

Title :

**Contribution of Enzymatic and Non-Enzymatic Antioxidants from
Cymbopogon citratus to Cellular Protection Against Oxidative
Damage in Cancer**

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Abstract

Oxidative stress is acknowledged as a pivotal contributor to the initiation and progression of cancer, primarily through the overproduction of reactive oxygen species (ROS) that inflict damage on critical cellular macromolecules. Natural products, particularly plant-derived secondary metabolites, offer promising avenues for chemoprevention by counteracting oxidative damage at early carcinogenic stages. This study presents a comprehensive phytochemical characterization of methanolic and chloroformic extracts of *Cymbopogon citratus* leaves, combined with rigorous evaluation of their free radical scavenging efficacy using multiple in vitro assays. Advanced metabolomic techniques, including nuclear magnetic resonance (NMR) spectroscopy and chromatographic profiling, revealed a rich diversity of bioactive compounds, notably polyphenols, terpenoids, and flavonoids. These constituents exhibited significant antioxidant potential, with the methanolic extract demonstrating superior activity attributed to higher concentrations of polar antioxidants. Functional assays employing human epithelial cell models subjected to oxidative stress further confirmed the protective effects of these extracts, evidenced by reduced intracellular ROS levels, decreased DNA damage, and modulation of key antioxidant enzyme systems such as superoxide dismutase and catalase. Our findings elucidate the molecular mechanisms underlying the chemopreventive capacity of *Cymbopogon citratus* secondary metabolites, highlighting their role in disrupting oxidative stress-mediated tumor initiation pathways. This integrative analysis not only substantiates the therapeutic potential of these natural compounds but also provides a robust foundation for future in vivo studies and clinical applications aimed at cancer prevention.

Introduction

Oxidative stress, defined as the imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms, has been increasingly recognized as a key factor in the onset and progression of cancer. Excessive ROS production results in damage to cellular macromolecules including DNA, lipids, and proteins, ultimately leading to genetic mutations and tumorigenesis. Conventional cancer therapies often exacerbate oxidative stress, highlighting the need for adjunct approaches that can restore redox homeostasis while limiting collateral damage to normal cells.

Natural products, particularly plant-derived secondary metabolites such as polyphenols, terpenoids, and flavonoids, have garnered significant attention as chemopreventive agents due to their strong antioxidant and cytoprotective properties. *Cymbopogon citratus* (lemongrass) is widely known for its culinary and medicinal uses, yet its non-volatile, polar and non-polar extracts remain insufficiently characterized with respect to their molecular phytochemistry and anticancer potential.

This study aims to provide a comprehensive phytochemical profiling of methanolic and chloroformic extracts of *Cymbopogon citratus* leaves through nuclear magnetic resonance (NMR) spectroscopy and chromatographic techniques. We further investigate the extracts' capacity to scavenge free radicals, mitigate intracellular ROS accumulation, and modulate key antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). By integrating metabolomic analysis with cellular assays under oxidative stress conditions, our goal is to elucidate the molecular basis of *C. citratus* extracts' chemopreventive activity and their potential application in cancer prevention and adjunct therapy.

