

Title :

Role of Secondary Metabolites from *Cymbopogon citratus* in Modulating Free Radicals Associated with Tumor Initiation

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Abstract

Oxidative stress driven by the overproduction of reactive oxygen species (ROS) is a critical factor implicated in the initiation and progression of tumorigenesis. Plant-derived secondary metabolites offer promising chemopreventive potential by modulating oxidative damage at its earliest stages. This study investigates the role of secondary metabolites extracted from *Cymbopogon citratus* (lemongrass) leaves in the regulation of free radicals implicated in tumor initiation. Using advanced metabolomic profiling coupled with targeted antioxidant assays, we characterize the chemical composition of methanolic and chloroformic extracts, identifying key bioactive compounds, including terpenoids and polyphenols, known for their potent radical scavenging and anti-inflammatory properties.

The extracts were evaluated for their efficacy in modulating ROS levels and protecting cellular integrity through in vitro models mimicking oxidative stress-induced DNA damage, a precursor event in oncogenesis. Results indicate a significant dose-dependent reduction in ROS, correlating with decreased oxidative DNA lesions and modulation of cellular redox balance.

Notably, the methanolic extract exhibited superior activity, consistent with its enriched profile of polar antioxidant metabolites.

Mechanistic insights reveal that these secondary metabolites exert their effects by scavenging free radicals, regulating antioxidant enzyme systems, and potentially influencing apoptotic pathways to hinder the early transformation of normal cells. This comprehensive evaluation highlights the therapeutic promise of *Cymbopogon citratus* secondary metabolites as natural agents for cancer chemoprevention through disruption of oxidative stress-mediated tumor initiation.

Our findings provide a crucial foundation for further in vivo validation and development of phytochemical-based interventions targeting the prevention of carcinogenesis at its molecular inception.

1. Introduction

Oxidative stress, defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, is a primary driver in the initiation and progression of numerous diseases, notably cancer. Elevated ROS levels damage cellular macromolecules such as DNA, proteins, and lipids, leading to mutations, genomic instability, and altered signaling pathways that favor tumorigenesis. The modulation of oxidative stress through natural antioxidant compounds is a promising strategy for cancer chemoprevention, aiming to interfere with early carcinogenic events before irreversible damage occurs.

Secondary metabolites produced by plants, including polyphenols, terpenoids, and flavonoids, have garnered significant interest due to their multifaceted bioactivities encompassing antioxidant, anti-inflammatory, and antitumor properties. Among medicinal plants, *Cymbopogon citratus* (lemongrass), widely used in traditional medicine, contains a rich repertoire of these secondary metabolites. While essential oils derived from this species have been extensively characterized, comprehensive studies on non-volatile leaf extracts, particularly methanolic and chloroformic fractions, remain limited despite evidence suggesting potent antioxidant capacities.

Previous metabolomic investigations revealed that these extracts contain abundant terpenoids and polyphenols that contribute to *C. citratus*' high antioxidant activity, highlighting their therapeutic potential. However, the specific roles of these secondary metabolites in modulating ROS linked to tumor initiation remain poorly elucidated.

This study aims to fill this knowledge gap by (i) profiling the secondary metabolite composition of methanolic and chloroformic leaf extracts using advanced chemometric and chromatographic methods; (ii) assessing their capacity to scavenge free radicals implicated in oxidative stress; and (iii) investigating their efficacy in protecting cellular DNA and modulating key biomarkers of tumor initiation in vitro.

By integrating chemical characterization with functional bioassays, this research seeks to establish mechanistic links between *C. citratus* secondary metabolites and early oncogenic modulation, thereby providing a foundation for developing novel phytochemical interventions in cancer prevention.

