

Phytochemical Study and Evaluation of the Antioxidant Activity of *Cymbopogon citratus* by Different Tests (*TAC, DPPH, ABTS*)

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How to cite this paper: Kamissoko, A., Kourouma, K., Traore, Z., Diallo, A. and Sakho, A.M. (2025) Phytochemical Study and Evaluation of the Antioxidant Activity of *Cymbopogon citratus* by Different Tests (*TAC, DPPH, ABTS*). *Journal of Agricultural Chemistry and Environment*, **14**, 295-306.

<https://doi.org/10.4236/jacen.2025.143020>

Received: June 3, 2025

Accepted: July 12, 2025

Published: July 15, 2025

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Abstract

Cymbopogon citratus is widely recognized for its many virtues, particularly in the fields of nutrition and health. It is frequently used in infusions, decoctions and essential oils, as well as for its antioxidant properties. The main aim of this study was to determine the chemical composition of *Cymbopogon citratus* extracts, as well as their antioxidant potential, both in terms of aqueous extracts and essential oil. To do this, *C. citratus* leaves were dried at room temperature, then pulverized using a grinder. The powder obtained was used to prepare three types of aqueous extracts in the form of: macerate, infused and decocted in a ratio of 10 g powdered sample to 100 ml water with an extraction yield of: (12.2%); (11.63%) and (9.25%) respectively. Physicochemical analysis using photo-colorimetric techniques, including Folin-Ciocalteu and aluminum chloride methods, allowed to know the phytochemistry of the various *C. citratus* aqueous extracts prepared, with the marked presence of several bioactive compounds such: alkaloids, flavonoids, tannins, coumarins, saponosides, leucocyanines, sterols and triterpenes, carotenoids, fixed oils and fats and the absence of anthraquinones, anthocyanins, gums and mucilages and emodins. Total polyphenols, measured in gallic acid equivalents (mg EAG/100 of RP), showed a higher level of content in the infused (822.17) followed by the macerate (481.59). Flavonoids and tannins in milligrams of catechin equivalents (mg EC/100g of RP) are high in the infused and with: (283.04) and (235.83) respectively. However, tannins in mg EC/100g of RP in the macerate are high with (3.28) and (3.24) in the decoction. The DPPH (Diphenylpicrylhydrazyl),

ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and TAC (Total Antioxidant Capacity) methods showed that the extract richest in polyphenols showed the highest antioxidant activity.

Keywords

Phytochemistry, *Cymbopogon citratus*, Extracts, Antioxidant

1. Introduction

Cymbopogon citratus, commonly known as citronella, belongs to the Poaceae family. Native to south-west Asia, in particular southern India and Sri Lanka, this species is now widespread throughout the world, particularly in tropical and sub-tropical areas [1]. It is one of the most extensively cultivated aromatic and medicinal plants in these areas due to its recognized therapeutic properties. *C. citratus* has been used both as a culinary herb and in natural medicine for the treatment and prevention of various ailments. Recent research has confirmed its multiple bioactive properties, including antibacterial, antifungal, antioxidant, antiseptic, anti-inflammatory, analgesic, and antipyretic effects, making it a valuable subject in phytotherapeutic studies [2] [3]. Aromatic and medicinal plants are potential sources of natural antioxidants thanks to the secondary metabolites they contain, such as polyphenols, carotenoids and essential oils [4]. Despite its traditional use as an herbal tea in local communities, *C. citratus* remains underexploited in the beverage industry in Guinea, where the market is currently dominated by synthetic formulations of varying and often uncertain quality. This study aims to conduct a phytochemical screening and evaluate the antioxidant potential of aqueous extracts of *C. citratus*, with the goal of promoting its integration into beverage products. Its major advantages include easy accessibility and a low risk of adverse effects. In addition to its use in infusions, the entire plant is often incorporated into traditional dishes for its distinctive lemony aroma and flavor [5]. Our findings highlight the plant's richness in phytochemicals and significant antioxidant potential. Among various extraction methods, infusion proved to be the most effective for yielding extracts high in polyphenols and flavonoids [5] [6]. Antioxidants, even in small concentrations, play a crucial role in inhibiting or regulating oxidation processes that contribute to food spoilage and the development of degenerative diseases [7]. Polyphenols are compounds renowned for their ability to neutralise free radicals, thereby mitigating oxidative stress and inflammation. According to [6], their study suggests that *C. citratus* extracts and their polyphenols have the capacity to prevent the absorption of cholesterol in the intestine by micellar destruction. In our study, various tests were used to determine antioxidant activity: DPPH, or 1,1-diphenyl-2-picrylhydrazyl, is a stable free radical widely used in tests to assess antioxidant activity [8]. This method is used to test the effectiveness of antioxidants present in food extracts, drinks and natural compounds, by measuring