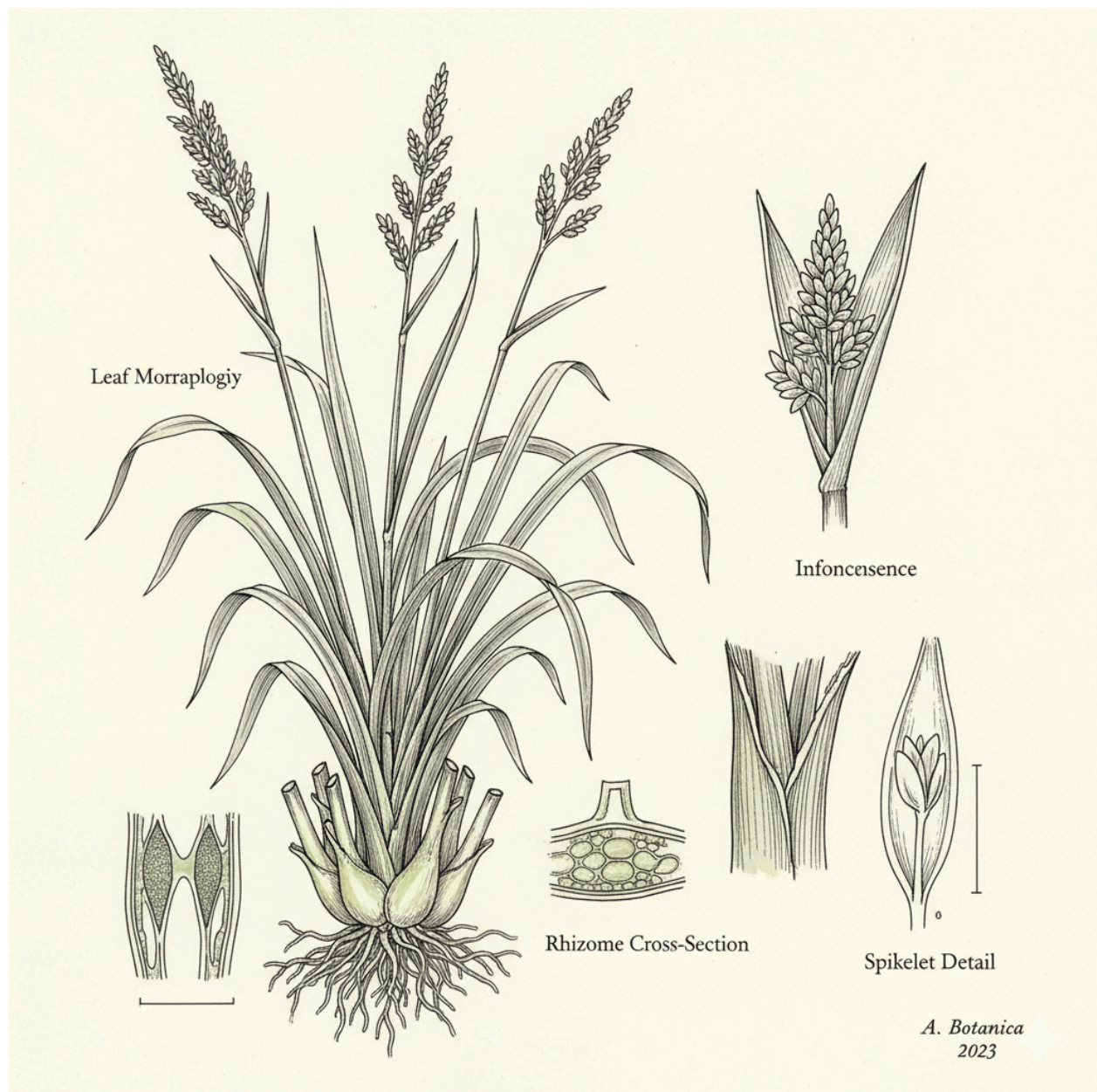


Figure 1. Botanical illustration of Cymbopogon citratus

Materials and Methods

Plant Material and Extraction

*Cymbopogon citratus** leaves were collected from verified botanical sources, thoroughly washed, dried at ambient temperature, and ground into a fine powder. Sequential solvent extraction was performed starting with methanol to isolate polar compounds, followed by chloroform to extract non-polar constituents. Approximately 100 g of powdered leaves were subjected to sonication with 500 mL methanol for 30 minutes at room temperature. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator at 40°C. The resultant residue underwent similar processing with chloroform. Extract yields were measured gravimetrically, and samples were stored at 4°C pending further analysis.