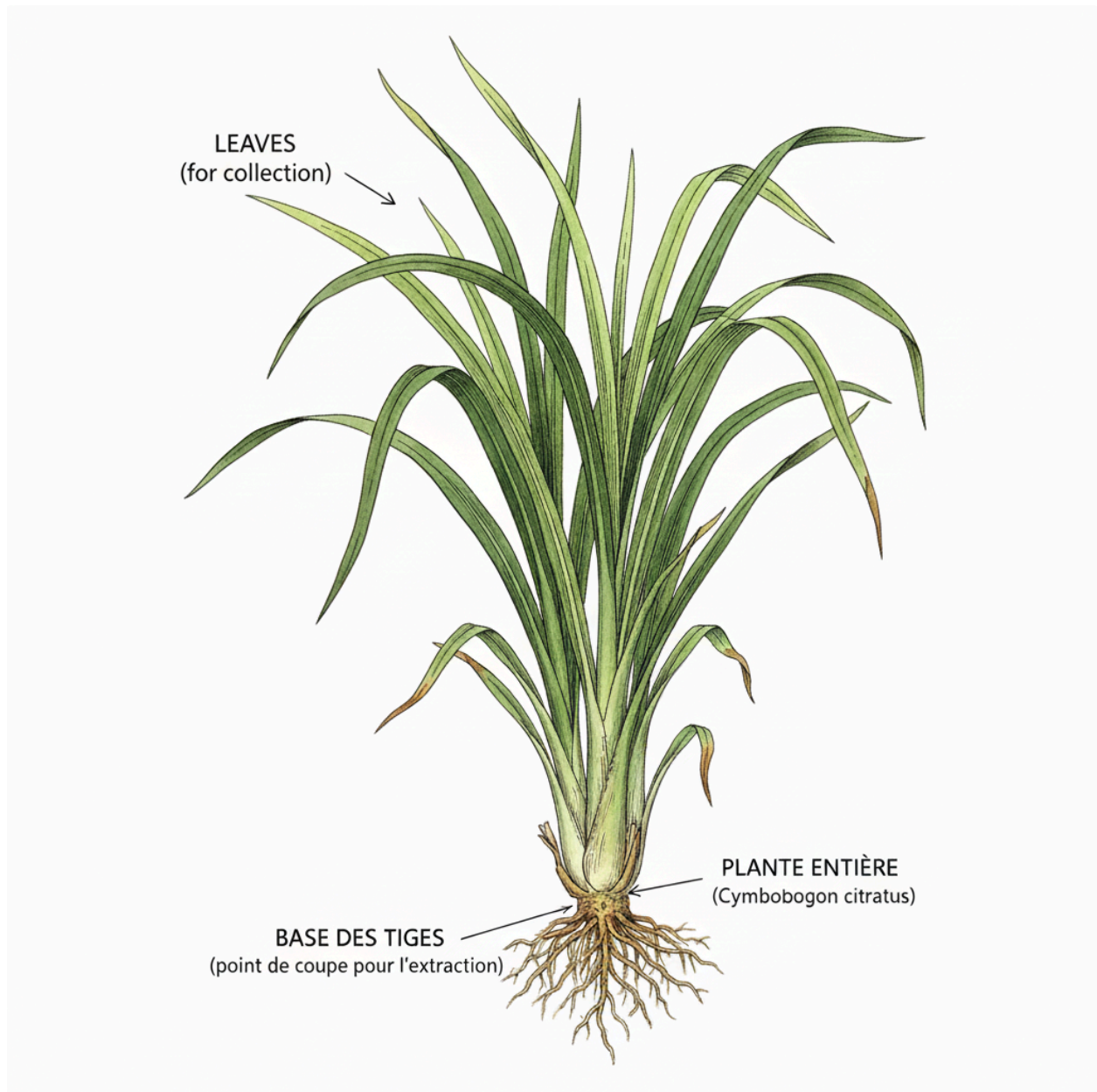


Figure 1. Botanical image of *Cymbopogon citratus*

2. Materials and Methods

2.1. Plant Material Collection and Preparation

Fresh leaves of *Cymbopogon citratus* were collected from a local site in the Kinshasa region, Democratic Republic of Congo. The leaves were carefully washed with distilled water to remove dirt and impurities, then air-dried at room temperature until consistent weight was achieved. The dried plant material was finely ground using a mechanical grinder to obtain a homogenous powder suitable for extraction.

2.2. Extraction Procedure

Sequential solvent extraction was performed to obtain both polar and non-polar metabolite fractions. Approximately 100 g of powdered leaves were subjected to sonication-assisted extraction using methanol (polar solvent) and chloroform (non-polar solvent), respectively. Each extraction lasted 30 minutes at ambient temperature to facilitate efficient release of bioactive compounds. Extracts were filtered through Whatman No.1 paper and concentrated under reduced pressure at 40°C using a rotary evaporator, yielding crude methanolic and chloroformic extracts. Extracts were stored at 4°C until further analyses.

2.3. Antioxidant Activity Assessment

The antioxidant capacity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Serial dilutions of the extracts (ranging from 10 to 200 µg/mL) were incubated with a stable DPPH solution (0.1 mM in methanol) in the dark for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of radical scavenging activity was calculated relative to a control (methanol only), and the half-maximal inhibitory concentration (IC₅₀) was determined by plotting percentage inhibition against extract concentration. Ascorbic acid was used as a positive control. Experiments were performed in triplicate for reliability.

2.4. Metabolomic Profiling and Chemical Characterization

Comprehensive metabolomic profiling was conducted via proton (¹H) and carbon (¹³C) nuclear magnetic resonance (NMR) spectroscopy at 600 MHz. Samples (10 mg of each extract) were dissolved in deuterated solvents (CD₃OD for methanolic, CDCl₃ for chloroformic extracts) and analyzed. Spectra were processed and annotated using Chenomx NMR Suite, referencing known chemical shifts to identify major metabolite classes, especially terpenoids, polyphenols, and flavonoids.