their ability to reduce this free radical [7] [9]. ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) is a chemical compound commonly used in antioxidant capacity evaluation tests. It is often used in the form of a cationic radical (ABTS-+), which allows it to react with antioxidants present in samples, enabling their ability to neutralise free radicals to be measured [10] [11]. TAC (total antioxidant capacity), on the other hand, is an analyte frequently used to assess the antioxidant status of biological samples and enables the antioxidant response against free radicals produced in a given disease to be evaluated [11]. Our results demonstrated that the extract with the highest polyphenol content also exhibited the greatest antioxidant activity across all tests, reinforcing the therapeutic and nutritional potential of *C. citratus*.

2. Material and Methods

2.1. Presentation of the Study Area

The prefecture of Coyah, located in the west of Guinea in the natural region of Guinea Maritime, is part of the administrative region of Kindia. It covers an area of around 2166 km² and is 50 km from the capital, Conakry. Its geographical coordinates are 9°42' N, 13°23' W. In 2016, the population was estimated at 281,757, with a density of around 130 inh./km². The capital is the town of Coyah, the administrative and economic center of the prefecture. Coyah is marked by mountainous terrain, notably Mount Kakoulima, the highest point in the area. Administratively, the prefecture is divided into four sub-prefectures: Coyah-Centre, Kouriah, Manéah and Wonkifong. The main ethnic groups are Soussous, Peuls and Malinkés. Coyah enjoys a strategic position thanks to its road network linking Conakry to the interior of the country and to Sierra Leone.

2.2. Framework of Studies

The Food Biochemistry and Natural Substances Laboratory, Faculty of Science and Technology, University of Science, Technology, and Engineering of Bamako served as a framework for studies.

2.3. Sample Collection

The plant material consisted of *C. citratus* leaves harvested in the prefecture of Coyah in August 2024. The samples were shade-dried at ambient temperature (25 - 30 °C) for 10 days, then ground using a Floria®-type mill. The resulting powder was used for extract preparation and subsequent analyses.

2.4. Extract Preparation

Extracts were prepared in three aqueous forms: maceration, infusion and decoction [12].

Decoction: Place 10 g of sample in 100 ml (10%) of distilled water in a beaker. Boil the mixture for 15 min, then filter. Add 50 ml of distilled water to the residue (pellet) and boil again for 15 min, then filter. Repeat with another 50 ml of distilled

water. Add the filtrates and concentrate (*i.e.* reduce the volume, if necessary) using a water bath or rotary evaporator maintained at 45 °C to obtain a volume of 60 ml, then freeze-dry.

Infusion: Place 10 g of sample in a beaker containing 100 ml of boiling distilled water. Leave the mixture at room temperature for approximately 15 - 20 min, then filter. Add 50 ml of boiling distilled water to the residue (pellet) and follow the same steps. Repeat the operation once with another 50 ml of boiling distilled water. Add the filtrates and concentrate (*i.e.* reduce the volume, if necessary) using a water bath or rotary evaporator maintained at 45 °C to obtain a volume of 60 ml, then freeze-dry.

Maceration: Place 10 g of sample in a beaker containing 100 ml of distilled water. Place the mixture in an ultrasonic bath (type P 30 H) at room temperature for approximately 15 - 20 min, then filter. Repeat the same operation 2 times with 50 ml distilled water. Add the filtrates and concentrate (*i.e.* reduce the volume if necessary) using a water bath or rotary evaporator maintained at 45 °C. Add the filtrates and concentrate (*i.e.* reduce the volume, if necessary) using a water bath or rotary evaporator (Bucchi CCT C21-901) maintained at 45 °C to obtain a volume of 60 ml, then freeze-dry.

$$\text{Extraction yield (\%)} = (\text{Freeze-dried extracts})/(\text{Test sample}) * 100$$

Essential oil

The essential oil of *C. Citratus* leaves was extracted by hydrodistillation using a Clevenger-type apparatus. A quantity of 200 g of dried leaves cut into small pieces was introduced into the flask. The hydrodistillation process was carried out for 3 h. The hydrolysate and essential oil (EO) were separated by decantation [13]. The essential oil (EO) was recovered in a glass tube and stored at room temperature. Yields were calculated using the extraction formula: $\text{EO} = (\text{EO (g)})/(\text{Test sample}) * 100$.

