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In Vitro Comparative Analysis of Antioxidant Activities in Ethanolic Extracts of Centella Asiatica Linn., Ocimum Basilicum, Ocimum Sanctum, and Camellia Sinensis

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ABSTRACT

Introduction: To comparatively evaluate the antioxidant potential of ethanolic extracts of Centella asiatica Linn. (CA), Ocimum basilicum Linn. (OB), Ocimum sanctum (OS), and Camellia sinensis (CS) using multiple in vitro assays.

Materials and Methods: Ethanolic extracts of CA, OB, OS, and CS were prepared and subjected to in vitro antioxidant analysis, including total antioxidant capacity, DPPH radical scavenging, nitric oxide radical scavenging, reducing power, and superoxide anion radical scavenging assays using UV spectrophotometry. Antioxidant activities were recorded at concentrations up to 1000 µg/ml. All assays were performed across a concentration range of 10–1000 µg/ml using serial dilutions.

Results: Total antioxidant activity (109.40 ± 0.15 % inhibition), DPPH scavenging (107.88 ± 9.32 % inhibition), nitric oxide scavenging (98.27 ± 9.48 % inhibition), reducing power (absorbance: 93.12 ± 9.12 at 700 nm), and superoxide scavenging (88.93 ± 8.14 % inhibition). The differences between groups were statistically significant ($p < 0.001$).

Conclusion: CA demonstrated superior antioxidant activity compared to OB, OS, and CS. Its high antioxidant potential suggests its promise as a natural therapeutic agent for preventing oxidative stress–related diseases.

Keywords: Antioxidants; Centella asiatica; Camellia sinensis; Ocimum basilicum; Ocimum sanctum; Oxidative stress.

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Introduction

Free radicals, or reactive oxygen species (ROS), are unstable molecules produced during normal metabolic processes and in response to environmental factors. Excessive ROS generation leads to oxidative stress, resulting in cellular damage and dysfunction [1]. Common triggers include inflammation, pollution, UV exposure, smoking, infections, malignancy, and degenerative diseases [2,3]. Antioxidants are compounds capable of neutralizing free radicals, thereby preventing or minimizing oxidative damage [4]. These can be endogenously synthesized or acquired exogenously from dietary and medicinal plant sources [5,6]. Natural antioxidants are favored over synthetic variants due to concerns regarding the carcinogenicity and toxicity of the latter [7].

Several medicinal plants, including Centella asiatica, Ocimum basilicum, Ocimum sanctum, and Camellia sinensis, have long been recognized for their antioxidant and therapeutic propertie [5,8]. Centella asiatica exhibits neuroprotective, anti-inflammatory, and antimicrobial activities. Ocimum basilicum and Ocimum sanctum, rich in phenolic compounds, show potent antioxidant and adaptogenic effects, while Camellia sinensis (tea) is well-established for its polyphenol-based free radical–scavenging properties [9,10]. Given the pivotal role of oxidative stress in chronic diseases, this study aimed to comparatively assess the antioxidant activities of ethanolic extracts of these four medicinal plants using in vitro assays, including total antioxidant capacity, DPPH radical scavenging, nitric oxide scavenging, reducing power, and superoxide radical scavenging.

Materials and Methods

Chemicals and Reagents

All analytical-grade reagents and solvents, including sulfuric acid, ammonium molybdate, DPPH, ascorbic acid, sodium nitroprusside, phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, nitro blue tetrazolium, NADH, phenazine methosulphate, quercetin, and absolute ethanol were procured from HiMedia® and Sigma-Aldrich®.

Plant Material

Fresh leaves of CA, OB, and OS were collected locally from Moradabad, India, while CS leaves were obtained from Assam. Botanical authentication was performed at the Department of Pharmacy, and voucher specimens (TMU/PH/PG/2022/012) were deposited.

Preparation of Plant Extracts

Leaves were washed, oven-dried at 37°C for 7 days, and pulverized into a coarse powder. The powdered material was macerated in ethanol at a solvent-to-plant ratio of 6:1 (v/w) for 72 hours at room temperature with intermittent shaking. The extracts were then filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator at 50°C. The concentrated extracts were stored in a desiccator until further use.