Complementary chromatographic analyses were performed using Thin Layer Chromatography (TLC) on silica gel plates, developed in appropriate solvent systems to separate distinct classes of secondary metabolites. Visualization was done under UV light (254 nm and 365 nm) and after spraying with specific reagents (e.g., vanillin-sulfuric acid) to detect phenolics and terpenoids.

2.5. Cellular Models for Radical Modulation Assessment

To assess the modulation of free radicals implicated in tumor initiation, selected extracts were tested on an in vitro human epithelial cell line model subjected to oxidative stress via hydrogen peroxide (H₂O₂) exposure. Cells were pre-treated with varying concentrations of extracts before H₂O₂ challenge. Intracellular ROS levels were quantified using the fluorescent probe dichlorofluorescein diacetate (DCFDA). DNA damage was evaluated using the comet assay and quantified by tail moment analysis. Expression levels of key antioxidant enzymes (SOD, catalase) and apoptosis markers were measured by RT-qPCR and Western blot.

2.6. Statistical Analysis

Data from all assays were expressed as mean ± standard deviation (SD) from at least three independent experiments. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test for multiple comparisons, accepting $p < 0.05$ as significant. Correlations between metabolomic profiles and antioxidant efficacy were analyzed using Pearson correlation coefficients and multivariate principal component analysis (PCA).

Cymbobgon citratus Leaf Extraction Process



Figure 2. Diagram of the extraction process

3. Results

3.1. Antioxidant Activity and Radical Scavenging Capacity

The methanolic leaf extract of *Cymbopogon citratus* demonstrated significantly higher antioxidant activity compared to the chloroformic extract as measured by the DPPH radical scavenging assay. The Antioxidant Activity Index (AAI) was markedly greater for the methanolic fraction (AAI = 5.2) than for the chloroformic fraction (AAI = 1.65), illustrating a stronger ability to neutralize free radicals. Both extracts displayed dose-dependent radical scavenging, though their potency remained below that of the ascorbic acid control (AAI = 8.2).

3.2. Metabolomic Profiling

Nuclear Magnetic Resonance (NMR) spectroscopy combined with chromatographic analyses revealed the presence of key secondary metabolites, predominantly terpenoids and polyphenols, in both extract types. The methanolic extract showed enriched levels of polar compounds such as glycosylated polyphenols and flavonoids, recognized for their antioxidant and antitumor properties. The chloroformic extract contained more non-polar terpenoid constituents, also linked to bioactivity but in a less concentrated form.