2.5. Phytochemical Screening

Phytochemical constituents were characterized by tube staining and precipitation reactions, using conventional reagents according to the qualitative test methods described by [14] [15]. The substances identified include: alkaloids, tannins, flavonoids, coumarins, saponins, sterols and triterpenes (Lieberman-Burchardt reaction), free anthraquinones (Bornträger reaction), terpenoids (Salkowski test), phytosterols (acetic anhydride test), carotenoids (Carr-Price test), gums and mucilages (alcohol test), leucoanthocyanins (iso-amyl alcohol test), emodins (anthraquinone group: caustic soda test) and quinones (HCl test).

2.6. Determination of Total Polyphenols

The determination of polyphenols was carried out using the colorimetric method described in [16]. A volume of 200 µl of extract or solution of gallic acid (20, 40, 60, 80 and 100 µg/l) was added to a tube, followed by the addition of 200 µl of Folin-Ciocalteu reagent. The mixture was diluted with 1 ml of distilled water and

incubated for 5 minutes. Next, 600 µl of sodium carbonate (Na_2CO_3) was added, and the mixture was incubated in the dark for 2 hours. Absorbances were measured against a blank prepared with distilled water under the same conditions at 750 nm using a spectrophotometer. The polyphenol content was expressed in mg of gallic acid equivalent per gram of plant material (g GAE/100 g DW).

2.7. Determination of Flavonoids

Total flavonoids were determined using the colorimetric method of [12]. A volume of 200 µl of diluted extract or catechin solution (20, 40, 60, 80 and 100 mg/l) was added to a tube containing 800 µl of distilled water. Next, 50 µl of a 5% sodium nitrite (NaNO_2) solution was added, followed by 50 µl of 10% aluminium chloride after 5 minutes. A volume of 400 µl of 1 M sodium hydroxide was added and the mixture stirred for 6 minutes. The solution was diluted with 1 ml of distilled water and shaken vigorously. Absorbance was measured against a blank prepared with distilled water at 510 nm using a spectrophotometer. Total flavonoid content was expressed as mg of catechin equivalents per gram of plant material (g EC/100 g DW).

2.8. Determination of Total Tannins

Total tannins were determined using the Folin Ciocalteu method reported by [17]. To a volume of 1 ml of extract (alcoholic or aqueous) or solution of gallic acid at different concentrations (100-200-300-400-500 µg/ml) in a test tube, 1 ml of distilled water was added, then 200 µl of ethanol was added, then 100 µl of Folin's reagent was added. The solution was homogenised and left to stand for 5 min. Next, 200 µl of 7% ammonium carbonate was added. Absorbances were read at 725 nm against a blank previously prepared with distilled water under the same conditions after one hour's incubation in the dark. Total tannin content was expressed as mg gallic acid equivalent per gram of plant material (g GAE/100 g DW).

2.9. Phosphomolybdate Test (TAC)

The Phosphomolybdate method was used to assess the total antioxidant capacity (TAC) of our extracts [12]. A volume of 100 µl of each extract was introduced into a tube and 900 µl of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was then added. The tubes were tightly closed and incubated at 95°C for 90 min.

Absorbances were read at 695 nm against a negative control which was prepared by mixing 900µl of the reagent solution and 100 µl of water and incubated under the same conditions as the sample. The results were expressed as milligram ascorbic acid equivalents per gram of crude powder (mg EAA/100 g DW).

2.10. DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was evalu-

ated according to the protocol described in (10). Using a 100 µg/ml extract solution, a calibration range was established. A volume of 50 µl of each extract at different concentrations was added to 1.95ml of DPPH metal solution (0.024 g/L). The absorbances of the solutions were measured at 515 nm using a spectrophotometer after 30 min incubation in the dark at room temperature against a negative control containing 50 µl of methanol and 1.95 ml of DPPH methanolic solution. Standard antioxidant molecules (ascorbic acid, quercetin, BHT) were used as a positive control. The free radical scavenging activity of DPPH (or percentage free radical inhibition) was calculated as follows: Inhibition (%) = [1 - (HE (g) Absorbance of sample)/(Absorbance of negative control)] Concentrations inhibiting 50% (IC50) of free radicals were deduced from the linear regression equations obtained.