Preparation of Extract Solutions

Extract stock solutions (10 mg/ml) were prepared in dimethyl sulfoxide and serially diluted with distilled water to obtain concentrations ranging from 10 to 1000 µg/ml.

Antioxidant Assays

Total Antioxidant Activity

All antioxidant assays were performed across the full concentration range of 10–1000 µg/ml for each extract to ensure uniform comparative evaluation. Extracts (0.2 ml) were mixed with reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate), incubated at 95°C for 90 min, and absorbance was measured at 695 nm. Standard antioxidants such as Quercetin served as a standard antioxidant control for comparison

DPPH Radical Scavenging Assay

Extracts (0.1 ml) were reacted with 1.9 ml of 200 µM DPPH, incubated for 20 min, and absorbance was recorded at 517 nm. Quercetin was used as a control.

Nitric Oxide Scavenging Assay

Extracts (1 ml) were incubated with 0.5 ml sodium nitroprusside (10 mM) at 37°C for 3 hours. After adding Griess reagent, absorbance was measured at 546 nm.

Reducing Power Assay

Extracts (1 ml) were combined with 2.5 ml potassium ferricyanide (1%) and 2.5 ml phosphate buffer (pH 6.6), incubated at 50°C for 20 min, then treated with 2.5 ml 10% TCA. The supernatant was mixed with distilled water and ferric chloride (0.1%) and absorbance measured at 700 nm.

Superoxide Anion Scavenging Assay

Extracts (1 ml) were mixed with 0.5 ml Tris-HCl buffer (16 mM, pH 8.0), 0.3 mM NBT, 0.12 mM phenazine

methosulphate, and incubated for 5 min. Absorbance was read at 560 nm.

Statistical Analysis

Assays were performed in triplicate. Results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA followed by Student's t-test via SPSS version 22.0. A p-value < 0.001 was considered statistically significant

Results

The assays were carried out in triplicate, and the mean and standard deviation were calculated using a one-way analysis of variance and Student's t-test. The results were considered statistically significant with a p-value less than 0.001. The antioxidant activity of the three ethanolic extracts was analyzed using the SPSS 22.0 version software. The ethanolic extracts of highest at 1000 µg/ml, with all assays consistently performed across concentrations ranging from 10 to 1000 µg/ml CA, OB, OS, and CS showed the presence of antioxidant activity. CA, OS, and OB exhibited increased antioxidant activity with increasing concentrations in a dose-dependent manner; highest at 1000 µg/ml. CA demonstrated the highest activity through the total antioxidant assay, DPPH assay, nitric oxide scavenging activity, reducing power assay, and, superoxide radical scavenging activity followed by OS and OB. CS had the lowest antioxidant activity among all groups (Table and graph 1-5).

In our investigation, we focused on CA, particularly at a concentration of 1000 µg/ml, and measured the highest activity than any other plant extract with total antioxidant activity of 109.40 ± 0.15 % inhibition, DPPH free radical scavenging of 107.88 ± 9.32 % inhibition, nitric oxide radical scavenging of 98.27 ± 9.48 % inhibition, reducing power (absorbance: 93.12 ± 9.12 at 700 nm), and superoxide anion radical scavenging of 88.93 ± 8.14 % inhibition. These findings were not only substantial but also statistically significant with a p-value less than 0.001. At this specific concentration, these findings highlight the potent antioxidant abilities of CA. These results hold promise for potential applications in addressing issues related to oxidative stress.

Table 1. Intergroup comparison of total antioxidant activity in ethanolic extracts of different groups using one-way ANOVA.

Concentration	Group I (CA)	Group II (OS)	Group III (OB)	Group IV (CS)	P value	Significance
1000 µg	109.40 ± 0.15	80.66 ± 0.23	73.55 ± 0.12	93.70 ± 0.14	0.001	Significant
800 µg	107.30 ± 0.67	78.74 ± 0.34	65.86 ± 0.45	93.55 ± 0.13	0.001	Significant
400 µg	95.60 ± 0.34	66.43± 0.21	61.82 ± 0.37	84.32 ± 0.15	0.001	Significant
200 µg	94.20 ± 0.45	61.20 ± 0.26	49.51 ± 0.28	86.05 ± 0.19	0.001	Significant
100 µg	47.20 ± 0.11	36.24 ± 0.19	30.81 ± 0.12	36.05 ± 0.18	0.001	Significant
50 µg	44.10 ± 0.17	28.55 ± 0.11	13.16 ± 0.13	33.16 ± 0.12	0.001	Significant
10 µg	41.80 ± 0.16	17.40 ± 0.14	3.55 ± 0.17	32.40 ± 0.13	0.001	Significant

Table 2. Intergroup comparison of DPPH assay in ethanolic extracts of different groups using one-way ANOVA.