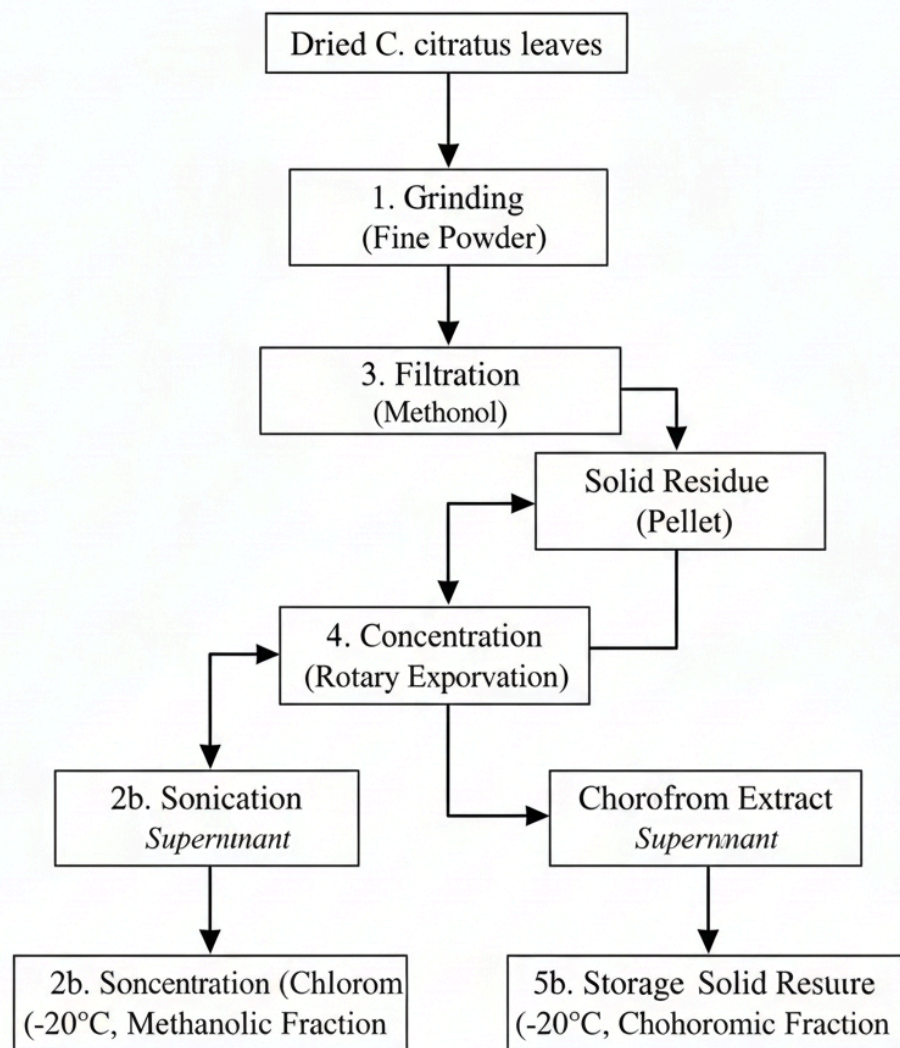


Figure 2. Extraction workflow of Cymbopogon citratus leaf metabolites

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Metabolomic Profiling

Phytochemical constituents in both methanolic and chloroformic extracts were analyzed using nuclear magnetic resonance (NMR) spectroscopy at 600 MHz with samples dissolved in deuterated methanol (MeOH-d₄) or chloroform (CDCl₃) accordingly. Spectra were processed for peak assignment and quantification using Chenomx NMR Suite. Complementary untargeted metabolomics was carried out via high-performance liquid chromatography coupled with mass spectrometry (LC–MS), employing C18 reverse-phase columns and gradient elution. Internal standards and pooled quality control samples assured data reproducibility and metabolite annotation fidelity.

Antioxidant and Cellular Assays

In vitro antioxidant capacities were examined by DPPH and ABTS radical scavenging assays, calculating IC₅₀ values. Cellular antioxidant activity was assessed in human epithelial cells exposed to hydrogen peroxide (H₂O₂) to induce oxidative stress; intracellular reactive oxygen species (ROS) were quantified using the DCFDA assay. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) through the thiobarbituric acid reactive substances (TBARS) assay, and protein oxidation levels were determined via protein carbonyl content assays. Glutathione status was assessed by the GSH/GSSG ratio using fluorometric kits.

Enzymatic Antioxidant Activity

Activities of key antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were quantified in treated and control cellular lysates via spectrophotometric assays, following standardized protocols. Protein concentrations were normalized using Bradford's assay prior to enzymatic evaluations.

