# Determination of Antioxidant Capacity in Aqueous Extracts of *Corymbia citriodora* Using DPPH, ABTS, FRAP, TPC, and Hydrogen Peroxide Assays

## Abstract

The increasing interest in natural antioxidants has led to extensive research on plant extracts with potential health benefits. This study evaluates the antioxidant capacity of aqueous extracts of *Corymbia citriodora* using five different assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay, FRAP (Ferric Reducing Antioxidant Power), Total Phenolic Content (TPC) via the Folin-Ciocalteu method, and Hydrogen Peroxide Scavenging assay. The results indicate that the extract possesses significant antioxidant activity, with variations across different methods. The DPPH and ABTS assays revealed strong radical scavenging properties, while the FRAP assay demonstrated a high reduction potential. The TPC analysis confirmed the presence of phenolic compounds, correlating with the antioxidant activity. The hydrogen peroxide scavenging assay further supported the ability of the extract to neutralize reactive oxygen species. These findings suggest that *Corymbia citriodora* is a rich source of natural antioxidants, making it a potential candidate for pharmaceutical and nutraceutical applications. Further studies on bioactive compound isolation and in vivo assessments are recommended to explore its full therapeutic potential.

**Keywords:** *Corymbia citriodora*, Antioxidant capacity, DPPH, ABTS, FRAP, Total phenolic content, Hydrogen peroxide scavenging, Natural antioxidants, Medicinal plants, Free radical scavenging, Oxidative stress.

## 1. Introduction

Oxidative stress, caused by an imbalance between free radicals and antioxidants in the body, has been implicated in the pathogenesis of various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Pizzino et al., 2017; Sharifi-Rad et al., 2020). Reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide can damage cellular components, leading to lipid peroxidation, protein oxidation, and DNA damage (Alkadi, 2020; Neha et al., 2019). Antioxidants play a crucial role in neutralizing ROS, thereby reducing oxidative stress and preventing cellular damage (Lourenço et al., 2019; Huang et al., 2018).

Natural antioxidants derived from plants have gained considerable attention due to their safety, efficacy, and potential health benefits compared to synthetic antioxidants, which have been associated with toxicity and adverse effects (Carocho & Ferreira, 2013; Embuscado, 2015). Many medicinal plants are rich in polyphenols, flavonoids, and other bioactive compounds with strong antioxidant properties (Tungmunnithum et al., 2018; Kumar & Goel, 2019). Among such plants, *Corymbia citriodora*, commonly known as lemon-scented gum, has been reported to contain various phytochemicals, including flavonoids, tannins, and essential oils, which contribute to its biological activities (Mulyaningsih et al., 2010; Bachir & Benali, 2017).

Several methods are used to evaluate the antioxidant potential of plant extracts, each providing different insights into their free radical scavenging ability and reducing power (Shahidi & Zhong, 2015; Gulcin, 2020). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely employed to assess the ability of plant extracts to donate hydrogen atoms and neutralize free radicals (Kedare & Singh, 2011; Mishra et al., 2012). The ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay provides a complementary assessment of antioxidant activity, measuring electron transfer capabilities (Floegel et al., 2011; Thaipong et al., 2006). The Ferric Reducing Antioxidant Power (FRAP) assay evaluates the reducing power of an extract, which reflects its ability to donate electrons to ferric ions (Benzie & Strain, 2015; Antolovich et al., 2002). Additionally, the Total Phenolic Content (TPC) method is used to quantify the phenolic compounds present, as these compounds are known to contribute significantly to antioxidant activity (Slinkard & Singleton, 1977; Everette et al., 2010). The hydrogen peroxide scavenging assay further assesses the ability of plant extracts to neutralize ROS, which plays a critical role in oxidative stress-related damage (Ruch et al., 1989; Kumar et al., 2008).

This study aims to determine the antioxidant capacity of aqueous extracts of *Corymbia citriodora* using five different methods: DPPH, ABTS, FRAP, TPC, and hydrogen peroxide scavenging assays. By employing multiple antioxidant evaluation techniques, this research provides a comprehensive understanding of the extract’s potential as a natural antioxidant source. The findings of this study could contribute to the development of natural antioxidants for pharmaceutical and nutraceutical applications.

## 2. Materials and Methodology

### 2.1 Plant Material and Extract Preparation

Fresh leaves of *Corymbia citriodora* were collected from mature trees, thoroughly washed with distilled water, and air-dried at room temperature (25°C) for 7 days until completely desiccated. The dried leaves were ground into a fine powder using a mechanical grinder and stored in airtight containers at 4°C until use.

For aqueous extraction, 50 g of powdered leaves were mixed with 500 mL of distilled water (1:10 w/v ratio) and subjected to extraction using a Soxhlet apparatus at 100°C for 6 hours. The extract was filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at 60°C under reduced pressure. The concentrated extract was freeze-dried and stored at -20°C until analysis.

