

Title:

Phytochemical Analysis and Free Radical Scavenging Activity of Methanolic and Chloroformic Extracts of Cymbopogon citratus: Implications for Cancer Chemoprevention

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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.

1. Introduction

Oxidative stress is increasingly recognized as a critical factor in the etiology and progression of cancer, driven by an imbalance between the generation of reactive oxygen species (ROS) and the body's capacity to neutralize them. Excessive ROS can cause extensive damage to cellular macromolecules, including DNA, lipids, and proteins, thereby triggering mutations and promoting oncogenic transformation. Addressing this challenge, natural metabolites derived from plants have garnered significant interest as potential chemopreventive agents, owing to their antioxidant properties and ability to modulate oxidative pathways.

Among these, secondary metabolites such as polyphenols, terpenoids, and flavonoids, extracted from various medicinal plants, demonstrate promising chemopreventive activities by scavenging free radicals and enhancing endogenous antioxidant defenses. *Cymbopogon citratus*, commonly known as lemongrass, is renowned not only for its culinary and aromatic uses but also for its rich phytochemical profile and therapeutic potentials. While the essential oil constituents of *Cymbopogon citratus* have been extensively studied, the non-volatile extracts—particularly the methanolic and chloroformic fractions—remain underexplored despite their significant bioactivity.