2.11. ABTS Assay

A decolorization test applicable to aqueous and lipophilic extracts was used to carry out the trapping of the ABTS radical according to the method of [18] and updated by [12]. ABTS radicals were generated by mixing 7 mM ABTS and 2.45 mM potassium persulphate after incubation at room temperature (25°C) in the dark for 16 - 24 hours. The ABTS stock solution was prepared and its absorbance adjusted to 0.730. Then 50 µl of the extract was mixed with 1950 µl of the adjusted ABTS stock solution. After 2 h at room temperature in the dark, the absorbance at 734 nm was read. Standard antioxidant molecules (ascorbic acid, quercetin, BHT) were used as positive controls. ABTS radical scavenging activity and IC50 were calculated using the procedures described in the DPPH protocol.

2.12. Statistical Analysis

All experiments were repeated three times and the data analysed using Excel Advanced.

3. Results

Results of Types of Extracts

Our extracts were prepared in three aqueous forms; the yields of substances are summarized in (Table 1). Different chemical groups were identified (Table 2). The concentrations of total polyphenols, flavonoids and tannins in the various extracts for the dry weight are expressed in mg and this was made possible by the absorbances obtained with gallic acid and catechin (Table 3). Some methods were used to determine the power of antioxidant, total antioxidant capacity (Table 4), DPPH and ABTS radical inhibition rates by leaf extracts (Table 5), values of IC50 values (µg/mL) for anti-DPPH and anti-ABTS standard antioxidants (Table 6), summary of IC50 values (µg/mL) for anti-DPPH and anti-ABTS standard antioxidants (Table 7), concentrations of extracts et standards (Figure 1).

Table 1. Extraction yield.

	Macerate (%)	Infused (%)	Decocted (%)
Extraction yield (%)	12.2 ± 0.05	11.63 ± 0.035	9.28 ± 0.035

Table 2. Phytochemical screening.

Chemical groups	Extracts		
	Decocted	Infused	Macerated
Alkaloids	–	–	+
Flavonoids	+	+	+
Tannins	+	+	+
Coumarins	+	+	+
Anthocyanins	–	–	–
Leucocyanins	+	+	+
Quinones	–	–	–
Saponosides	+	+	+
Free anthraquinones	–	–	–
Emodines	–	–	–
Phytosterols	–	+	–
Sterols and triterpenes	+	+	+
Gums and mucilages	–	–	–
Caroténoïde	+	+	+
Fixed oils and fats	+	+	+

Note:(+): Present; (–): Absent.

Table 3. Total polyphenol, flavonoid and tannin levels in leaf extracts.

Extracts	Polyphenols (mg EAG/100 g DW)	Flavonoids (mg EC/100 g DW)	Tannins (mg EC/100 g DW)
Macerated	481.59 ± 32.22	199.44 ± 4.36	2.84 ± 0.01
Infused	822.17 ± 28.41	283.04 ± 3.67	3.24 ± 0.05
Decocted	279.25 ± 21.99	235.83 ± 1.79	3.28 ± 0.05

Table 4. Total antioxidant capacity (TAC) of extracts.

Extracts	Macerate	Infused	Decocted	HE
TAC (mg EAA/g PB)	8.32 ± 0.13	8.95 ± 0.38	7.64 ± 0.44	281.94 ± 5.29

Table 5. DPPH and ABTS radical inhibition rates by leaf extracts

Concentrations (µg/mL)		50	100	200	400	800	IC50 (µg/mL)
DPPH test	Macerated	34.17 ± 0.52	37.70 ± 0.73	41.52 ± 0.37	47.11 ± 1.54	55.69 ± 0.22	536.12 ± 7.27
		0.52	0.73	0.37	1.54	0.22	