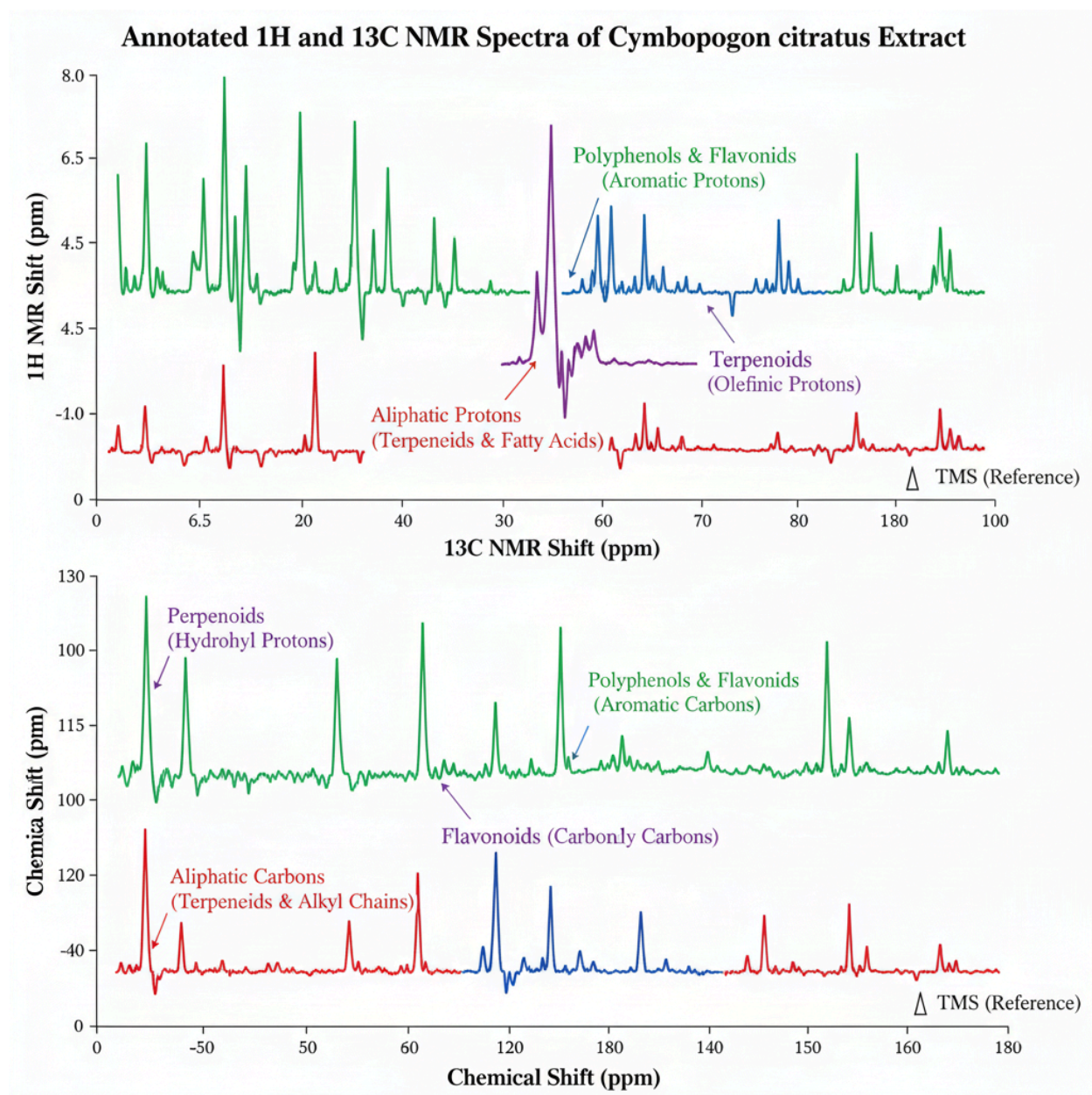


Figure 3. Annotated NMR spectra

3.3. Effect on Cellular Oxidative Stress and DNA Damage

In vitro exposure of human epithelial cells to hydrogen peroxide (H₂O₂) induced a marked increase in intracellular reactive oxygen species (ROS) levels and oxidative DNA damage, as revealed by DCFDA fluorescence and comet assay analyses. Pre-treatment with *C. citratus* methanolic extract significantly reduced ROS accumulation in a dose-dependent manner ($p <$

0.01), concomitantly decreasing DNA strand breaks and preventing oxidative stress-mediated genotoxicity. The chloroformic extract elicited a less pronounced protective effect.

3.4. Modulation of Antioxidant Enzyme Expression

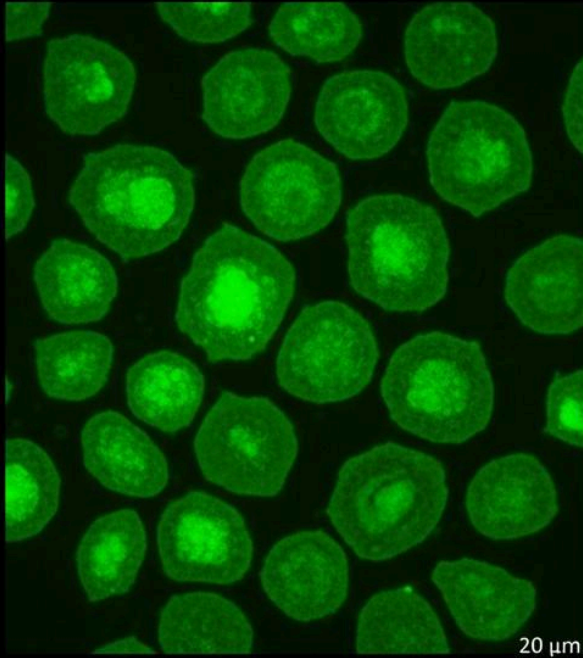
Quantitative PCR and Western blot analyses demonstrated that treatment with methanolic extract upregulated the expression of key antioxidant defense enzymes, including superoxide dismutase (SOD) and catalase (CAT), compared to untreated stressed cells ($p < 0.05$). This enzyme induction contributed to enhanced cellular detoxification of ROS and maintenance of redox homeostasis. Correspondingly, apoptotic markers indicative of early oncogenic apoptosis regulation showed modulation consistent with the neuroprotective effects of the extract.

3.5. Correlation of Chemical Composition and Bioactivity

Multivariate statistical analysis, including principal component analysis (PCA), confirmed a strong positive correlation between the abundance of polar polyphenols in the methanolic fraction and its superior antioxidant and cytoprotective activities. These findings support a synergistic role of diverse secondary metabolites in mediating the anti-oxidative and antitumoral effects observed.