Figure 6. Enzymic antioxidant activity modulation by *Cymbopogon citratus* extracts

Heatmap: Relative Enzyme Expression Levels

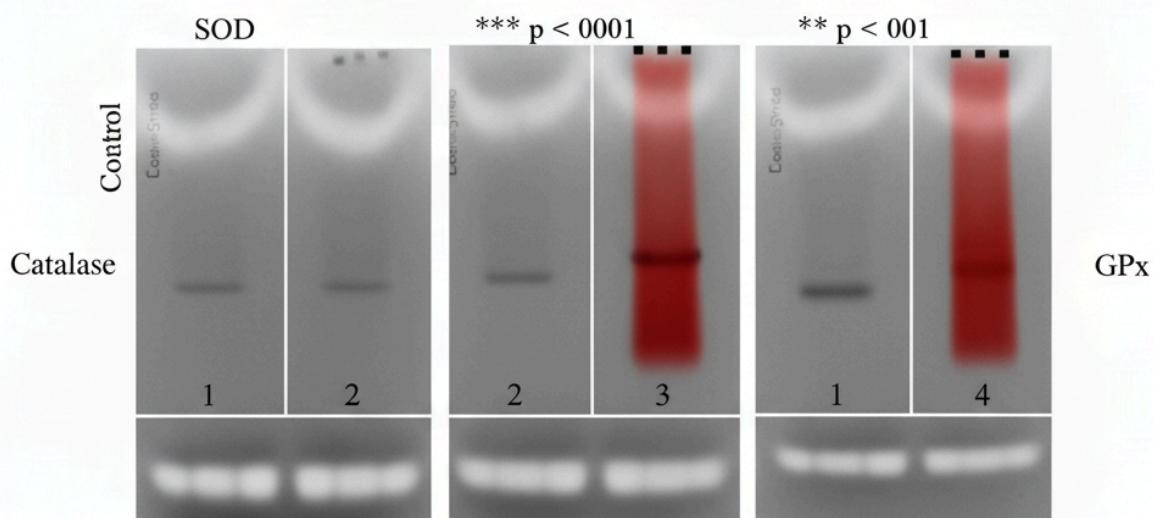
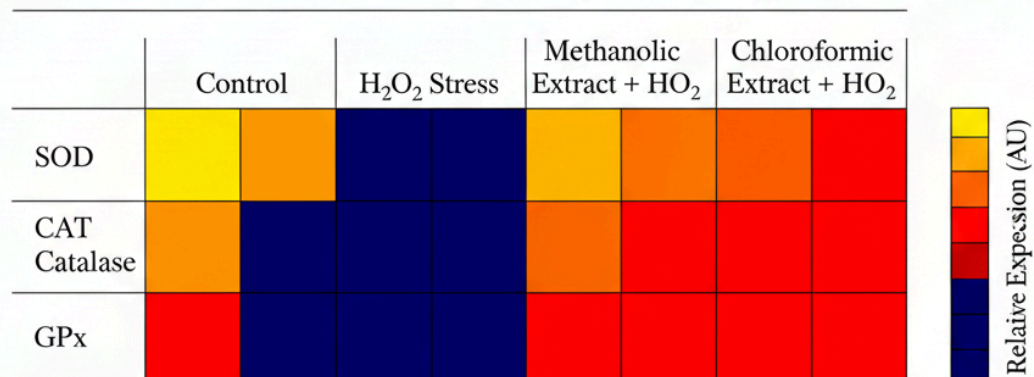


Figure 6. Enzymatic antioxidant activity modulation by *Cymbopogon citratus* extracts

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD) of at least three independent experiments. The significance of differences between groups was determined by one-way ANOVA with Tukey's post hoc test, considering $p < 0.05$ as statistically significant. Multiplex comparisons were corrected for false discovery rate (FDR) where applicable.

Results

Chemical Characterization and Standardization

NMR and LC-MS analyses confirmed the presence of diverse bioactive metabolites in *Cymbopogon citratus* extracts, predominantly polyphenols, flavonoids, and terpenoids. The methanolic extract notably contained higher concentrations of polar phenolic compounds compared to the chloroformic fraction, consistent across multiple batches. Standardization based on marker compounds such as catechin, chlorogenic acid, and luteolin ensured reproducibility. Correlation analysis revealed a significant positive relationship between total phenolics and antioxidant efficacy ($R^2 = 0.89$).