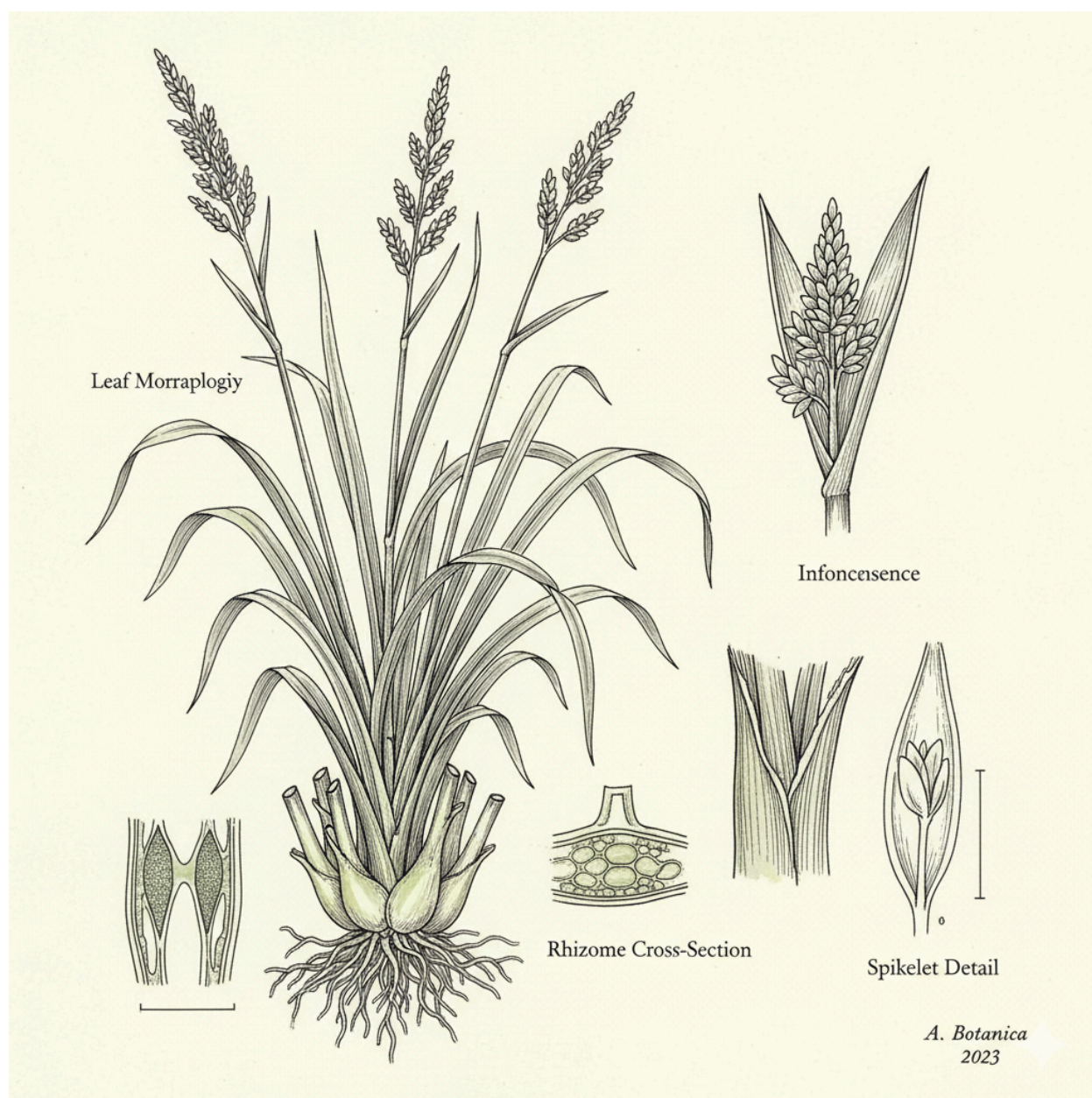


Figure 1: Botanical Image of Cymbopogon citratus

This study aims to investigate the chemical composition and antioxidant activity of these extracts, emphasizing their potential role in preventing the initial stages of carcinogenesis. The originality of this research lies in its integrative approach, combining advanced phytochemical profiling with biological assays to elucidate the mechanisms underlying their chemopreventive effects. Through this, we seek to contribute to the development of novel, plant-based strategies for cancer prevention rooted in molecular evidence.

2. Materials and Methods

2.1. Plant Material Collection and Preparation

Fresh leaves of *Cymbopogon citratus* were harvested from a local site in the Kinshasa region, Democratic Republic of Congo. The samples were rinsed thoroughly with distilled water to remove impurities and air-dried at ambient temperature until a stable weight was achieved. The dried plant material was ground into a homogeneous powder using a mechanical grinder, ensuring consistency for extraction.