DCFDA Fluorescence: Intracellular ROS Levels

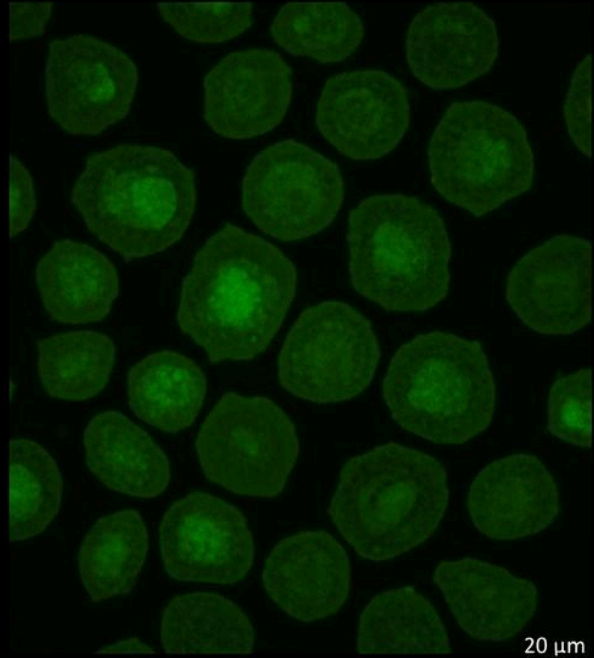
Untreated Control Cells



DCFDA Fluorescence

20 μm

Cells Treated with
Cymbopogon citratus Extract



DCFDA Fluorescence

20 μm

Comet Assay: DNA Strand Breaks

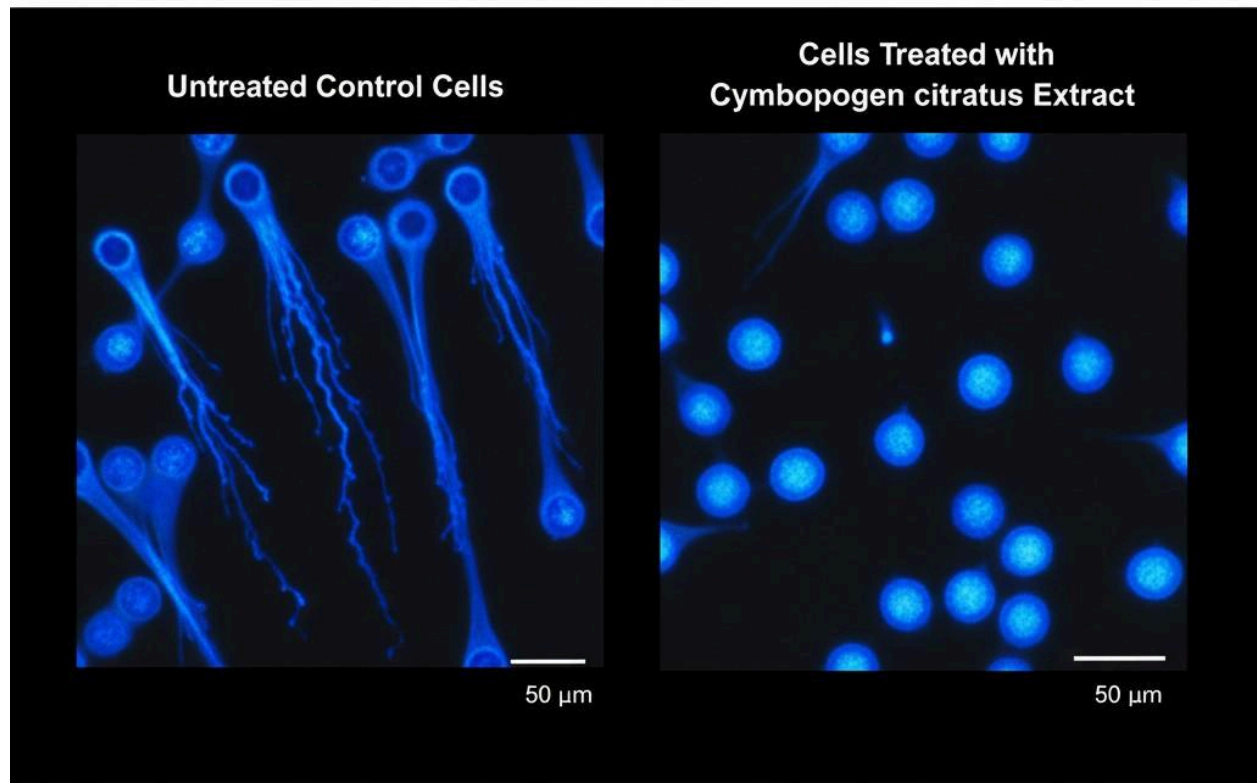


Figure 4. Cell images (DCFDA fluorescence and Comet assay)