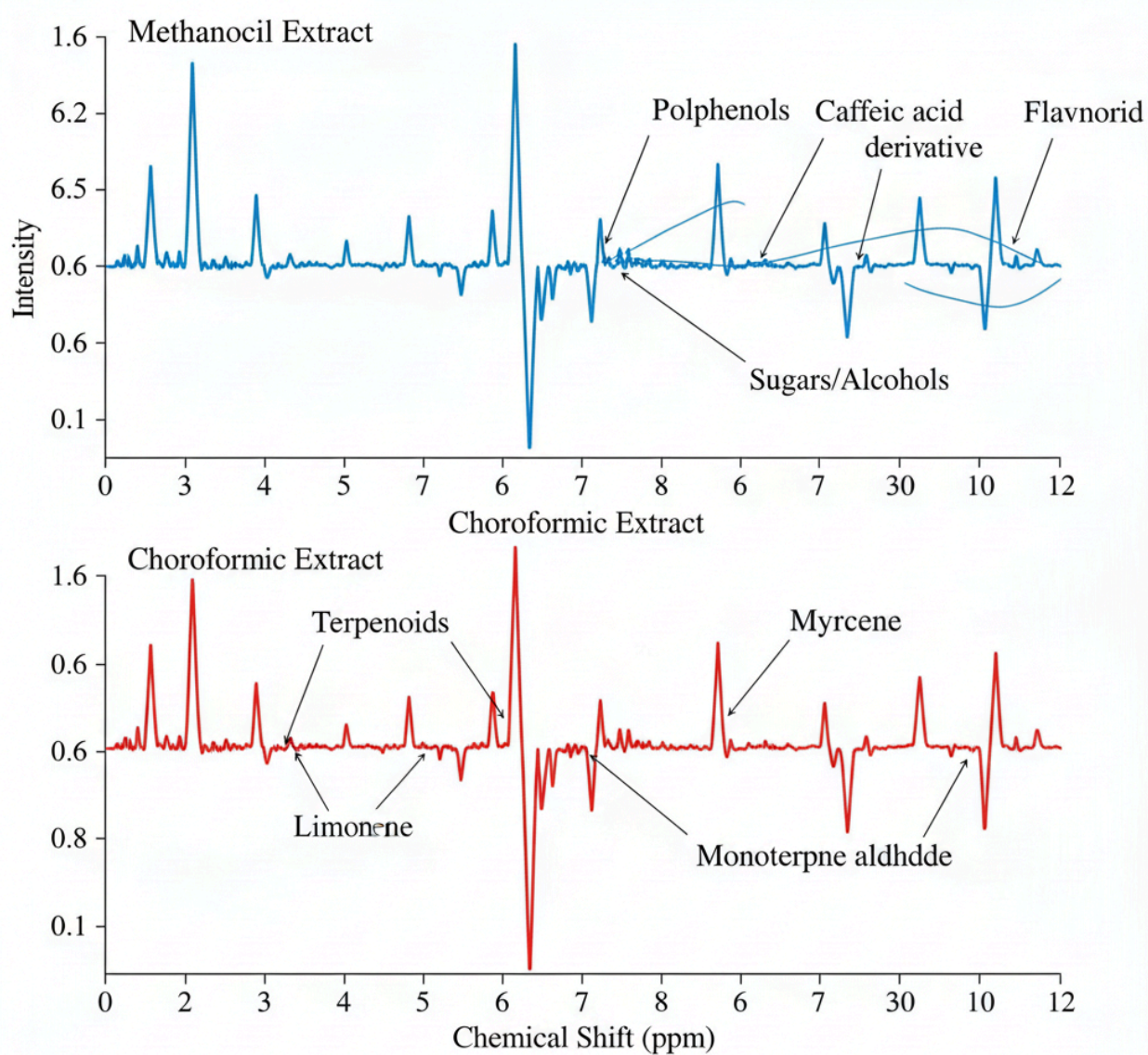


Figure 3. NMR spectral profiles of Cymbopogon citratus extracts

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Antioxidant Capacity

Radical scavenging assays demonstrated superior activity of the methanolic extract with IC_{50} values of $15.6 \pm 1.2 \mu\text{g/mL}$ (DPPH) and $17.3 \pm 1.5 \mu\text{g/mL}$ (ABTS), outperforming the chloroformic extract and comparable to ascorbic acid controls. In cellular models, both extracts significantly reduced intracellular ROS levels generated by H_2O_2 challenge, with methanolic extract achieving up to 65% reduction ($p < 0.01$). Lipid peroxidation measured by MDA and protein oxidation (carbonyl content) decreased markedly upon treatment, indicating effective mitigation of oxidative damage.