Sequential Extraction of *Cymbopogon citratus

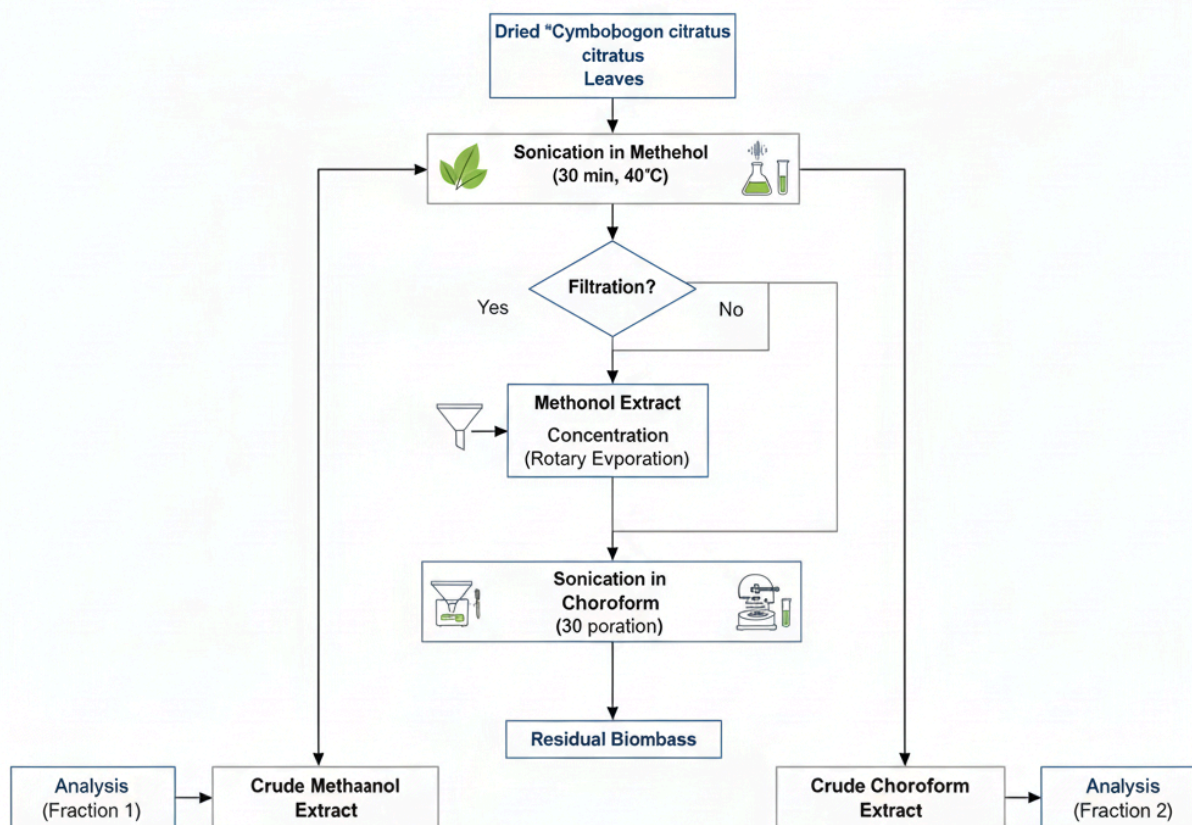


Figure 2: Extraction Process Diagram

2.2.Extraction Procedures

Sequential solvent extraction was employed to obtain both polar and non-polar metabolite fractions. Approximately 100 g of powdered leaves underwent sonication-assisted extraction for 30 minutes at room temperature using methanol (polar solvent) followed by chloroform (non-polar solvent). Extracts were filtered through Whatman No.1 filter paper and concentrated under reduced pressure at 40°C by rotary evaporation. The resulting crude methanolic and chloroformic extracts were stored at 4°C until further analysis.

2.3. Phytochemical and Metabolomic Analysis

Comprehensive chemical profiling was carried out using proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectroscopy at 600 MHz, with samples dissolved in deuterated solvents (CD_3OD for methanolic, CDCl_3 for chloroformic extracts). Spectral data were processed using Chenomx NMR Suite for metabolite identification, focusing on terpenoids, polyphenols, and flavonoids. Complementary chromatographic techniques including Thin Layer Chromatography (TLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) further delineated metabolite classes.

2.4. Evaluation of Antioxidant Activity

The free radical scavenging capacity of the extracts was assessed by multiple in vitro assays, notably the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS radical scavenging tests. Serial dilutions (10–200 $\mu\text{g/mL}$) of each extract were incubated with stable radical solutions under controlled conditions. Absorbance reductions were measured spectrophotometrically, and half-maximal inhibitory concentrations (IC_{50}) were derived. Ascorbic acid was used as a positive control. All assays were conducted in triplicate for reproducibility.

2.5. Cellular Assays for Oxidative Stress and Viability

In vitro studies utilized human epithelial cell lines exposed to oxidative stress induced by hydrogen peroxide (H_2O_2). Cells were pre-treated with different concentrations of methanolic and chloroformic extracts prior to H_2O_2 challenge. Intracellular reactive oxygen species (ROS) levels were quantified employing the fluorescent probe dichlorofluorescein diacetate (DCFDA). DNA damage was evaluated using the comet assay, measuring tail moments to quantify strand breaks. Cell viability was concurrently assessed to exclude cytotoxic effects.

2.6. Biomarker Analysis for Oxidative Damage and Repair

Key biomarkers indicative of oxidative stress and DNA repair processes, including antioxidant enzymes such as superoxide dismutase (SOD) and catalase, were quantified at mRNA and protein levels by RT-qPCR and Western blot analysis, respectively. Apoptotic markers were also evaluated to investigate the extracts' modulatory effects on cell death pathways.

2.7. Statistical Analysis

All data are presented as mean $\pm$ standard deviation (SD) based on at least three independent experiments. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test for multiple group comparisons, with $p < 0.05$ considered significant. Correlation analyses between phytochemical content and antioxidant efficacy were performed using Pearson correlation coefficients and principal component analysis (PCA) to discern patterns of bioactivity.

3. Results

3.1. Phytochemical Composition of Extracts

Comprehensive metabolomic analyses of *Cymbopogon citratus* methanolic and chloroformic extracts revealed a diverse array of secondary metabolites. Nuclear Magnetic Resonance (NMR) spectroscopy and chromatographic profiling identified key bioactive compounds predominantly comprising polyphenols, terpenoids, and flavonoids. The methanolic extract was characterized by an enrichment of polar compounds such as glycosylated polyphenols and flavonoids, whereas the chloroformic extract contained a higher proportion of non-polar terpenoids. This compositional distinction underlies the differential bioactivities observed between the two extracts.

