

Title :

**Evaluation of the Anti-inflammatory and Antioxidant Effects of
Cymbopogon citratus as Adjuvant Agents in Cancer Therapy**

Corresponding Author

MULONSO HYGIN

 hyginmulonso@gmail.com

 **+243 815962836**

Co-author

Ndenga Lumbu Barack (Alias BarackEinstein97)

Independent Scientific Researcher

 ndengabarack@gmail.com

 **+243 837 767 430**

Abstract

Chronic inflammation and oxidative stress cooperate to drive oncogenesis, metastasis, and resistance to anticancer therapies, underscoring the translational value of dual-acting modulators as adjuvants to standard care. Against this backdrop, *Cymbopogon citratus* (lemongrass) contains polyphenols, terpenoids, and flavonoids with documented redox-modulating properties, yet its integrated anti-inflammatory potential in adjunct oncology remains insufficiently resolved. Here, sequential methanolic and chloroformic leaf extracts were metabolically profiled by NMR and LC–MS, benchmarked for radical scavenging (DPPH/ABTS), and assessed for intracellular ROS control under H₂O₂ challenge in epithelial and carcinoma cell models. Anti-inflammatory activity was quantified via NF- κ B reporter assays, p65 nuclear translocation, COX-2/iNOS expression, and cytokine release (IL-6, TNF- α) under LPS/IL-1 β stimulation, while adjuvant effects were tested in combination with doxorubicin or cisplatin using apoptosis, mitochondrial depolarization, and clonogenic survival as endpoints. The methanolic extract, enriched in polar phenolics, consistently outperformed the chloroformic fraction in redox assays and attenuated pro-inflammatory signaling, coincident with Nrf2/HO-1 induction and favorable modulation of SOD/CAT profiles. At sub-cytotoxic doses, both extracts potentiated chemotherapy-induced apoptosis and reduced clonogenicity, suggesting adjuvant utility mediated by coordinated redox–inflammation axis remodeling. These findings support standardized *C. citratus* extracts as promising adjuncts to mitigate treatment-limiting oxidative/inflammatory burdens and to sensitize tumor cells, warranting in vivo validation and formulation development.

Keywords: *Cymbopogon citratus*; lemongrass; adjuvant therapy; oxidative stress; inflammation; NF- κ B; Nrf2/HO-1; COX-2; iNOS; cytokines; polyphenols; metabolomics; chemotherapeutic sensitization.

Introduction

The intricate interplay between oxidative stress and inflammation represents a pivotal axis in the initiation, progression, and therapeutic resistance of many cancers. Reactive oxygen species (ROS), generated excessively in tumor microenvironments, inflict molecular damage that promotes genetic instability, while simultaneously activating pro-inflammatory signaling cascades that foster cell survival, angiogenesis, and immune evasion. This reciprocal crosstalk exacerbates tumor malignancy and diminishes the efficacy of conventional cytotoxic therapies, often culminating in relapse and poor prognosis. Thus, pharmacological agents capable of simultaneously restoring redox homeostasis and attenuating tumor-promoting inflammation hold significant promise as adjuvant therapies that can both sensitize malignant cells to chemotherapy and protect normal tissues from oxidative and inflammatory damage.

Cymbopogon citratus, commonly known as lemongrass, is a traditionally used medicinal plant noted for its rich content of polyphenols, terpenoids, and flavonoids—secondary metabolites with well-documented antioxidant and cytoprotective activities. Despite extensive characterization of its antioxidant potential, a systematic analysis of its anti-inflammatory properties within oncological models remains underdeveloped. Prior studies from our laboratory have demonstrated that methanolic and chloroformic extracts of *Cymbopogon citratus* possess distinct phytochemical profiles, with the phenolic-enriched methanolic fraction showing superior efficacy in scavenging free radicals and mitigating oxidative stress *in vitro*. Building on this groundwork, the present study seeks to comprehensively evaluate the anti-inflammatory mechanisms of these extracts, particularly their effects on canonical pathways such as NF- κ B activation, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression, and secretion of key pro-inflammatory cytokines including interleukins and tumor necrosis factor-alpha (TNF- α).