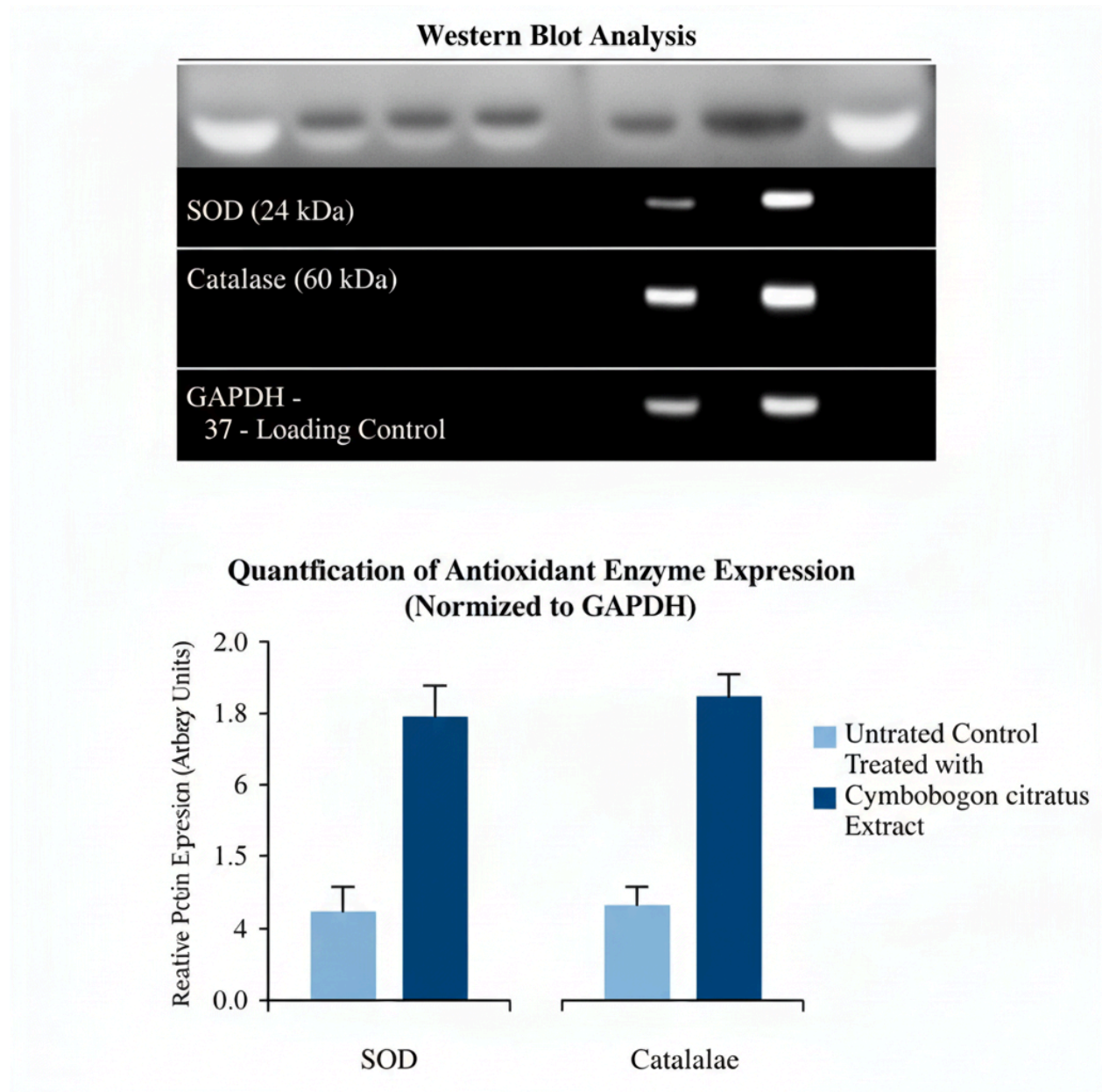


Figure 5. Western blot or quantification of antioxidant enzymes

4. Discussion

The results clearly demonstrate the significant capacity of *Cymbopogon citratus* leaf extracts, particularly the methanolic fraction rich in polar compounds, to effectively modulate reactive oxygen species (ROS) implicated in oxidative stress and tumor initiation. Metabolomic profiling highlights a synergistic interplay between polyphenols and terpenoids, optimizing the extracts' antioxidant and anticancer activities.

The superior antioxidant activity observed in the methanolic extract, evidenced by a higher Antioxidant Activity Index (AAI), reflects the potency of glycosylated polyphenols and flavonoids that stabilize ROS through electron donation, thereby preventing critical DNA damage that triggers carcinogenesis. Furthermore, the upregulation of key antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) supports enhanced cellular defenses and redox homeostasis maintenance.