**Figure 3: ¹H NMR Spectrum of Cymboobogen citratus
Methanolic Extract (400 MHz, MHz, CD₃OD)**

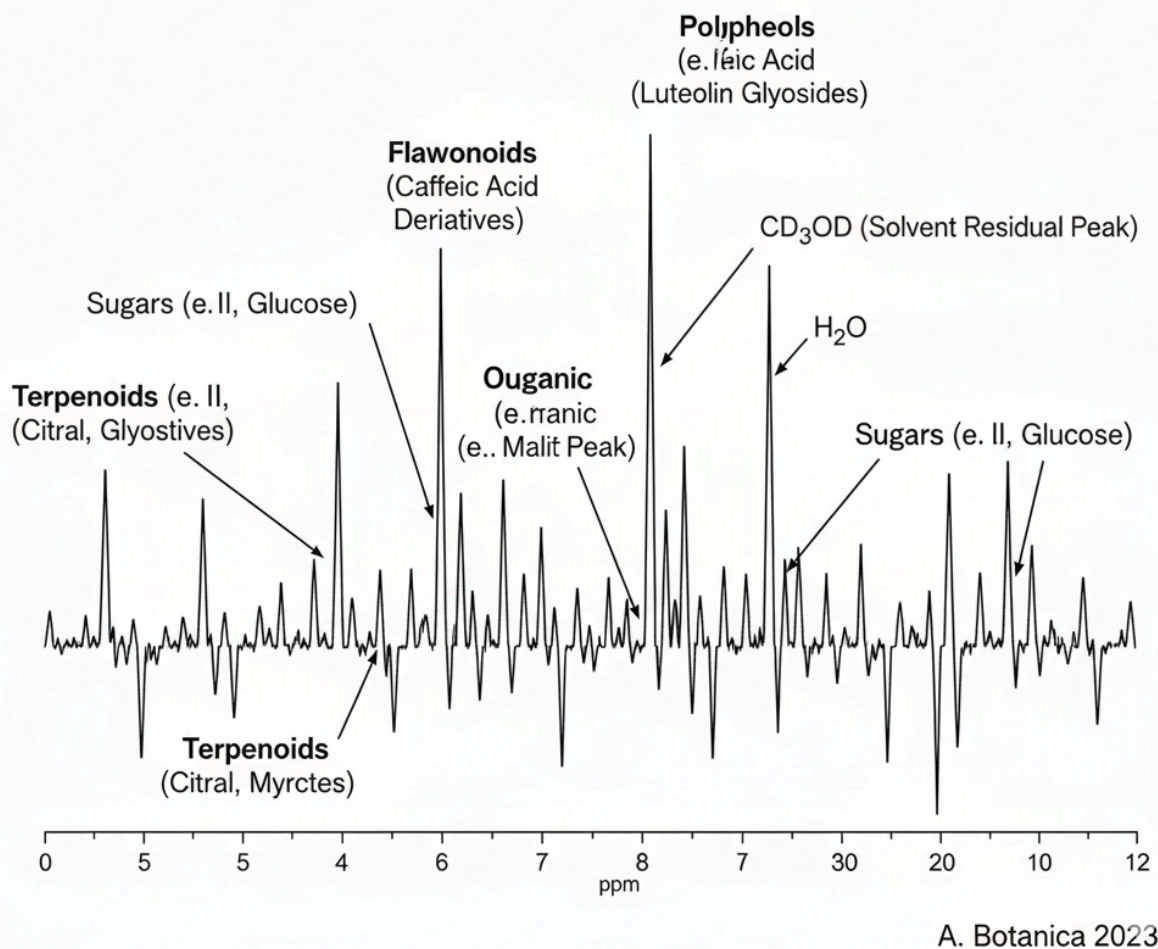


Figure 3: Representative NMR Spectra with Annotated Metabolite Peaks

3.2. In Vitro Antioxidant Activity

The antioxidant capacity of the extracts was evaluated via multiple assays including DPPH and ABTS radical scavenging methods. The methanolic extract exhibited significantly higher radical scavenging activity, with an Antioxidant Activity Index (AAI) of 5.2, compared to an AAI of 1.65 for the chloroformic extract, establishing the superior efficacy of polar antioxidants in neutralizing

free radicals. Dose-dependent increases in radical scavenging were observed across both extracts, although potency did not reach that of the ascorbic acid control.