Continued

DPPH test	Infused	34.56 ± 0.74	40.05 ± 0.98	44.36 ± 0.56	50.69 ± 1.53	68.68 ± 0.82	388.96 ± 18.40
	Decocted	32.89 ± 0.47	38.87 ± 0.98	45.10 ± 1.06	72.21 ± 0.64	78.48 ± 0.08	266.34 ± 6.04
	Macerated	11.19 ± 0.78	16.37 ± 0.59	26.28 ± 0.53	50.90 ± 0.92	88.62 ± 1.47	403.65 ± 4.95
ABTS test	Infused	17.93 ± 1.40	30.83 ± 1.84	33.61 ± 2.65	53.00 ± 0.31	88.28 ± 1.40	366.06 ± 2.02
	Decocted	4.98 ± 1.12	13.87 ± 1.83	25.50 ± 0.51	51.49 ± 0.82	89.84 ± 0.59	407.23 ± 3.64

Table 6. Rates of inhibition of DPPH and ABTS radicals by leaf Extracts.

Concentrations (µg/mL)	0.06	0.46	3.71	14.84	29.69	IC50 (µg/mL)
DPPH test	35.88 ± 0.59	42.50 ± 1.62	50.00 ± 0.29	66.32 ± 0.74	74.41 ± 0.88	5.20 ± 0.87
ABTS test	8.21 ± 1.25	25.40 ± 1.25	45.53 ± 1.12	55.11 ± 0.81	69.81 ± 1.52	19.19 ± 0.38

Table 7. Summary of IC50 values (µg/mL) for anti-DPPH and anti-ABTS standard antioxidants.

Standards	IC50 values (µg/ml)		
	Ascorbic acid	Quercetin	BHT
DPPH test	53.68 ± 2.09	19.19 ± 0.29	48.41 ± 0.78
ABTS test	88.78 ± 1.24	24.46 ± 0.50	46.18 ± 0.74

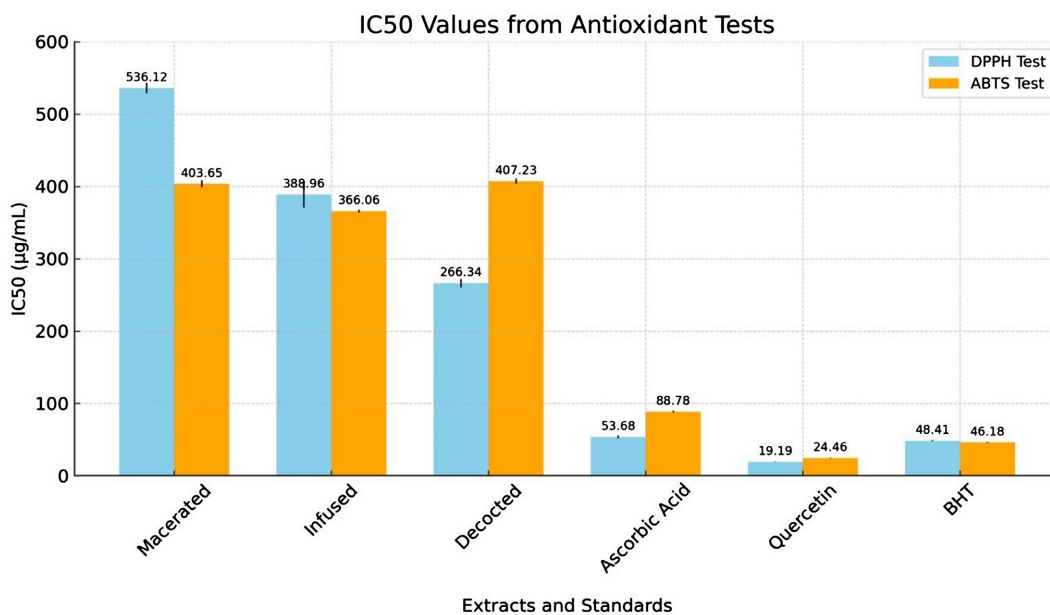


Figure 1. Values of extracts and standards concentrations.