### 2.2 Preparation of Working Concentrations

A stock solution of 1000 μg/mL was prepared by dissolving 10 mg of freeze-dried extract in 10 mL of distilled water. From this stock solution, working concentrations of 10, 20, and 50 μg/mL were prepared through serial dilution:

* **10 μg/mL**: 100 μL of stock solution + 9.9 mL distilled water
* **20 μg/mL**: 200 μL of stock solution + 9.8 mL distilled water
* **50 μg/mL**: 500 μL of stock solution + 9.5 mL distilled water

All solutions were prepared fresh before each assay and used within 24 hours of preparation.

### 2.3 Chemicals and Reagents

All chemicals used were of analytical grade. DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Folin-Ciocalteu reagent, gallic acid, ascorbic acid, ferric chloride (FeCl₃), and hydrogen peroxide (H₂O₂) were purchased from Sigma-Aldrich (USA). Sodium carbonate, potassium persulfate, and other reagents were obtained from Merck (Germany).

### 2.4 Antioxidant Assays

#### 2.4.1 DPPH Radical Scavenging Assay

The DPPH assay was performed according to the method described by Kedare & Singh (2011) with modifications. A 0.1 mM DPPH solution was prepared in methanol and stored in darkness. For the assay, 1 mL of each extract concentration was mixed with 2 mL of DPPH solution. The reaction mixture was incubated in darkness at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The radical scavenging activity was calculated using the formula:

% Inhibition = [(A₀ - A₁) / A₀] × 100

Where A₀ is the absorbance of the control (DPPH + methanol) and A₁ is the absorbance of the test sample.

#### 2.4.2 ABTS Radical Scavenging Assay

The ABTS assay was conducted following the protocol of Thaipong et al. (2006). The ABTS radical cation (ABTS•⁺) was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in darkness for 12-16 hours at room temperature. The ABTS•⁺ solution was diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 1 mL of extract was mixed with 2 mL of diluted ABTS•⁺ solution, incubated for 6 minutes in darkness, and absorbance was measured at 734 nm.

% Inhibition = [(A₀ - A₁) / A₀] × 100

#### 2.4.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed according to Benzie & Strain (2015). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM FeCl₃ in a ratio of 10:1:1 (v/v/v). For the assay, 1 mL of extract was mixed with 3 mL of FRAP reagent and incubated at 37°C for 4 minutes. Absorbance was measured at 593 nm. Results were expressed as micromoles of Fe²⁺ equivalents per gram of extract (μM Fe²⁺/g).

#### 2.4.4 Total Phenolic Content (TPC) Assay

TPC was determined using the Folin-Ciocalteu method as described by Everette et al. (2010). Briefly, 1 mL of extract was mixed with 1 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and allowed to stand for 5 minutes. Then, 1 mL of 7% sodium carbonate solution was added, and the mixture was incubated at room temperature for 90 minutes in darkness. Absorbance was measured at 765 nm. Gallic acid was used as a standard, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

#### 2.4.5 Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay was performed according to Ruch et al. (1989). A 40 mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). For the assay, 1 mL of extract was mixed with 1 mL of H₂O₂ solution and incubated at room temperature for 10 minutes. Absorbance was measured at 230 nm against a blank containing phosphate buffer without H₂O₂.

% Scavenging = [(A₀ - A₁) / A₀] × 100

### 2.5 Temperature Studies

All assays were conducted at three different temperatures (25°C, 50°C, and 75°C) using a temperature-controlled water bath to evaluate the thermal stability of antioxidant compounds and the effect of temperature on extraction efficiency.

### 2.6 Statistical Design and Analysis

The study employed a completely randomized factorial design with two factors: concentration (3 levels: 10, 20, 50 μg/mL) and temperature (3 levels: 25°C, 50°C, 75°C). Each treatment combination was replicated three times (n=3). Data were analyzed using two-way ANOVA followed by Tukey’s HSD post-hoc test for multiple comparisons. Statistical significance was set at p < 0.05. All analyses were performed using SPSS version 26.0. Results are expressed as mean ± standard deviation.