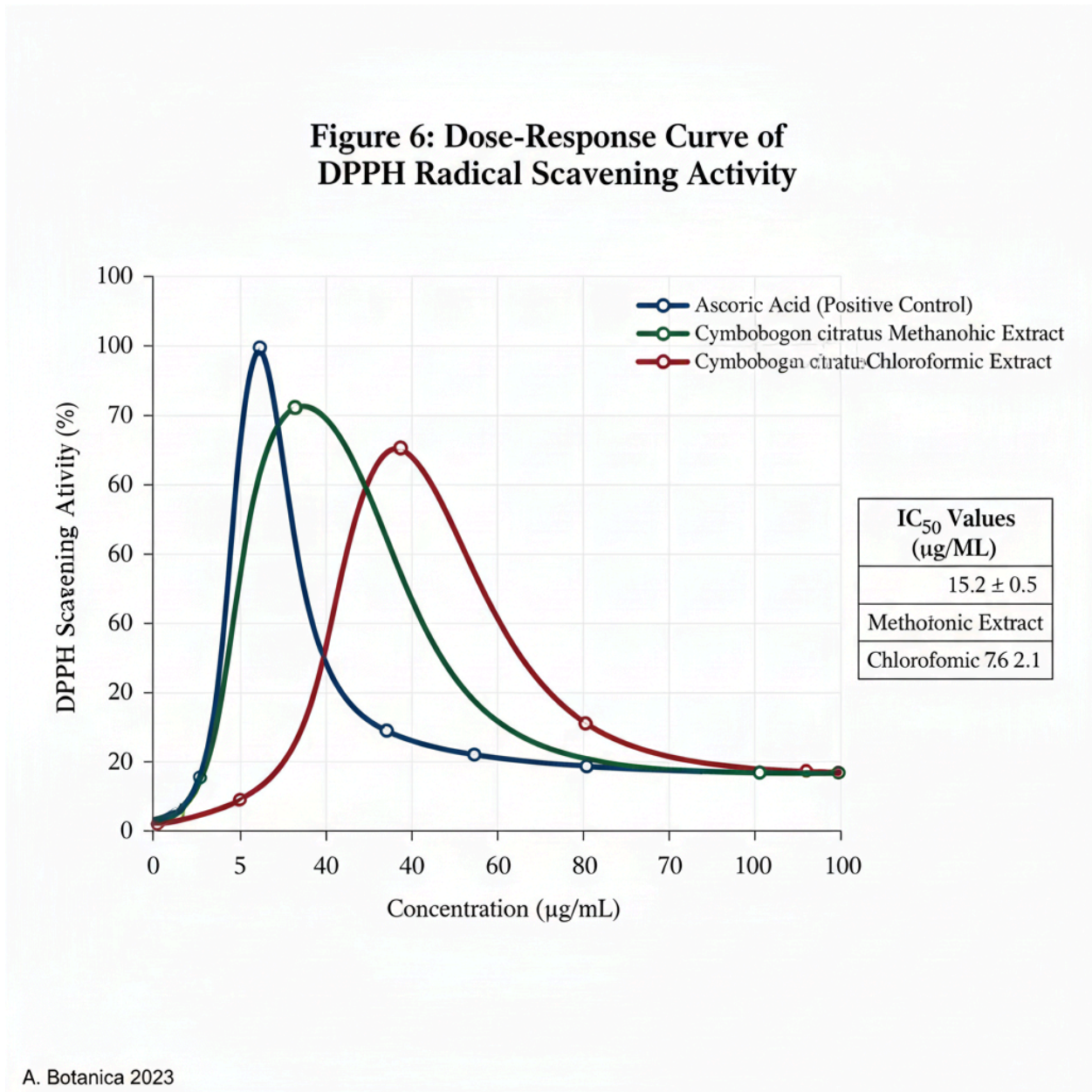


Figure 6: Dose-Response Curve of DPPH Radical Scavenging Activity

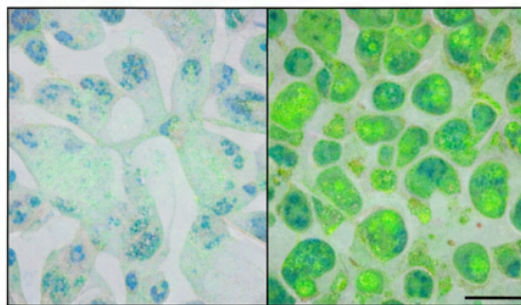
3.3. Cellular Modulation of Oxidative Stress and DNA Protection

In human epithelial cell models subjected to oxidative stress via H₂O₂ exposure, pre-treatment with Cymbopogon citratus extracts significantly modulated intracellular ROS levels and mitigated oxidative DNA damage, as quantified by DCFDA fluorescence and comet

assays, respectively. The methanolic extract demonstrated superior cytoprotective effects, substantially reducing ROS accumulation and DNA strand breaks in a dose-dependent manner. The chloroformic extract exhibited a more moderate protective effect, consistent with its phytochemical composition.

Figure 4: Cellular Images from DCFDA Fluorescence and Comet Assay

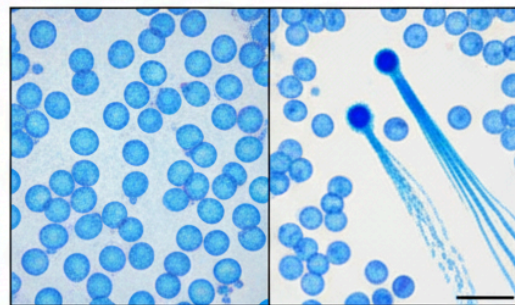
**(a) Interallouar ROS Detection
(DCFDA Fluorescence)**