4. Discussion

The results obtained in this study confirm that *Cymbopogon citratus* has notable antioxidant potential, which supports its traditional use in natural medicine. The comparative analysis of the three extraction methods (maceration, infusion, and decoction) revealed that infusion is the most effective method for extracting bioactive compounds, particularly polyphenols and flavonoids. These results are consistent with those of several recent studies, including that of [19] which showed that infusion promotes a better release of phenolic compounds from other medicinal plants used in infusions. The presence of many phytochemical groups as flavonoids, tannins, coumarins, saponosides, leucocyanins, carotenoids, sterols and triterpenes, fixed oils and fats were found in our three (3) extracts. However, alkaloids and phytosterols were absent in two (2) extracts, and free anthraquinones, emodins, gums and mucilages were completely absent (Table 2). The widespread presence of those phenolics compounds confirms the phytochemical richness of *C. Citratus*. The absence of alkaloids and certain potentially toxic compounds (such as free anthraquinones or emodins) is a positive aspect that enhances the safety of using this plant in food products or dietary supplements. Although the aqueous extract of *C. citratus* is commonly consumed as an aromatic beverage, its biological activity is concentration-dependent, consistent with findings from previous studies [5]. In terms of antioxidant activity, the tested extracts showed promising results in the DPPH and ABTS assays. The decocted extract exhibited particularly high radical inhibition, with a DPPH IC₅₀ of $266.34 \pm 6.04 \mu\text{g/mL}$. This result may seem paradoxical, given that the decoction had lower polyphenol content compared to the infusion. However, it is possible that certain specific compounds, released or transformed under prolonged heat, are more effective against free radicals. Studies such as that of [20] have emphasized that antioxidant effects depend not only on the quantity of polyphenols but also on their chemical structure and their ability to interact with specific radicals. Antioxidants are substances capable of inhibiting specific oxidative enzymes, interacting with oxidative agents to prevent damage to other molecules, or even repairing biological systems, such as iron-carrying proteins [21]. In addition, experimental results revealed that the overall antioxidant capacity of *C. citratus* leaf extracts was comparable to that of ascorbic acid, a reference control, suggesting antioxidant activity attributable to the presence of phenolic compounds [22].

The evaluation of total antioxidant capacity (TAC) further confirms the superiority of the infusion, followed by the maceration and decoction. These results highlight the importance of the extraction method in obtaining an optimal bioactive extract. Additionally, the IC₅₀ values obtained for the extracts remain higher (and therefore less potent) than those for standard antioxidants such as quercetin (DPPH; IC₅₀) or ascorbic acid, which is expected since these pure molecules are highly concentrated. Nevertheless, the antioxidant activity of the natural extracts remains significant, especially considering their safety profile and accessibility.

Moreover, the inhibition rates of the ABTS radical show a marked progression

with increasing concentration, and the tested extracts reach values close to those of high-concentration standards. This confirms their potential as ingredients for functional plant-based beverage formulations.

Phytochemical screening highlighted the chemical diversity of the extracts, suggesting a probable synergy between the different groups of secondary metabolites that may enhance the overall antioxidant effect. This concept of synergy between polyphenols, saponins, and other natural antioxidants is supported by several recent studies, including that of [23] on tropical plant extracts.

5. Conclusions

The extracts of *Cymbopogon citratus* have demonstrated a broad spectrum of phenolic compounds, alongside significant antioxidant and free radical-neutralizing activities. The present study revealed that the antioxidant effects of *C. citratus* extracts are concentration-dependent, exhibiting notable inhibitory effects on oxidative stress markers. These findings underscore the potential of *C. citratus* as a valuable source of bioactive compounds with antioxidant properties, attributed to the presence of various phytochemicals such as polyphenols, flavonoids, alkaloids, saponins, tannins, and anthraquinones. These compounds likely act synergistically to neutralize free radicals, thus mitigating cellular damage associated with oxidative stress and potentially preventing the onset of several degenerative diseases.

The results from free radical scavenging assays, including DPPH and ABTS, provide strong evidence for the antioxidant efficacy of *C. citratus* leaf extracts, with efficacy comparable to that of ascorbic acid, a well-known antioxidant reference. While the current findings are promising, further research is essential to elucidate the mechanisms underlying the antioxidant action of these compounds, their bioavailability, and their long-term therapeutic effectiveness. Additionally, clinical trials are warranted to confirm the therapeutic potential of *C. citratus* extracts in real-world applications.

Future studies should focus on the detailed characterization of the bioactive compounds in *C. citratus*, their interactions within complex biological systems, and their effectiveness *in vivo*. These advancements will contribute significantly to the integration of *C. citratus* in both traditional and modern therapeutic frameworks, broadening its application in health and nutrition and enhancing its reputation as a plant with substantial medicinal value.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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