**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 (Lobo et al., 2010; Halliwell & Gutteridge, 2015). 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 (Gülçin, 2012; Oboh et al., 2014). Antioxidants play a crucial role in neutralizing ROS, thereby reducing oxidative stress and preventing cellular damage (Prior et al., 2005; Wang et al., 2011).

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 (Atawodi, 2005; Zheng & Wang, 2001). Many medicinal plants are rich in polyphenols, flavonoids, and other bioactive compounds with strong antioxidant properties (Cai et al., 2004; Mensor et al., 2001). 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 (Ayoola et al., 2008; Oladimeji et al., 2019).

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 (Brand-Williams et al., 1995; Rezaeizadeh et al., 2011). 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 (Rahman et al., 2010; Oboh et al., 2014). The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay provides a complementary assessment of antioxidant activity, measuring electron transfer capabilities (Ou et al., 2001; Sun et al., 2002). The Ferric Reducing Antioxidant Power (FRAP) assay evaluates the reducing power of an extract, which reflects its ability to donate electrons to ferric ions (Tadhani et al., 2007; Sowndhararajan & Kang, 2013). 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 (Singleton et al., 1999; Prior et al., 2005). 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 (Wink, 2015; Sarikurkcu 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.

## ****II Materials and Methodology****

The study was conducted to evaluate the antioxidant activity of Corymbia citriodora aqueous extracts using DPPH, ABTS, FRAP, TPC, and Hydrogen Peroxide Scavenging assays. The plant leaves were collected, dried, and extracted using distilled water. The extract was filtered and concentrated before use. Chemicals such as DPPH, ABTS, Folin-Ciocalteu reagent, sulfuric acid, ammonium molybdate, sodium phosphate, and hydrogen peroxide were obtained from standard suppliers. A UV-Vis spectrophotometer was used to measure absorbance for each assay.

The antioxidant activity of the extracts was determined using five assays. The DPPH and ABTS assays measured the radical scavenging ability by quantifying color changes at 517 nm and 734 nm, respectively. The FRAP assay assessed the reducing power of the extract by converting ferric (Fe³⁺) to ferrous (Fe²⁺), with absorbance measured at 593 nm. The TPC was determined using the Folin-Ciocalteu method, where the reaction of phenolic compounds with the reagent produced a blue-colored complex measured at 765 nm. The hydrogen peroxide scavenging assay evaluated the ability of the extract to neutralize H₂O₂, measured at 230 nm. All assays were conducted at varying concentrations (10, 20, and 50 µg/mL) and temperatures (25°C, 50°C, and 75°C) to assess the effect of these parameters on antioxidant activity. The results were expressed as ascorbic acid equivalents (AAE) and analyzed statistically using ANOVA with a significance level of p < 0.05.

## ****III Results****

The antioxidant activity of Corymbia citriodora aqueous extracts was evaluated using DPPH, ABTS, FRAP, TPC, and Hydrogen Peroxide Scavenging assays at different concentrations (10, 20, and 50 µg/mL) and temperatures (25°C, 50°C, and 75°C). The results, expressed as ascorbic acid equivalents (AAE), are presented in the tables below.

### **Table 1: DPPH and ABTS Radical Scavenging Activity of Corymbia citriodora Extract**

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

The DPPH and ABTS assays evaluate the free radical scavenging potential of antioxidants in plant extracts. The results clearly show that both assays produced a concentration-dependent increase in antioxidant activity. At the lowest concentration (10 µg/mL), DPPH inhibition ranged from 45.6% to 50.7%, and ABTS from 42.8% to 46.9%, depending on temperature. At the highest concentration (50 µg/mL), DPPH inhibition peaked at 73.2% and ABTS at 72.1%. This trend strongly suggests that the antioxidant components in the extract become more effective as their availability increases.

Temperature also positively influenced the radical scavenging capacity, with activity increasing consistently from 25°C to 75°C at each concentration level. This suggests that elevated temperatures may enhance the solubility or release of antioxidant phytochemicals within the extract. For instance, at 50 µg/mL, the increase from 67.5% inhibition at 25°C to 73.2% at 75°C in the DPPH assay points to the beneficial effects of mild thermal processing. The ABTS assay mirrored this pattern, supporting the extract’s stability and potential for use in thermally processed products.

Overall, the results indicate that **Corymbia citriodora** extracts possess robust radical scavenging activity, which is sensitive to both concentration and heat. The parallel increase in DPPH and ABTS inhibition reflects the presence of a diverse group of antioxidants, likely including polyphenols and flavonoids, capable of donating electrons or hydrogen atoms to neutralize free radicals. This dual reactivity also highlights the extract’s potential applicability across various antioxidant-dependent mechanisms.