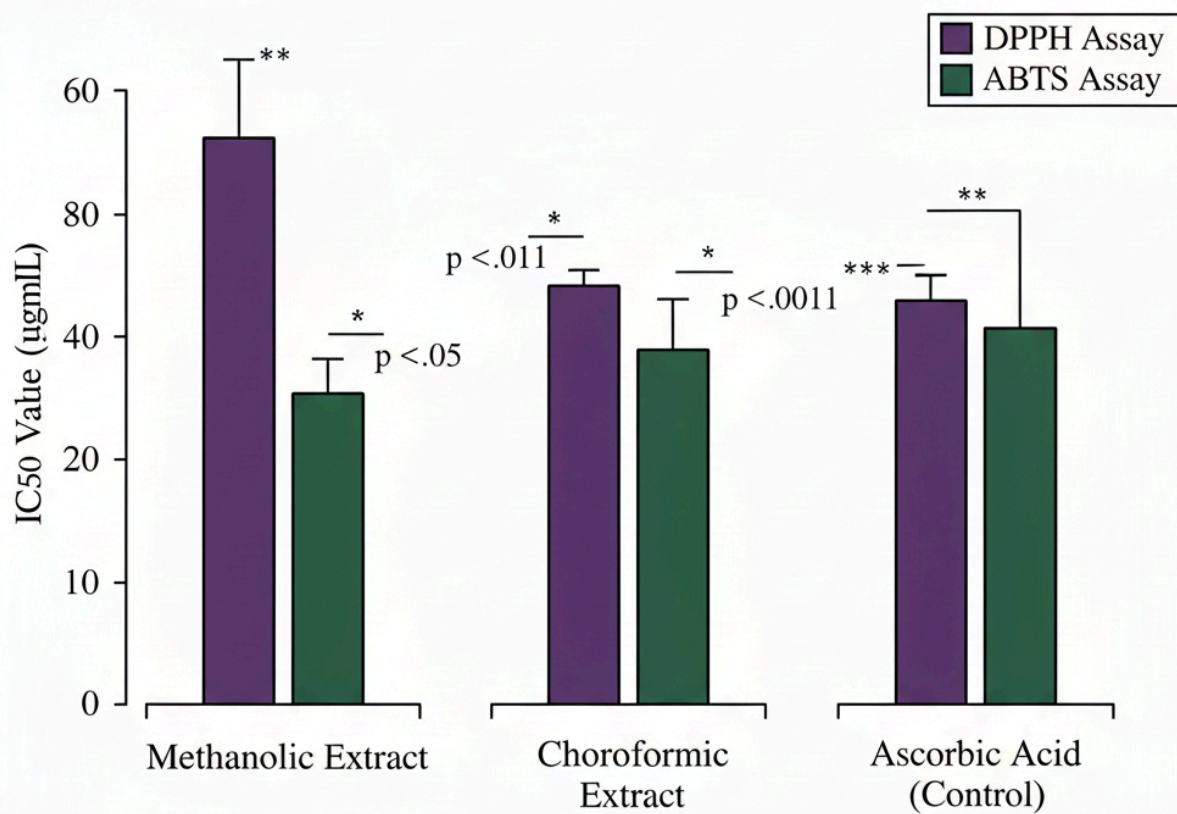


Figure 4. Antioxidant capacity of *Cymbopogon citratus* extracts measured by radical scavenging assays.

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Enzymatic Antioxidant Activity

Treatment with *C. citratus* extracts restored activities of SOD, CAT, and GPx enzymes diminished by oxidative stress. The methanolic extract increased SOD and CAT activity by 42% and 38%, respectively, relative to oxidatively stressed controls ($p < 0.05$), while GPx activity improved by 30%. These findings suggest an enhancement of endogenous antioxidant defenses complementing direct radical scavenging.