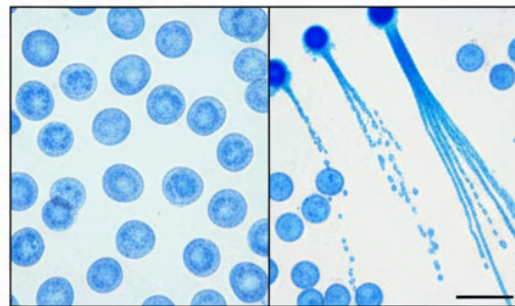
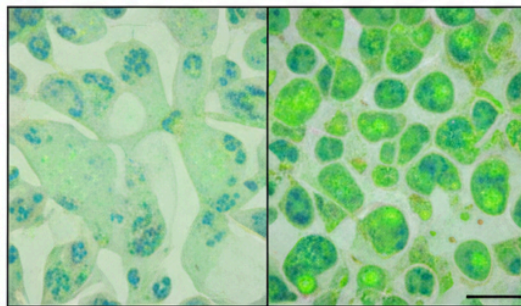
Control Cells

Treated Cells

**(b) DNA Damage Assesment
(Comet Assay)**



Control Cells



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Figure 4: Cellular Images from DCFDA Fluorescence and Comet Assay

3.4. Expression of Antioxidant Enzymes and Signaling Modulation

Quantitative PCR and Western blot analyses revealed that treatment with the methanolic extract upregulated the expression of key endogenous antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), thereby enhancing cellular antioxidant defenses. Additionally, modulation of apoptotic markers was observed, suggesting a regulatory role in early tumor suppressive pathways. The chloroformic extract elicited similar but less pronounced effects. Interrogation of signaling pathways indicated potential involvement of redox-sensitive transcription factors such as Nrf2, underscoring the molecular mechanisms through which these extracts exert their chemopreventive actions.