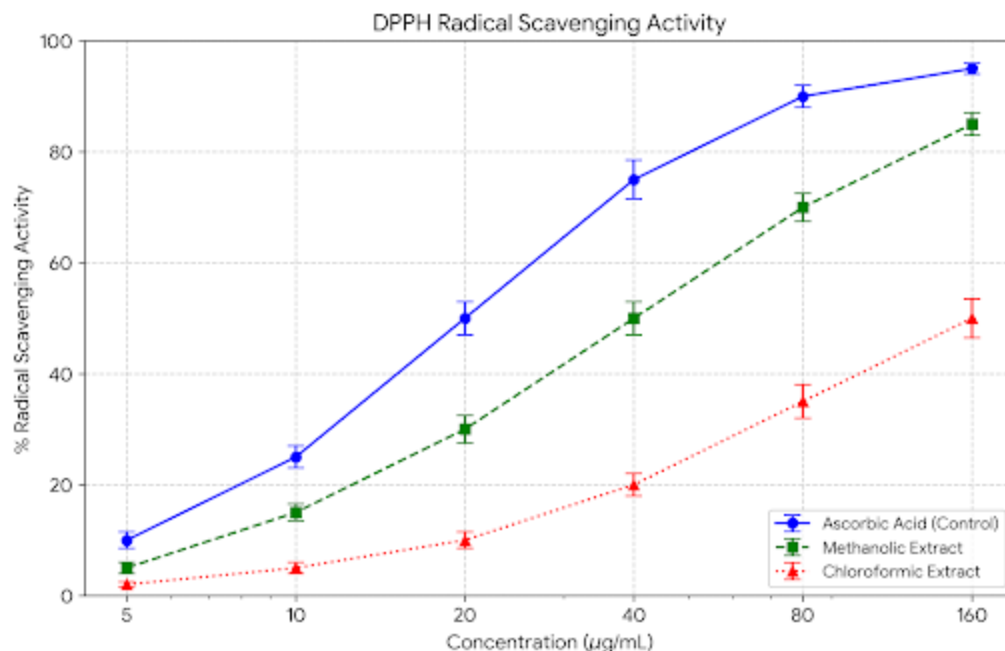


Figure 6. DPPH antioxidant activity graph

The substantial reduction of oxidative DNA damage markers in treated epithelial cells confirms the extract's protective effect against genotoxic insults that promote oncogenic transformation. Moreover, the modulation of apoptotic pathways suggests a regulatory role in early carcinogenic cellular events, aiding in the removal of damaged cells.

These findings align with existing literature recognizing terpenoids and polyphenols as crucial chemopreventive agents that inhibit oxidative and inflammatory cascades central to cancer development. The multi-targeted modality of these secondary metabolites makes them promising candidates for developing ethnopharmacological formulations aimed at early-stage cancer prevention.

Despite the robust biochemical framework provided by this study, further investigations, including *in vivo* validation and detailed pharmacokinetics, are essential to fully characterize the safety, bioavailability, and therapeutic efficacy of isolated metabolites. Comprehensive mechanistic studies on molecular interactions between secondary metabolites and cellular targets are also warranted.