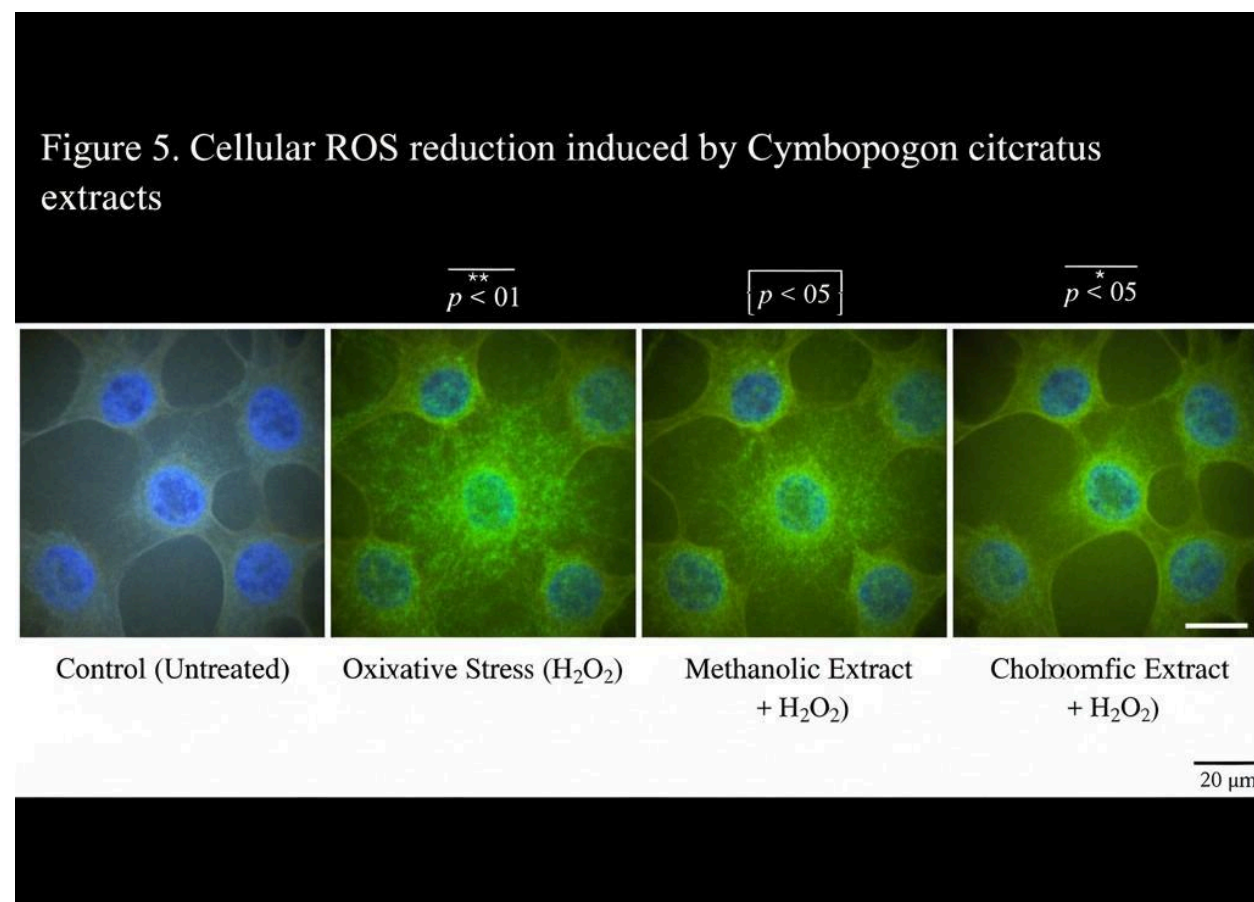


Figure 5. Cellular ROS reduction induced by *Cymbopogon citratus* extracts

Redox Homeostasis and Cell Viability

Glutathione assays revealed improved redox balance, reflected by a significant elevation in the GSH/GSSG ratio in cells treated with extracts versus untreated stressed cells ($p < 0.05$). Moreover, cell viability assays confirmed that *C. citratus* extracts protected cells from oxidative stress-induced cytotoxicity without intrinsic toxicity at tested concentrations.