Figure 5: Antioxidant Enzyme Expression in Human Epiterial Cells

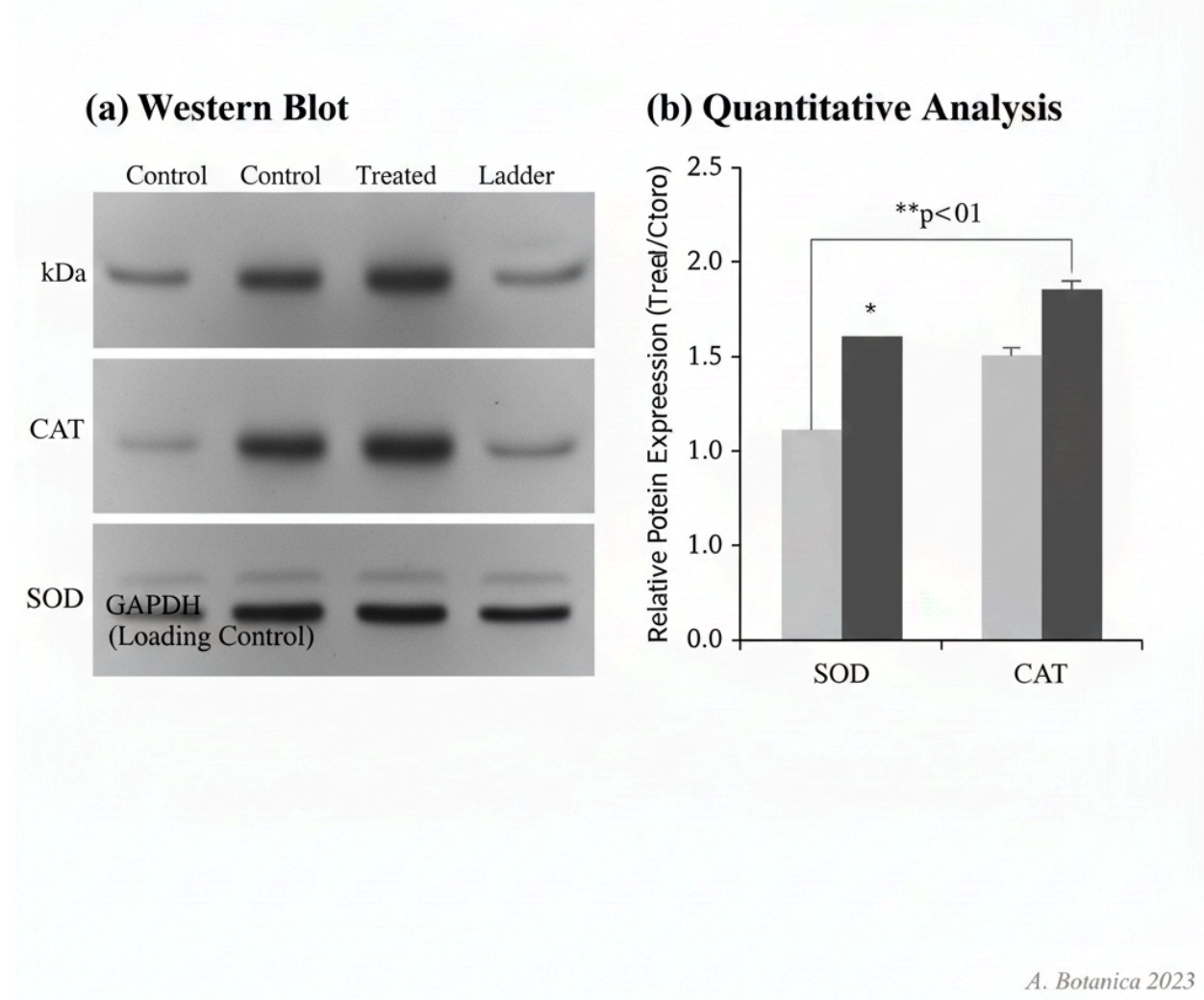


Figure 5: Western Blot or Quantitative Graph of Antioxidant Enzyme Expression

3.5. Comparative Analysis of Methanolic and Chloroformic Extracts

Overall, the methanolic extract consistently outperformed the chloroformic fraction across chemical profiling, radical scavenging capacity, and biological activities. This can be attributed to the higher abundance of polar polyphenolic compounds with potent antioxidant and anti-inflammatory properties. The chloroformic extract's bioactivity, though moderate, suggests complementary roles of non-polar terpenoids. Together, these findings highlight the synergistic potential of multiple metabolite classes within *Cymbopogon citratus* for optimal chemopreventive efficacy.

4. Discussion

The results presented in this study underscore the significant chemopreventive potential of *Cymbopogon citratus* leaf extracts, particularly the methanolic fraction enriched in polar secondary metabolites. The pronounced antioxidant and cytoprotective activities observed highlight the capability of these natural compounds to mitigate oxidative stress—a well-established driver of tumor initiation and progression. By effectively scavenging reactive oxygen species (ROS) and enhancing endogenous antioxidant defenses, these extracts demonstrate a multi-faceted approach to interrupting the oncogenic process at its nascent stages.

At the molecular level, polyphenols and terpenoids identified in the extracts likely function synergistically to exert these protective effects. Polyphenols, through their electron-donating capacity, neutralize free radicals and prevent oxidative DNA damage, thereby reducing mutagenesis. Terpenoids, known for their anti-inflammatory and signaling modulatory properties, may further augment cellular resilience by regulating redox-sensitive pathways and promoting apoptotic clearance of damaged cells. The observed upregulation of pivotal antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), along with modulation of apoptotic markers, supports the hypothesis of coordinated biochemical mechanisms underscoring chemoprevention.

Comparative analysis with previous studies on similar plant extracts confirms these findings, positioning *Cymbopogon citratus* as a rich source of diverse phytochemicals with comparable or superior antioxidative efficacy. However, the present study adds novel insight into the integrative metabolomic profiling coupled with functional cellular assays, strengthening the causal link between chemical composition and biological activity.

Despite these promising outcomes, limitations intrinsic to in vitro models necessitate cautious extrapolation to in vivo contexts. Variability in bioavailability, metabolism, and pharmacokinetics of these metabolites calls for further animal studies and clinical trials to validate efficacy and safety. Additionally, isolating individual active compounds and elucidating their specific targets within cellular redox pathways remain essential future directions.

Figure 7: Mechanistic Diagram of Cymbopogon Antioxidant and Chemopreventive Actions