Concentration	Concentration	Concentration	Concentration	Concentration	Concentration	Concentration
1000 µg	1000 µg	1000 µg	1000 µg	1000 µg	1000 µg	1000 µg
800 µg	800 µg	800 µg	800 µg	800 µg	800 µg	800 µg
400 µg	400 µg	400 µg	400 µg	400 µg	400 µg	400 µg
200 µg	200 µg	200 µg	200 µg	200 µg	200 µg	200 µg
100 µg	100 µg	100 µg	100 µg	100 µg	100 µg	100 µg
50 µg	50 µg	50 µg	50 µg	50 µg	50 µg	50 µg

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Table 3. Intergroup comparison of nitric oxide radical scavenging assay in ethanolic extracts of different groups using one-way ANOVA.

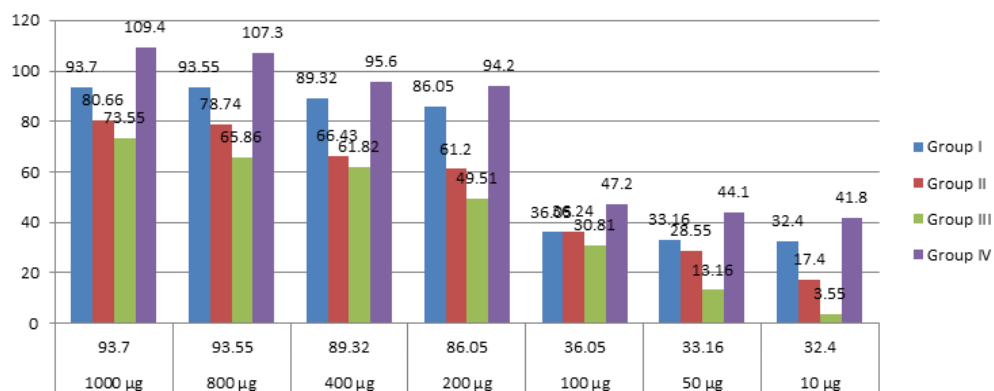
Concentration	Group I (CA)	Group II (OS)	Group III (OB)	Group IV (CS)	P value	Significance
1000 µg	98.27 ± 9.48	78.18 ± 8.21	69.93 ± 6.74	83.60 ± 7.61	0.001	Significant
800 µg	90.23 ± 7.89	65.95 ± 5.67	60.45 ± 5.61	71.76 ± 7.76	0.001	Significant
400 µg	68.00 ± 5.62	59.22 ± 5.69	35.37 ± 8.61	63.41 ± 6.74	0.001	Significant
200 µg	59.65 ± 5.67	37.20 ± 4.58	32.31 ± 6.12	45.67 ± 4.21	0.001	Significant
100 µg	51.70 ± 3.17	35.58 ± 4.51	27.82 ± 3.87	42.62 ± 4.32	0.001	Significant
50 µg	41.70 ± 3.19	28.15 ± 1.46	22.62 ± 3.45	38.93 ± 2.12	0.001	Significant
10 µg	36.19 ± 2.17	21.73 ± 0.65	15.89 ± 2.23	29.04 ± 0.98	0.001	Significant

Table 4. Intergroup comparison of reducing power in ethanolic extracts of different groups using one-way ANOVA.