### **Table 2: FRAP and Total Phenolic Content (TPC) of Corymbia citriodora Extract**

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

The FRAP assay reflects the reducing power of a sample, while TPC indicates the concentration of phenolic compounds. In this study, both measures increased with rising extract concentration and temperature. FRAP values ranged from 110.2 µM Fe²⁺/g at 10 µg/mL and 25°C to 160.9 µM at 50 µg/mL and 75°C. Similarly, TPC values rose from 24.3 mg GAE/g to 49.3 mg GAE/g across the same conditions. These trends confirm that phenolic compounds are the major contributors to the extract’s reducing potential.

Temperature appeared to facilitate the extraction or activation of phenolic antioxidants, as seen by the steady increase in both TPC and FRAP values at higher temperatures. For example, at 20 µg/mL, TPC increased from 31.1 mg GAE/g at 25°C to 37.4 mg at 75°C, while FRAP increased from 125.6 to 135.2 µM. This suggests that moderate heating enhances the antioxidant potential of the extract without degrading its phenolic content—a valuable insight for industrial processing.

The strong correlation between FRAP and TPC results supports the hypothesis that polyphenols are the principal active compounds behind the observed antioxidant effects. These findings underline the extract’s capacity to act through electron donation (reducing agents) and provide a quantitative basis for comparing its antioxidant strength to other plant-based extracts. It further strengthens the claim that **Corymbia citriodora** is a rich source of natural, thermally stable antioxidants.

### **Table 3: Hydrogen Peroxide Scavenging Activity of Corymbia citriodora Extract**

|  |  |  |
| --- | --- | --- |
| **Concentration (µg/mL)** | **Temperature (°C)** | **H₂O₂ Scavenging (% Inhibition)** |
| 10 | 25 | 38.4 ± 1.1 |
| 10 | 50 | 41.2 ± 1.3 |
| 10 | 75 | 44.8 ± 1.2 |
| 20 | 25 | 50.1 ± 1.3 |
| 20 | 50 | 53.6 ± 1.1 |
| 20 | 75 | 57.4 ± 1.2 |
| 50 | 25 | 65.2 ± 1.2 |
| 50 | 50 | 68.9 ± 1.3 |
| 50 | 75 | 72.6 ± 1.4 |

The hydrogen peroxide scavenging assay evaluates the extract’s ability to neutralize H₂O₂, a reactive oxygen species implicated in oxidative cell damage. As with the other assays, there was a clear, concentration-dependent increase in activity. At 10 µg/mL, the inhibition ranged from 38.4% to 44.8%, while at 50 µg/mL it increased to 65.2–72.6%. This indicates that the extract effectively detoxifies hydrogen peroxide, especially at higher concentrations.

Temperature also positively influenced scavenging activity. Across all concentrations, the increase from 25°C to 75°C led to improved performance. For instance, at 20 µg/mL, activity improved from 50.1% to 57.4% as temperature rose. These findings may suggest that heating facilitates the release of antioxidant compounds from the plant matrix or activates certain enzymatic processes that enhance scavenging activity.

This assay highlights the extract’s specific ability to counter oxidative damage from peroxides, which complements the radical scavenging and reducing abilities demonstrated in the earlier tables. The consistent performance across assays affirms the multi-mechanistic antioxidant properties of **Corymbia citriodora**. Its capacity to eliminate hydrogen peroxide enhances its therapeutic potential, particularly for conditions where peroxides play a central pathological role, such as inflammation, aging, and neurodegeneration.

## ****IV Discussion****

The results of this study demonstrate that the aqueous extract of Corymbia citriodora exhibits significant antioxidant activity, which varies with concentration and temperature. The extract showed strong free radical scavenging activity in the DPPH and ABTS assays, high ferric ion reducing power in the FRAP assay, substantial total phenolic content (TPC), and effective hydrogen peroxide scavenging capacity. These findings suggest that Corymbia citriodora is a promising source of natural antioxidants.

The DPPH and ABTS assays, which measure free radical scavenging activity, indicated that antioxidant potential increased with both concentration and temperature. The highest activity was observed at 50 µg/mL and 75°C, suggesting that the extract contains heat-stable antioxidants. These results align with previous studies reporting that plant polyphenols, flavonoids, and tannins contribute to radical scavenging activity (Brand-Williams et al., 1995; Rezaeizadeh et al., 2011). The significant increase in ABTS radical inhibition at higher temperatures suggests enhanced solubility and release of antioxidant compounds, similar to findings in other medicinal plants (Sarikurkcu et al., 2008).

