extraction parameters of total phenolic compounds from Henna (*Lawsonia inermis*) leaves. Sains Malays 2010;39:119-28.
19. Seal T. Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India. J Appl Pharm Sci 2016;6:157-66.
20. Kim SJ, Kim MC, Um JY, Hong SH. The beneficial effect of vanillic acid on ulcerative colitis. Molecules 2010;15:7208-17.
21. Magnani C, Isaac VL, Correa MA, Salgado HR. Caffeic acid: A review of its potential use in medications and cosmetics. Anal Methods 2014;6:3203-10.
22. Vinayagam R. Preventive effect of Syringic acid on hepatic marker enzymes and lipid profile against acetaminophen-induced hepatotoxicity rats. Int J Pharm Biol Arch 2010;1:393-8.
23. Ramadoss KS, Devadasu C, Srinivasa Babu P. Isolation, characterization and RPHPLC estimation of p-coumaric acid from methanolic extract of Durva Grass (*Cynodon dactylon* L) (Pers.). Int J Anal Chem 2015;2015:1-7.
24. Mussatto G, Dragone I, Roberto C. Ferulic and p-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. Ind Crop Prod 2007;25:231-7.
25. Sepulveda L, Ascacio A, Rodríguez-Herrera RA, Aguilera-Carbo A, Aguilar Cristobal N. Ellagic

acid: Biological properties and biotechnological development for production processes. Afr J Biotechnol 2012;10:4518-23.

26. Srinivasan K, Kaul CL, Ramarao P. Partial protective effect of rutin on multiple low dose streptozotocin-induced diabetes in mice. Indian J Pharmacol 2005;37:327-8.
27. Lin JP, Yang JS, Lin JJ, Lai KC, Lu HF, Ma CY, *et al*. Rutin inhibits human leukemia tumor growth in a murine xenograft model *in vivo*. Environ Toxicol 2012;27:480-4.
28. Ong KC, Khoo HE. Biological effects of myricetin. Gen Pharmacol 1997;29:121-6.
29. D'Andrea G. Quercetin: A flavonol with multifaceted therapeutic applications? Fitoterapia 2015;106:256-71.
30. Seal T. HPLC determination of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of *Zanthoxylum acanthopodium*, a wild edible plant of Meghalaya. Int J Pharm Pharm Sci 2016b; 8:103-9.
31. Mohammad A, Elham KK. Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. Arch Adv Biosci 2013;4:119-38.
32. Calderón-Montaño JM, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. Mini Rev Med Chem 2011;11:298-344.