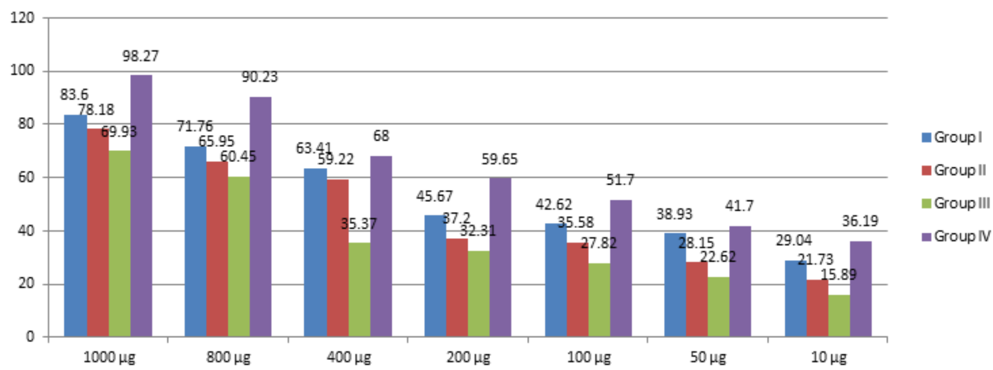
Concentration	Group I (CA)	Group II (OS)	Group III (OB)	Group IV (CS)	P value	Significance
1000 µg	93.12 ± 9.12	75.28 ± 5.38	68.36 ± 7.65	88.36 ± 7.62	0.001	Significant
800 µg	85.47 ± 8.12	72.78 ± 3.39	63.93 ± 6.59	82.59 ± 8.51	0.001	Significant
400 µg	72.97 ± 7.21	57.01 ± 2.01	48.51 ± 6.19	67.47 ± 4.32	0.001	Significant
200 µg	65.21 ± 6.42	45.47 ± 1.62	35.09 ± 5.08	60.01 ± 3.78	0.001	Significant
100 µg	56.00 ± 5.28	24.51 ± .45	18.55 ± 2.34	37.59 ± 3.98	0.001	Significant
50 µg	38.11 ± 3.21	22.43 ± 1.65	15.01 ± 2.15	29.97 ± 2.92	0.001	Significant
10 µg	26.71 ± 2.13	11.43 ± 1.35	8.63 ± 1.52	14.51 ± 1.47	0.001	Significant

Table 5. Intergroup comparison of superoxide radical scavenging assay in ethanolic extracts of different groups using one-way ANOVA.

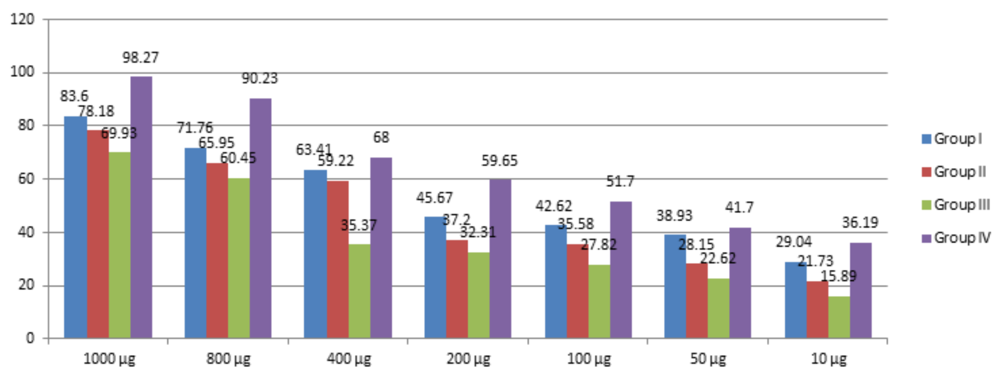
Concentration	Group I (CA)	Group II (OS)	Group III (OB)	Group IV (CS)	P value	Significance
1000 µg	88.93 ± 8.14	71.85 ± 5.21	53.74 ± 3.87	77.59 ± 4.64	0.001	Significant
800 µg	87.97 ± 9.15	57.84 ± 6.32	44.13 ± 5.43	71.82 ± 6.74	0.001	Significant
400 µg	82.59 ± 8.98	59.15 ± 4.84	50.86 ± 4.97	69.51 ± 5.12	0.001	Significant
200 µg	80.66 ± 8.39	51.38 ± 4.51	42.78 ± 4.58	61.24 ± 5.84	0.001	Significant
100 µg	38.36 ± 2.90	30.75 ± 3.31	22.40 ± 3.20	35.32 ± 3.47	0.001	Significant
50 µg	10.09 ± 2.90	6.54 ± 1.37	4.78 ± 2.12	8.86 ± 1.21	0.001	Significant
10 µg	6.97 ± 1.38	3.95 ± 1.11	2.70 ± 1.65	5.82 ± 0.96	0.001	Significant