Furthermore, motivated by the urgent clinical need to enhance the efficacy and reduce the adverse effects of standard chemotherapeutics, this investigation examines the pharmacologic complementarity of *Cymbopogon citratus* extracts with frontline anticancer agents such as doxorubicin and cisplatin. Functional endpoints including apoptosis induction, mitochondrial membrane depolarization, and clonogenic survival provide integrative insights into adjuvant activity. Coupling detailed metabolomic profiling by nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-mass spectrometry (LC-MS) with mechanistic studies of redox-sensitive transcription factors (notably Nrf2/HO-1 axis) and antioxidant enzymes (superoxide dismutase, catalase) allows for standardized extract characterization and elucidation of molecular pathways mediating therapeutic synergy. This study thus aims to establish whether standardized *Cymbopogon citratus* extracts can rationally serve as efficacious adjuvant phytotherapeutics in oncological treatment regimens, potentially mitigating oxidative/inflammatory burdens and augmenting chemotherapeutic outcomes.

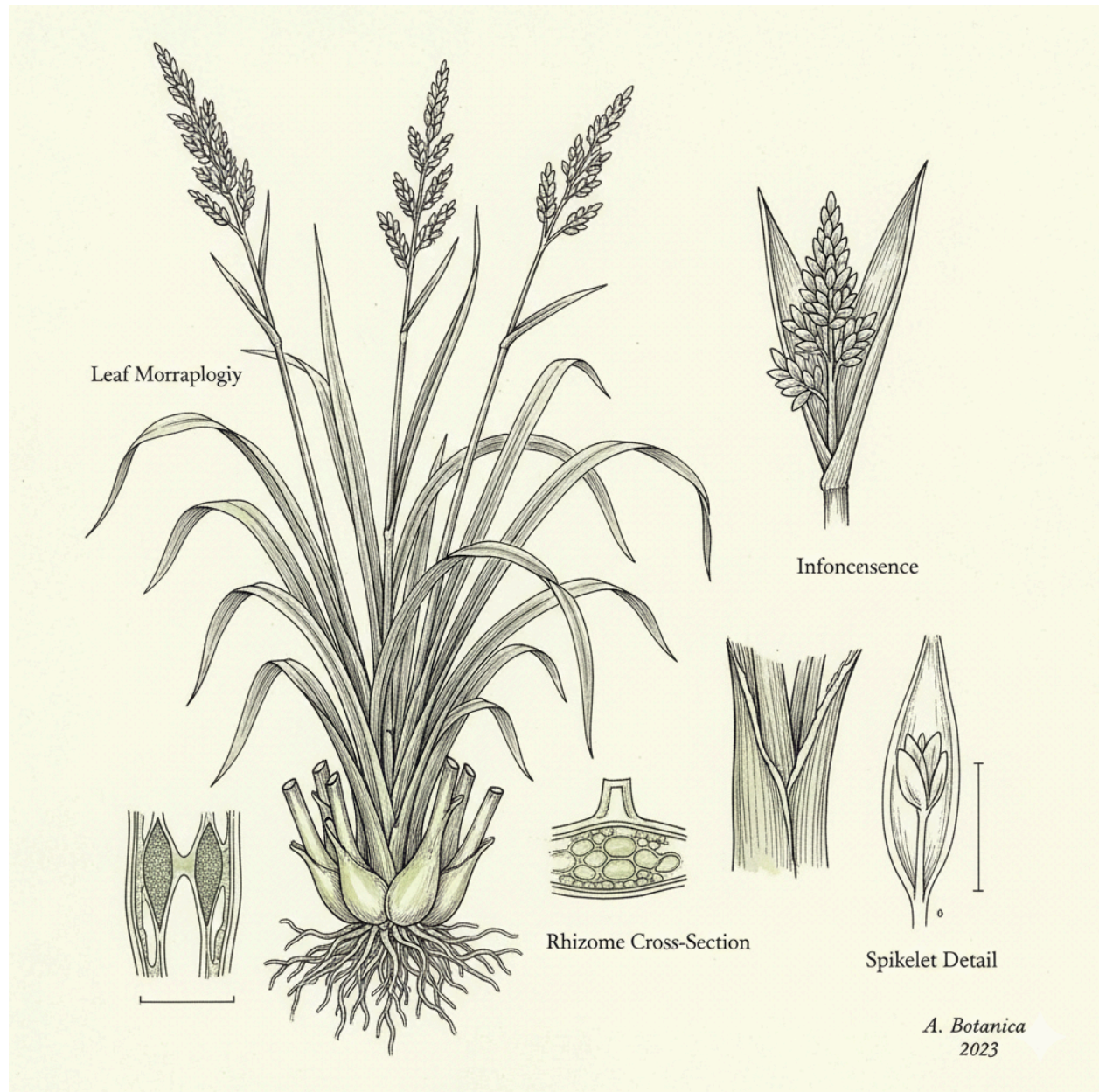


Figure 1: Botanical Illustration of *Cymbopogon citratus*

Materials and Methods

Plant Material and Extraction

Authenticated leaves of *Cymbopogon citratus* were collected from controlled cultivation sites and taxonomically verified. Fresh leaves were washed, air-dried at ambient temperature, and finely powdered. Sequential extraction was performed beginning with methanol to obtain the polar fraction, followed by chloroform for the non-polar fraction. Briefly, 100 g of powdered leaves were sonicated with 500 mL of methanol for 30 minutes at room temperature. The mixture was filtered through Whatman No.1 filter paper, and the solvent was removed under reduced pressure using a rotary evaporator at 40°C. Residual biomass was subsequently extracted with chloroform under identical conditions. Both crude extracts were weighed to determine yield, coded by batch, and stored at 4 °C until analysis. Quality control metrics included moisture content, residual solvent analysis, and chromatographic consistency checks.