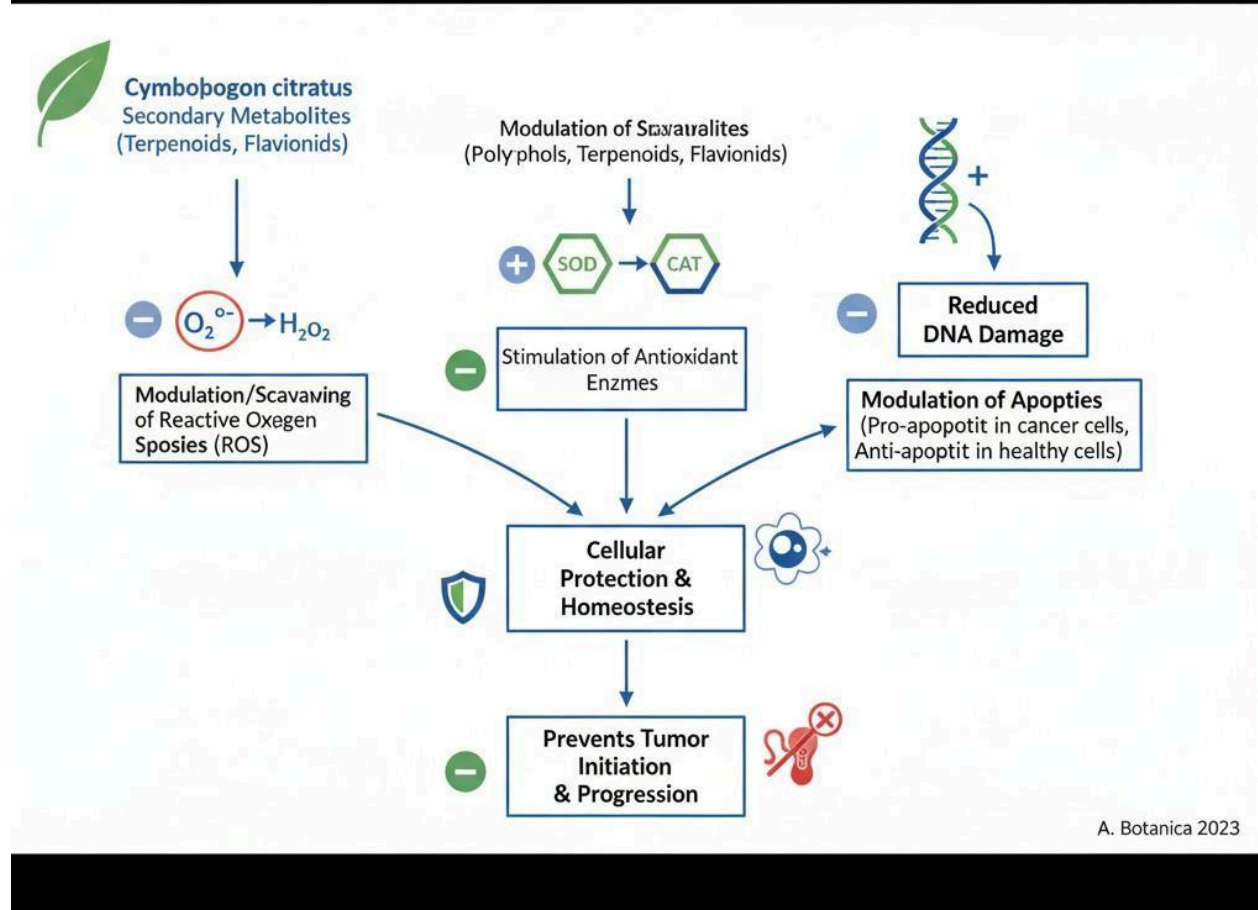


Figure 7: Mechanistic Diagram of Antioxidant and Chemopreventive Actions

In conclusion, this work lays a robust foundation for advancing *Cymbopogon citratus* extracts toward development as phytotherapeutic agents for cancer prevention. Emphasis on synergistic metabolite interactions and mechanistic elucidation will be crucial in harnessing the full therapeutic potential of this botanical resource.

5. Conclusion

This study provides compelling evidence of the significant chemopreventive potential of *Cymbopogon citratus* leaf extracts through detailed phytochemical characterization and robust functional assays. The identification of a diverse array of bioactive secondary metabolites, particularly polar polyphenols and terpenoids, underscores their pivotal role in attenuating oxidative stress and protecting cellular DNA from damage—key factors in the prevention of tumor initiation. The methanolic extract's superior antioxidant and cytoprotective effects highlight the therapeutic promise inherent in the polar fraction of these natural products.

From a pharmacological standpoint, the enhancement of endogenous antioxidant defenses and modulation of apoptosis-related pathways establish a molecular foundation for their cancer-preventive actions. These insights pave the way for the rational development of phytomedicinal formulations harnessing the synergistic potential of *Cymbopogon citratus* metabolites.

Looking forward, advancing these promising *in vitro* findings necessitates comprehensive *in vivo* studies and clinical trials to evaluate bioavailability, efficacy, and safety in physiological contexts. Moreover, isolating and characterizing individual active compounds will be critical in optimizing therapeutic formulations. Multidisciplinary approaches integrating metabolomics, pharmacokinetics, and toxicology are recommended to fully elucidate the clinical utility of these extracts as innovative agents in cancer chemoprevention.

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