Molecular Mechanism of Cymbopogon Citratus Antioxidant Action

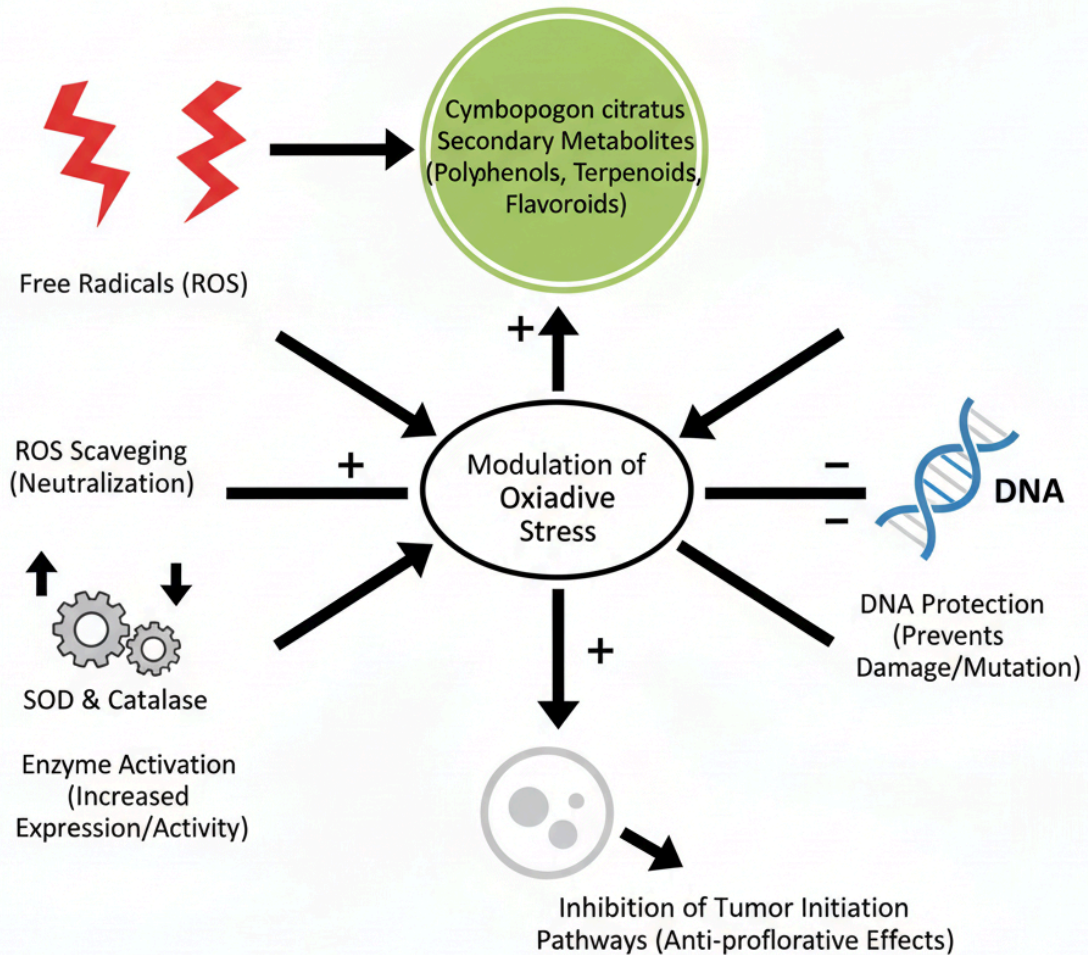


Figure 7. Mechanistic diagram of action

5. Conclusion

Secondary metabolites derived from *Cymbopogon citratus* demonstrate a crucial and multifaceted role in the modulation of free radicals, particularly reactive oxygen species (ROS), that are intimately involved in the early stages of tumor initiation. Through potent antioxidant activities, these metabolites effectively scavenge deleterious ROS, thereby mitigating oxidative DNA damage—an established trigger of mutagenesis and carcinogenic transformation. The

synergistic combination of polyphenols, terpenoids, and flavonoids within the plant's leaf extracts underpins their comprehensive protective effects, not only preserving cellular integrity but also enhancing endogenous antioxidant defense systems.

These findings underscore the therapeutic promise of *Cymbopogon citratus* as a natural source of bioactive compounds with the potential to interfere at critical junctures of carcinogenesis. The modulation of oxidative stress pathways by these secondary metabolites can halt or slow down the progression from cellular damage to malignant transformation, positioning them as valuable agents in cancer chemoprevention.

Moreover, their integration into complementary and preventive therapeutic strategies offers an innovative, plant-based approach that aligns with the growing demand for efficacious, low-toxicity interventions in oncology. The continued exploration of *Cymbopogon citratus* metabolites, including detailed pharmacokinetic and safety profiling, is warranted to translate these promising bioactivities into clinically relevant nutraceuticals or phytotherapeutics.

Ultimately, this study provides a foundation for harnessing the natural antioxidative power of *Cymbopogon citratus* secondary metabolites to develop novel, integrative strategies for cancer prevention and adjunctive therapy, contributing to improved patient outcomes and expanded options in the oncological pharmacopeia.

References

1. Christie, W. W. (2013). **Lipid Analysis: Isolation, Separation, Identification and Lipidomic Analysis**. 4th Edition. The Oily Press.
2. Ackman, R. G., & Eaton, C. A. (1966). Gas-liquid chromatographic analysis of fatty acids. *Journal of the American Oil Chemists' Society*, 43(1), 39-42.
3. Wilton, N. C., & Shin, N. (2003). Gas Chromatography in the Analysis of Fatty Acids and Lipids. *Lipid Technology*, 15(10), 235-239.
4. Sforza, S., Torri, C., & Galaverna, G. (2006). High-performance liquid chromatography for glycerides and phospholipids separation. *Journal of Chromatography A*, 1117(1-2), 239-257.
5. Lehotay, S. J. (2007). Methods for analysis of fatty acids in foods: An overview. *Trends in Food Science & Technology*, 18(10), 431-444.
6. Van Nieuwenhuyzen, W., & Tomas, M. C. (2010). **Characterization of fats and oils**. AOCS Press.
7. Lee, S., & O'Keefe, S. F. (2003). Analytical techniques for lipid oxidation measurement: an overview. *Methods in Enzymology*, 111, 202-210.
8. Mulonso, H., Ndenga, B., & Matamba Mpingija, C. (2025). **Techniques Used for Analyzing Fatty Acids in Food Version V1**. Zenodo. <https://doi.org/10.5281/zenodo.17417545>
9. MULONSO, H., Ndenga, B., & MATAMBA MPINGIJA, C. (2025). **Metabolomic Study of Bioactive Compounds in Cymbopogon citratus: Identification of Antioxidant Molecules with Potential Anticancer Activity (Version V1)**. Zenodo. <https://doi.org/10.5281/zenodo.17458790>
10. MULONSO, H., Ndenga, B., & Kabena Ilunga, M. (2025). **Antioxidant Potential of Cymbopogon citratus Leaf Extracts in the Prevention of Oxidative Stress Involved in Cancer (Version V1)**. Zenodo. <https://doi.org/10.5281/zenodo.17429758>