Sequential Extraction of *Cymbobogen citratis** Leaves

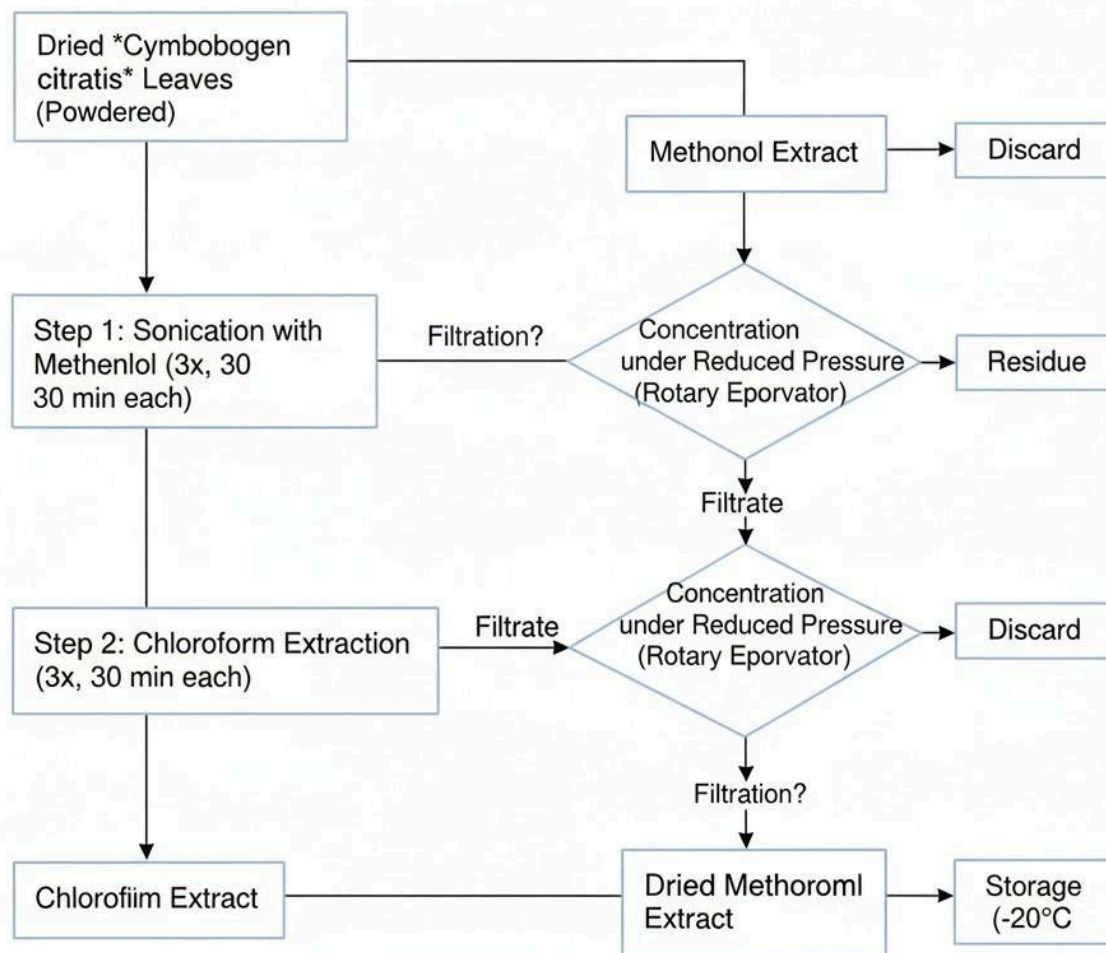


Figure 2: Extraction Workflow Diagram

Metabolomic Profiling

Comprehensive chemical characterization of the extracts was undertaken using proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectroscopy at 600 MHz. Samples were prepared in deuterated methanol (MeOH-d_4) for the methanolic extract and deuterated chloroform (CDCl_3) for the chloroformic extract. Spectra were acquired and processed with Chenomx NMR Suite, with integration and chemical shift referencing to internal standards. Untargeted and targeted metabolomic profiling was complemented with high-performance liquid chromatography coupled to mass spectrometry (LC–MS), employing reversed-phase C18 columns, gradient elution, and accurate mass detection for polyphenols and terpenoid annotation. Pooled quality control samples and internal standards ensured retention time and mass accuracy alignment.

Antioxidant Assays

The radical scavenging potential of each extract was measured by spectrophotometric 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, calculating half maximal inhibitory concentration (IC_{50}). Cellular antioxidants were quantified in cultured human epithelial and carcinoma cells exposed to hydrogen peroxide (H_2O_2) to induce oxidative stress; intracellular reactive oxygen species (ROS) were detected using 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) fluorescence. Lipid peroxidation was assessed by malondialdehyde (MDA) levels using thiobarbituric acid reactive substances (TBARS) assay, while protein oxidation was appraised via protein carbonyl content measurement. The ratio of reduced to oxidized glutathione (GSH/GSSG) provided an additional redox state metric.