The FRAP assay confirmed the reducing power of Corymbia citriodora extracts, with higher ferric ion reduction at elevated concentrations and temperatures. This indicates the presence of electron-donating antioxidants capable of reducing Fe³⁺ to Fe²⁺, a key mechanism in oxidative stress prevention (Prior et al., 2005). The strong correlation between FRAP and TPC values suggests that phenolic compounds play a major role in antioxidant activity. Phenolic compounds are well-known for their redox properties, allowing them to act as reducing agents and hydrogen donors (Singleton et al., 1999; Sowndhararajan & Kang, 2013).

The hydrogen peroxide scavenging assay further confirmed the extract’s ability to neutralize reactive oxygen species (ROS). Hydrogen peroxide is a major contributor to oxidative damage in biological systems, and its effective neutralization by Corymbia citriodora extracts supports the potential health benefits of this plant (Wink, 2015; Sun et al., 2002). The dose-dependent increase in scavenging activity suggests that higher concentrations of the extract may provide enhanced protection against oxidative stress.

The statistical analysis revealed a significant influence of both concentration and temperature on antioxidant activity, with a strong positive correlation between extract concentration and radical scavenging ability. This agrees with previous studies that reported enhanced antioxidant properties with increasing polyphenol content in plant extracts (Oboh et al., 2014; Tadhani et al., 2007). The findings also highlight the potential of mild heat treatment in improving the extraction efficiency of bioactive compounds without causing degradation (Cai et al., 2004). However, excessive heating beyond the optimal temperature may lead to thermal degradation of some heat-sensitive antioxidants, requiring further investigation.

Overall, these results support the potential application of Corymbia citriodora extracts as natural antioxidants in pharmaceutical, food, and cosmetic industries. The plant’s high radical scavenging and reducing power suggest that it could serve as an alternative to synthetic antioxidants, which are often associated with toxicity concerns (Lobo et al., 2010; Rahman et al., 2010). Further studies are needed to identify the specific bioactive compounds responsible for this antioxidant activity and to evaluate their stability in different formulations.

## ****V Conclusion****

The present study demonstrates that the aqueous extract of Corymbia citriodora possesses significant antioxidant activity, which is influenced by both concentration and temperature. The extract exhibited strong free radical scavenging ability in the DPPH and ABTS assays, high ferric ion reducing power in the FRAP assay, substantial total phenolic content (TPC), and effective hydrogen peroxide scavenging capacity. The results revealed that antioxidant activity increased with higher extract concentrations and elevated temperatures, suggesting that thermal processing enhances the release of bioactive compounds responsible for antioxidative effects.

The positive correlation between antioxidant capacity and total phenolic content suggests that phenolic compounds play a major role in the observed activity. The statistical analysis confirmed that both concentration and temperature significantly affected antioxidant performance, with optimal activity observed at 50 µg/mL and 75°C. These findings support the potential use of Corymbia citriodora as a natural antioxidant source with applications in food preservation, pharmaceuticals, and cosmetics.

Further research is recommended to isolate and characterize the specific bioactive compounds contributing to the antioxidant activity of Corymbia citriodora. Additionally, investigations into the stability and bioavailability of these compounds in different formulations and processing conditions will provide deeper insights into their potential industrial applications.

**Recommendation**

Based on the findings of this study, it is recommended that future research on Corymbia citriodora focuses on isolating and characterizing the specific bioactive compounds responsible for its antioxidant activity to better understand their molecular mechanisms and therapeutic potential. The stability of these compounds under various formulation and storage conditions should be examined to determine their practical applications in food, pharmaceutical, and cosmetic industries. To gain a more comprehensive understanding of its antioxidant capabilities, additional assays such as superoxide anion scavenging and lipid peroxidation inhibition should be conducted, along with comparative studies involving synthetic antioxidants to assess relative efficacy and safety. Investigations into the bioavailability, metabolic pathways, and potential synergistic effects with other natural antioxidants are also essential to evaluate its health benefits and enhance industrial applications. Furthermore, exploring various extraction techniques can help optimize the yield and potency of its antioxidant constituents. Given its possible antimicrobial properties, integrated studies could expand its utility in the pharmaceutical and food sectors. Finally, in vivo studies and clinical trials are necessary to confirm its effectiveness in mitigating oxidative stress-related diseases, while sustainable harvesting and cultivation practices should be encouraged to ensure long-term research and commercial exploitation of this valuable plant.

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