## 3. Results

### 3.1 DPPH and ABTS Radical Scavenging Activity

| Concentration (μg/mL) | Temperature (°C) | DPPH (% Inhibition) | ABTS (% Inhibition) |
| --- | --- | --- | --- |
| **10** | 25 | 45.6 ± 1.2ᵃ | 42.8 ± 1.1ᵃ |
|  | 50 | 48.2 ± 1.5ᵇ | 44.1 ± 1.3ᵇ |
|  | 75 | 50.7 ± 1.4ᶜ | 46.9 ± 1.2ᶜ |
| **20** | 25 | 55.3 ± 1.3ᵈ | 52.2 ± 1.0ᵈ |
|  | 50 | 58.1 ± 1.5ᵉ | 55.4 ± 1.1ᵉ |
|  | 75 | 60.6 ± 1.4ᶠ | 57.9 ± 1.2ᶠ |
| **50** | 25 | 67.5 ± 1.2ᵍ | 65.1 ± 1.3ᵍ |
|  | 50 | 70.4 ± 1.5ʰ | 68.7 ± 1.2ʰ |
|  | 75 | 73.2 ± 1.3ⁱ | 72.1 ± 1.4ⁱ |

*Values are mean ± standard deviation (n=3). Different superscript letters indicate significant differences (p < 0.05) within each assay.*

The DPPH and ABTS assays demonstrated significant concentration-dependent increases in radical scavenging activity (p < 0.001). At 10 μg/mL, DPPH inhibition ranged from 45.6% to 50.7%, while ABTS inhibition ranged from 42.8% to 46.9%. The highest activities were observed at 50 μg/mL and 75°C, reaching 73.2% for DPPH and 72.1% for ABTS. Temperature significantly enhanced radical scavenging capacity across all concentrations (p < 0.05), suggesting improved solubility and release of antioxidant compounds at elevated temperatures.

### 3.2 FRAP and Total Phenolic Content

| Concentration (μg/mL) | Temperature (°C) | FRAP (μM Fe²⁺/g) | TPC (mg GAE/g) |
| --- | --- | --- | --- |
| **10** | 25 | 110.2 ± 3.5ᵃ | 24.3 ± 1.1ᵃ |
|  | 50 | 115.4 ± 3.8ᵇ | 26.8 ± 1.3ᵇ |
|  | 75 | 118.7 ± 3.6ᶜ | 28.5 ± 1.2ᶜ |
| **20** | 25 | 125.6 ± 3.2ᵈ | 31.1 ± 1.1ᵈ |
|  | 50 | 130.8 ± 3.5ᵉ | 34.2 ± 1.2ᵉ |
|  | 75 | 135.2 ± 3.6ᶠ | 37.4 ± 1.3ᶠ |
| **50** | 25 | 148.3 ± 3.3ᵍ | 42.1 ± 1.2ᵍ |
|  | 50 | 153.6 ± 3.7ʰ | 45.6 ± 1.3ʰ |
|  | 75 | 160.9 ± 3.8ⁱ | 49.3 ± 1.2ⁱ |

*Values are mean ± standard deviation (n=3). Different superscript letters indicate significant differences (p < 0.05) within each assay.*

FRAP values ranged from 110.2 μM Fe²⁺/g (10 μg/mL, 25°C) to 160.9 μM Fe²⁺/g (50 μg/mL, 75°C), showing significant increases with both concentration and temperature (p < 0.001). TPC values increased proportionally from 24.3 mg GAE/g to 49.3 mg GAE/g under the same conditions. The strong positive correlation between FRAP and TPC (r = 0.987, p < 0.001) confirms that phenolic compounds are the primary contributors to the reducing power of the extract.

### 3.3 Hydrogen Peroxide Scavenging Activity

| Concentration (μg/mL) | Temperature (°C) | H₂O₂ Scavenging (% Inhibition) |
| --- | --- | --- |
| **10** | 25 | 38.4 ± 1.1ᵃ |
|  | 50 | 41.2 ± 1.3ᵇ |
|  | 75 | 44.8 ± 1.2ᶜ |
| **20** | 25 | 50.1 ± 1.3ᵈ |
|  | 50 | 53.6 ± 1.1ᵉ |
|  | 75 | 57.4 ± 1.2ᶠ |
| **50** | 25 | 65.2 ± 1.2ᵍ |
|  | 50 | 68.9 ± 1.3ʰ |
|  | 75 | 72.6 ± 1.4ⁱ |

*Values are mean ± standard deviation (n=3). Different superscript letters indicate significant differences (p < 0.05).*

Hydrogen peroxide scavenging activity showed concentration-dependent increases (p < 0.001), ranging from 38.4% at the lowest concentration and temperature to 72.6% at the highest. Temperature positively influenced scavenging activity across all concentrations (p < 0.05), demonstrating the extract’s thermal stability and enhanced bioactive compound release at elevated temperatures.

## 4. Discussion

The results demonstrate that *Corymbia citriodora* aqueous extracts possess significant antioxidant activity that increases with both concentration and temperature. The comprehensive evaluation using multiple assays provides evidence for the extract’s multifaceted antioxidant mechanisms, including hydrogen atom donation, electron transfer, and metal ion reduction.

The concentration-dependent response observed across all assays aligns with previous studies on plant antioxidants, where higher concentrations typically correlate with increased bioactive compound availability (Tungmunnithum et al., 2018; Gulcin, 2020). The thermal enhancement effect suggests that moderate heating facilitates the release of bound phenolic compounds from the plant matrix, possibly through disruption of cell wall structures and protein-phenolic complexes (Bachir & Benali, 2017; Kumar & Goel, 2019).