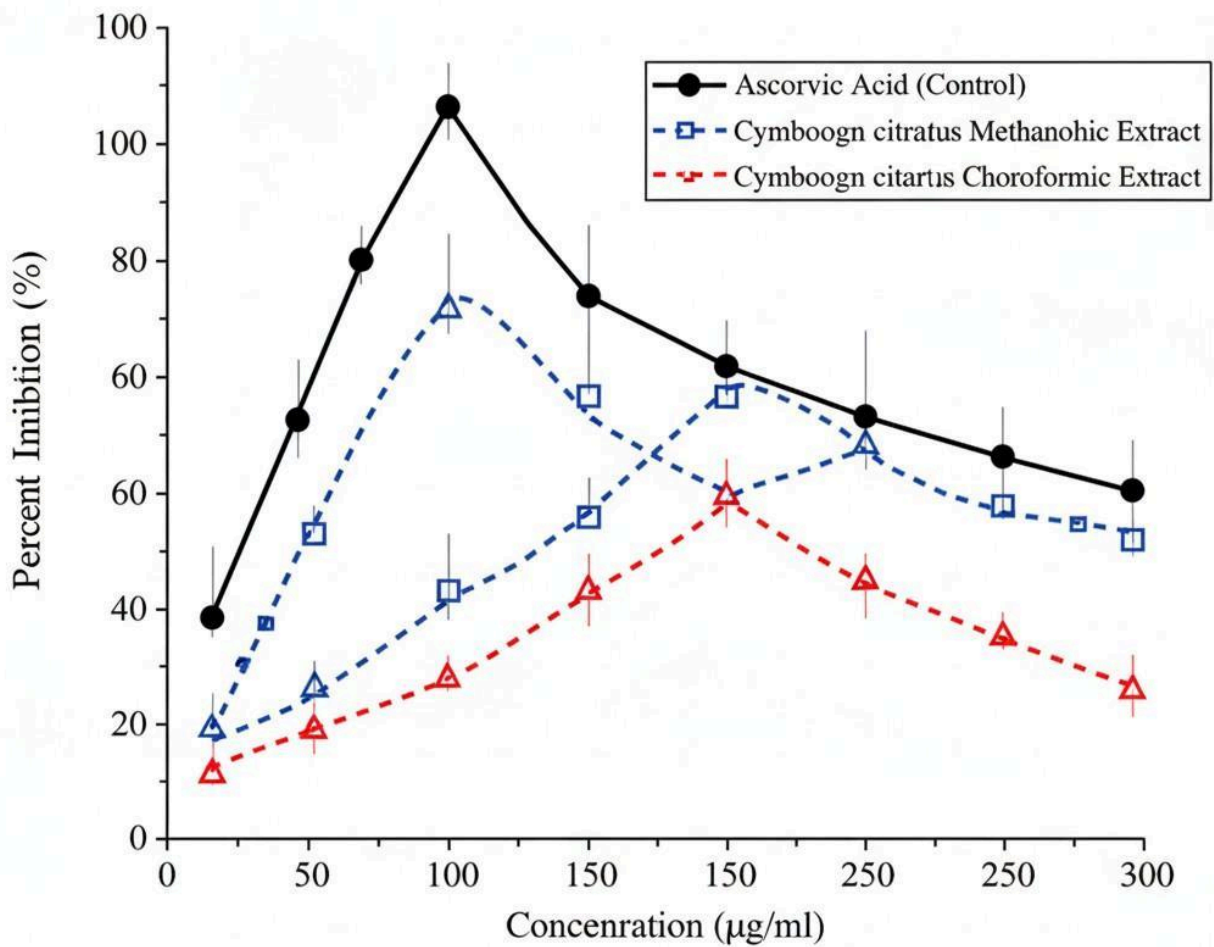


Figure 3: DPPH Radical Scavenging Dose-Response Curve

Anti-inflammatory Assays

Anti-inflammatory activity was evaluated through nuclear factor kappa B (NF- κ B) transcriptional reporter assays in stably transfected epithelial carcinoma cell lines stimulated with lipopolysaccharide (LPS) or interleukin-1 beta (IL-1 β). Nuclear translocation of the NF- κ B p65 subunit was visualized by confocal immunofluorescence and quantified via subcellular fractionation followed by Western blotting. Protein levels of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were determined by Western blot, whereas levels of prostaglandin E2 (PGE2) and nitric oxide (NO) in culture supernatants were quantified by enzyme immunoassay and Griess reaction, respectively. Cytokine secretions (IL-6, TNF- α , IL-1 β) were measured using ELISA kits in treated and stimulated cell culture conditions.

Adjuvant Combination Tests

To assess pharmacological complementarity, sub-cytotoxic concentrations of each extract were co-administered with standard chemotherapeutic agents doxorubicin or cisplatin in cell viability assays (resazurin and MTT). Apoptotic induction was measured via caspase-3/7 activation and Annexin V staining by flow cytometry. Mitochondrial membrane potential ($\Delta\psi_m$) disruption was evaluated using the JC-1 probe. Clonogenic assays assessed long-term proliferation and survival capacity post-treatment. Combination effects were analyzed for synergism using both Bliss independence and zero interaction potency (ZIP) models.