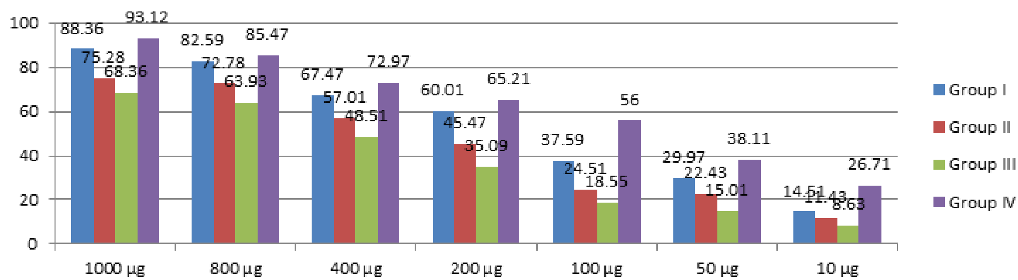
Graph 1. Comparison of total antioxidant activity in ethanolic extracts.



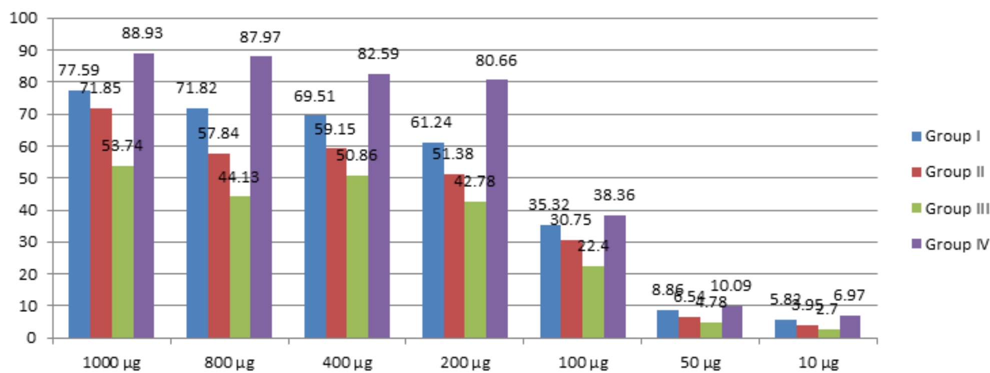
Graph 2. Intergroup comparison of DPPH assay in ethanolic extracts of different groups.



Graph 3. Intergroup comparison of nitric oxide radical scavenging assay in ethanolic extracts of different groups.



Graph 4. Intergroup comparison of reducing power in ethanolic extracts of different groups.



Graph 5. Intergroup comparison of superoxide radical scavenging assay in ethanolic extracts.

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Discussion

DNA damage is caused by oxidative stress on cellular organelles, membrane lipids, and proteins. This increases the risk of cardiovascular diseases. To protect cells from damage, maintaining high levels of intracellular antioxidants is essential. Several medications, including synthetic retinoids, steroids, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), have been used for the treatment of diseases, but they have a large number of positive side effects [13]. Currently, researchers are focusing on creating natural antioxidants that are effective in combating this scenario, which should be easy to obtain, inexpensive, and have few adverse effects. This study investigates the antioxidant activity of four plants- CA, OB, OS, and CS. The research evaluates and compares the antioxidant activity of these extracts using various models. The study employs ultraviolet (UV) spectrometry to measure the antioxidant activity of ethanolic extracts of these plants. The research demonstrates that these plants possess significant antioxidant capacity, with CA exhibiting the highest antioxidant activity, followed by OS and OB. CS, on the other hand, has the lowest antioxidant activity.