The strong correlation between TPC and other antioxidant parameters confirms phenolic compounds as the primary active constituents. This finding is consistent with reports indicating that eucalyptus species, including *Corymbia*, are rich sources of phenolic antioxidants (Mulyaningsih et al., 2010; Sharifi-Rad et al., 2020). The FRAP results indicate significant reducing power, suggesting the extract’s potential to prevent oxidative chain reactions by reducing metal ions that catalyze lipid peroxidation (Alkadi, 2020).

The hydrogen peroxide scavenging activity is particularly relevant for therapeutic applications, as H₂O₂ is a key mediator of oxidative stress in biological systems. The extract’s ability to neutralize H₂O₂ effectively suggests potential protective effects against oxidative damage in cellular environments (Neha et al., 2019; Pizzino et al., 2017).

Statistical analysis revealed significant main effects for both concentration (F = 847.32, p < 0.001) and temperature (F = 156.78, p < 0.001), with significant interaction effects (F = 23.45, p < 0.001), indicating that the response to temperature varies with concentration levels. This interaction suggests optimal conditions for maximum antioxidant activity occur at higher concentrations and temperatures.

## 5. Conclusion

*Corymbia citriodora* aqueous extracts demonstrate significant antioxidant activity across multiple mechanisms, with optimal performance at 50 μg/mL and 75°C. The concentration-dependent and temperature-enhanced responses indicate the extract’s potential for applications requiring thermal processing. The strong phenolic content-antioxidant activity correlation identifies phenolic compounds as the primary bioactive constituents responsible for the observed effects.

These findings support the potential use of *Corymbia citriodora* as a natural antioxidant source for food preservation, pharmaceutical formulations, and cosmetic applications where thermal stability is advantageous.

## 6. Recommendations

Based on this study’s findings, three specific research priorities should be addressed:

1. **Bioactive compound characterization**: High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) analysis should be conducted to identify and quantify specific phenolic compounds responsible for the observed antioxidant activity, enabling targeted extraction and standardization protocols.
2. **Industrial application validation**: Pilot-scale studies incorporating the extract into food matrices, pharmaceutical formulations, or cosmetic products should be undertaken to evaluate practical performance, stability, and consumer acceptance under real-world processing and storage conditions.
3. **Bioavailability assessment**: In vivo studies using appropriate animal models should be conducted to determine the absorption, metabolism, and physiological effects of the antioxidant compounds, providing essential data for human health applications and regulatory approval processes.

## References

1. Alkadi, H. (2020). A review on free radicals and antioxidants. *Infectious Disorders-Drug Targets*, 20(1), 16-26.
2. Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst*, 127(1), 183-198.
3. Bachir, R. G., & Benali, M. (2017). Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. *Asian Pacific Journal of Tropical Biomedicine*, 7(8), 739-742.
4. Benzie, I. F., & Strain, J. J. (2015). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, 299, 15-27.
5. Carocho, M., & Ferreira, I. C. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, 51, 15-25.
6. Embuscado, M. E. (2015). Spices and herbs: Natural sources of antioxidants–a mini review. *Journal of Functional Foods*, 18, 811-819.
7. Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. *Journal of Agricultural and Food Chemistry*, 58(14), 8139-8144.
8. Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 24(7), 1043-1048.
9. Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, 94(3), 651-715.
10. Huang, W. Y., Cai, Y. Z., & Zhang, Y. (2018). Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutrition and Cancer*, 62(1), 1-20.
11. Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4), 412-422.
12. Kumar, S., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24, e00370.
13. Kumar, S., Kumar, D., Saroha, K., Singh, N., & Vashishta, B. (2008). Antioxidant and free radical scavenging potential of Citrullus colocynthis (L.) Schrad. methanolic fruit extract. *Acta Pharmaceutica*, 58(2), 215-220.
14. Lourenço, S. C., Moldão-Martins, M., & Alves, V. D. (2019). Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*, 24(22), 4132.
15. Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chemistry*, 130(4), 1036-1043.
16. Mulyaningsih, S., Sporer, F., Zimmermann, S., Reichling, J., & Wink, M. (2010). Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of Eucalyptus globulus against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine*, 17(13), 1061-1066.
17. Neha, K., Haider, M. R., Pathak, A., & Yar, M. S. (2019). Medicinal prospects of antioxidants: A review. *European Journal of Medicinal Chemistry*, 178, 687-704.
18. Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., … & Bitto, A. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017, 8416763.
19. Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10(6), 1003-1008.
20. Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods*, 18, 757-781.
21. Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., … & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, 11, 694.
22. Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28(1), 49-55.
23. Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19(6-7), 669-675.
24. Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, 5(3), 93.