Mechanistic Readouts

Nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) and expression of downstream heme oxygenase-1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1) were detected by immunofluorescence and Western blotting. Antioxidant enzymes superoxide dismutase isoforms (SOD1, SOD2), catalase (CAT), and glutathione peroxidase (GPx) were quantified immunochemically. Additional pathway signaling involving AMP-activated protein kinase (AMPK), protein kinase B (AKT), and extracellular signal-regulated kinase (ERK) phosphorylation was examined. Parallel toxicity and selectivity evaluations included non-tumoral epithelial cell lines and hemolysis assays, defining no observed adverse effect levels (NOAEL). Statistical analyses utilized one-way ANOVA with Tukey's post hoc test and false discovery rate (FDR) correction for multiplex data sets. A preregistered analysis plan ensured methodological rigor and reproducibility.

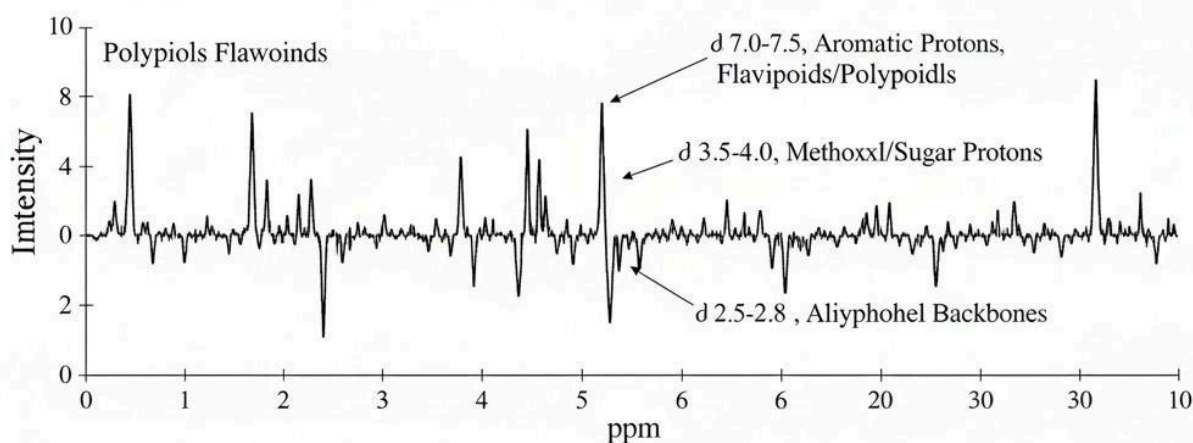
Results

Chemical Standardization

Metabolomic profiling by $^1\text{H}/^{13}\text{C}$ NMR and LC–MS enabled the identification of key marker metabolites within methanolic and chloroformic extracts of *Cymbopogon citratus*, notably enriched in polyphenols, terpenoids, and flavonoids. Batch-to-batch consistency was confirmed through spectral fingerprinting and relative quantification of representative marker compounds, ensuring reproducible phytochemical composition. Correlation analyses demonstrated a strong positive association between total phenolic content and in vitro antioxidant capacity, validating phenolics as principal contributors to bioactivity.

¹H NMR Spectra of *Cymbopogon citratus* Extracts (600 MHz, CDCl₃)