We found that all four ethanolic extracts of CA, OS, CS, and OB demonstrated significant antioxidant activity in a dose-dependent manner, with the maximum activity at maximum activity at 1000 μg/m. While many studies have examined the correlation between TPC and antioxidant activity, this study opted for different approaches. Radical scavenging of DPPH and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) are two common methods for evaluating antioxidant activity. Radical scavenging of DPPH and ABTS are two common methods for evaluating antioxidant activity. The study explains that oxidative stress significantly contributes to numerous diseases as it results from free radicals generated during regular metabolic processes. The antioxidant system protects cells from oxidant-induced damage during metabolism. Enzymatic and non-enzymatic antioxidants, such as SOD and ascorbic acid, remove free radicals and prevent cellular damage. Phenolic components also have anti-oxidant properties. CA contains active chemicals that prevent oxidative damage to essential organs [14]. The study shows that CA has more antioxidant potential than other plants. The ethanol extract of CA contains terpenes like madecassic acid, Asiatic acid, and three Asiaticosides, as well as phenolic constituents, most notably flavonoids and polyphenols, which explain the ethanol extract of CA's antioxidant activity. Phenolic compounds exhibit redox characteristics because of their ability to operate as hydrogen donors, reducers, and singlet oxygen quenchers. They also show strong metal chelation potential. Conversely, the oxidation-reduction abilities of polyphenols are crucial in neutralizing free radicals [15-17]. Natural antioxidants such as polyphenols found in fruits, vegetables, and herbs have been extensively studied for their health benefits. The findings of this study provide valuable insights into the potential practical applications of natural antioxidants in health and disease. By evaluating the antioxidant activity of various natural compounds, the study highlights their potential as preventive or therapeutic agents in reducing oxidative stress and preventing or managing diseases related to oxidative stress. The study suggests that incorporating a variety of antioxidant-rich foods into the diet can provide a wide range of health benefits, including reducing the risk of chronic diseases. Based on the findings of this study, several areas require further investigation to evaluate the potential of antioxidants.

The following text explores the gaps in our understanding of natural antioxidants and their potential application to improve human health. Firstly, there is a need for more research to determine the optimal dosage and formulation of natural antioxidants to achieve maximum bioavailability and efficacy. This research would also help identify the appropriate concentration and timing of antioxidant administration to achieve the desired health effects. Additionally, formulation studies can explore different delivery systems, such as encapsulation, to enhance the stability and bioavailability of antioxidants. Second, there is a need to compare and rank different natural antioxidants to identify the most potent and versatile antioxidants.

This comparative research would provide valuable information for the development of antioxidant-rich functional foods or dietary supplements. The study found that CA demonstrated higher antioxidant activity, likely due to its rich content of specific bioactive compounds such as terpenes, flavonoids, and polyphenols. These compounds possess strong antioxidant properties, capable of neutralizing free radicals and preventing oxidative damage to cells and tissues. Its high antioxidant potential suggests its promising role in preventing and managing diseases associated with oxidative stress, such as cancer, diabetes, and cardiovascular diseases. A limitation of the present study is the restricted comparison with a single standard antioxidant (quercetin). Inclusion of additional well-established controls such as ascorbic acid or butylated hydroxytoluene (BHT) would have provided a more

comprehensive benchmark for evaluating the relative antioxidant potency of the plant extracts. The comparative analysis performed in this study, evaluating CA, against OB, OS, and CS, reinforces earlier observations made in previous comparative studies [2,5,8,14-18]. We conclude that CA exhibited the highest antioxidant activity, which is consistent with previous research. The study hints at specific compounds within CA, such as Asiaticosides, flavonoids, and polyphenols, attributed to its superior antioxidant activity.

Conclusion

Our research found that the leaf extract of the CA plant has the highest level of antioxidant activity compared to extracts from other plants. This suggests that CA could be a valuable natural resource for developing new treatments for diseases caused by free radicals. However, as the present study is based on in vitro assays, the findings may not be directly extrapolated to in vivo conditions, and further animal and clinical studies are required to validate these results. More research is needed to identify which substances in CA are responsible for its antioxidant activity. Additionally, further in vivo and clinical studies are required to validate the therapeutic efficacy and safety of these plant extracts.

Conflict of Interest

There is no conflict of interest to declare.

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