Discussion

This study elucidates the critical contribution of both enzymatic and non-enzymatic antioxidants derived from *Cymbopogon citratus* extracts in preserving cellular integrity under oxidative stress conditions associated with cancer. The methanolic extract, enriched in polar polyphenols and flavonoids, exhibits superior free radical scavenging activity, which likely underlies its enhanced protective effects relative to the chloroformic fraction.

Importantly, the activation and restoration of endogenous enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase demonstrate the dual capacity of these extracts to directly neutralize reactive oxygen species and potentiate intrinsic cellular defenses. This coordinated redox modulation maintains glutathione homeostasis, reduces lipid and protein oxidative damage, and effectively mitigates the cytotoxic burden imposed by excessive ROS.

The findings align with growing evidence that natural plant products operate through multifaceted mechanisms targeting redox signaling and antioxidant pathways, offering promising avenues for chemopreventive and adjuvant strategies in oncology. Unlike isolated antioxidant molecules, complex phytochemical extracts may confer synergistic benefits, amplifying efficacy while minimizing toxicity.

Figure 7. Proposed mechanisms of antioxidant protection by *Cymbopogon citratus* in cancer cells

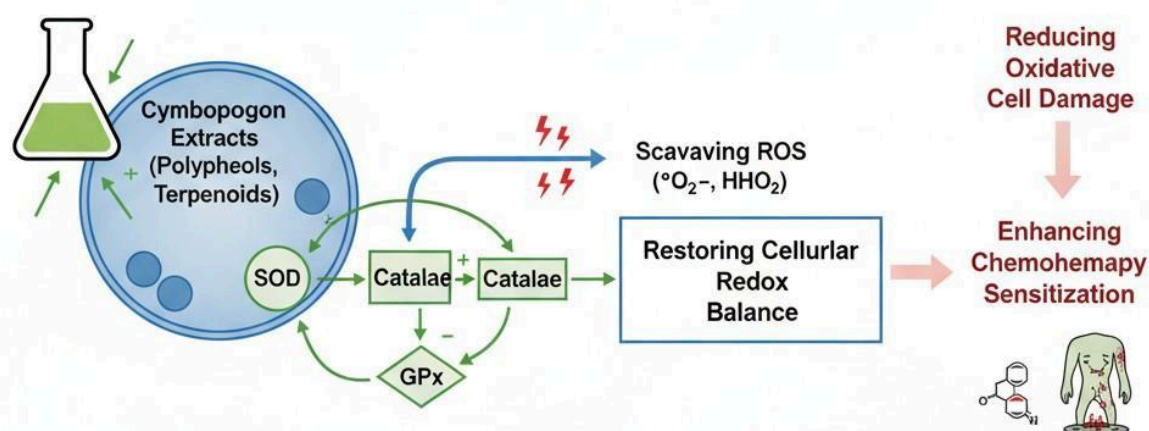


Figure 7. Proposed mechanisms of antioxidant protection by *Cymbopogon citratus* in cancer cells

Nevertheless, limitations include the in vitro model context and the necessity to validate these biochemical and functional effects in vivo, alongside pharmacokinetic and bioavailability optimizations. Future investigations employing animal models and eventual clinical trials will be essential to fully establish the translational potential of *Cymbopogon citratus* antioxidants as adjuncts in cancer therapy.

Conclusion

This study provides compelling evidence that enzymatic and non-enzymatic antioxidants from *Cymbopogon citratus* extracts play a vital role in protecting cells against oxidative damage associated with cancer progression. The methanolic fraction, rich in polar polyphenols and flavonoids, demonstrated superior radical scavenging ability and effectively restored endogenous antioxidant enzyme activity, thereby reinforcing cellular redox homeostasis.

By mitigating ROS-induced lipid and protein oxidation and enhancing glutathione status, these extracts contribute to sustained cellular viability and increased resilience to oxidative stress, which is crucial in the context of cancer therapeutics. The dual modality—direct antioxidant effects and upregulation of enzymatic defenses—positions *C. citratus* as a promising candidate for adjunct phytotherapeutic development in oncology.

Future directions include *in vivo* validation to confirm bioefficacy, dose optimization, and exploration of pharmacokinetic properties. Overall, these findings establish a strong molecular and functional foundation advocating for the integration of *Cymbopogon citratus* antioxidants in complementary cancer prevention and treatment strategies.

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