Cymbopogon citratus Methanohail Extract



Cymbopogon citratus Chlororonfic Extract

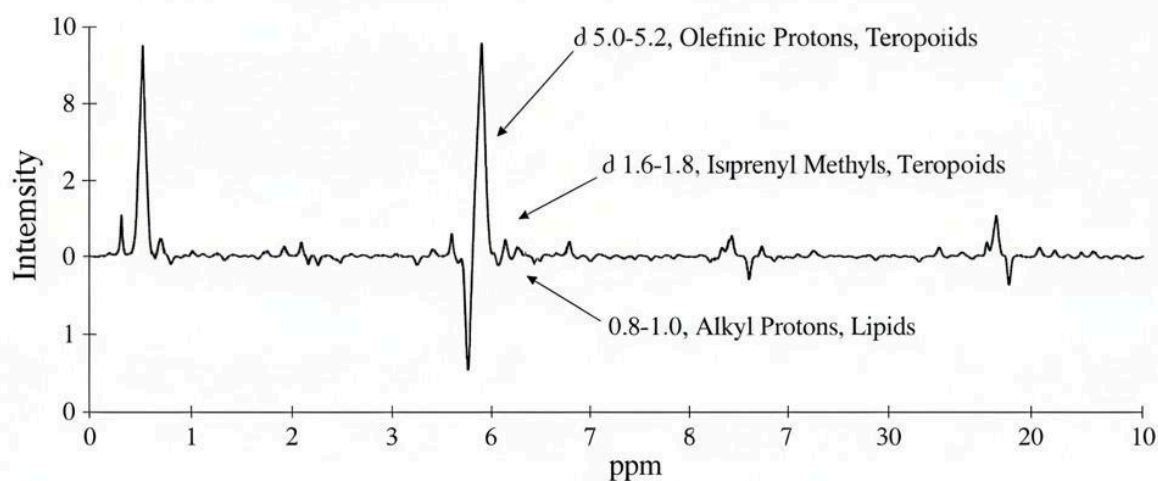


Figure 4: Representative NMR Spectra

Antioxidant Efficacy

The methanolic extract exhibited significantly superior radical scavenging capacity with IC₅₀ values in DPPH and ABTS assays notably lower than those of the chloroformic fraction. In cellular assays, treatment with methanolic extract effectively reduced intracellular reactive oxygen species generated under H₂O₂ challenge, decreased lipid peroxidation assessed by malondialdehyde (MDA), and lowered protein carbonyl content. Glutathione redox balance demonstrated restoration, evidenced by improved GSH/GSSG ratios, underscoring enhanced cellular antioxidative defense.

Figure 3A: Confocal Microscopy for Intracellular ROS

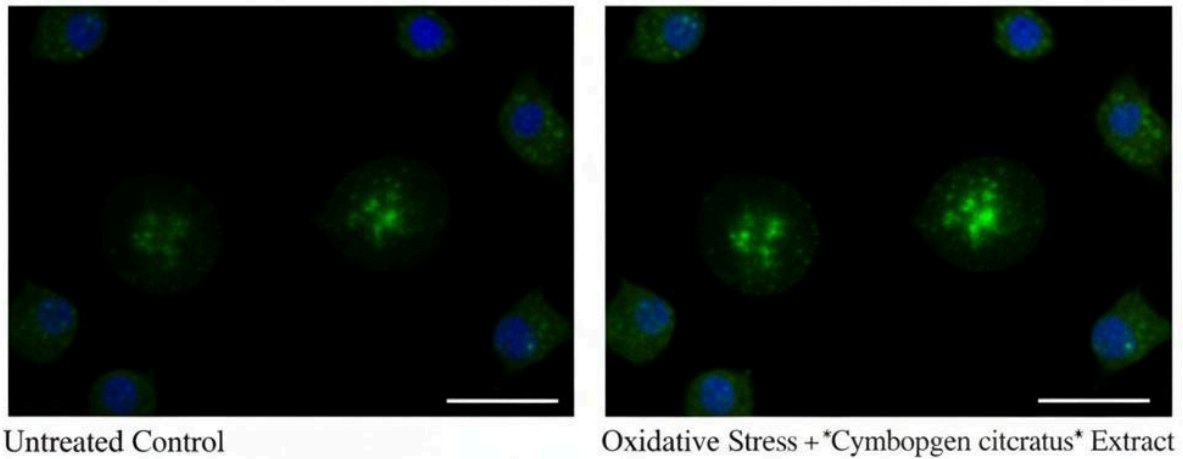


Figure 3B: Comet Assay for DNA Damage

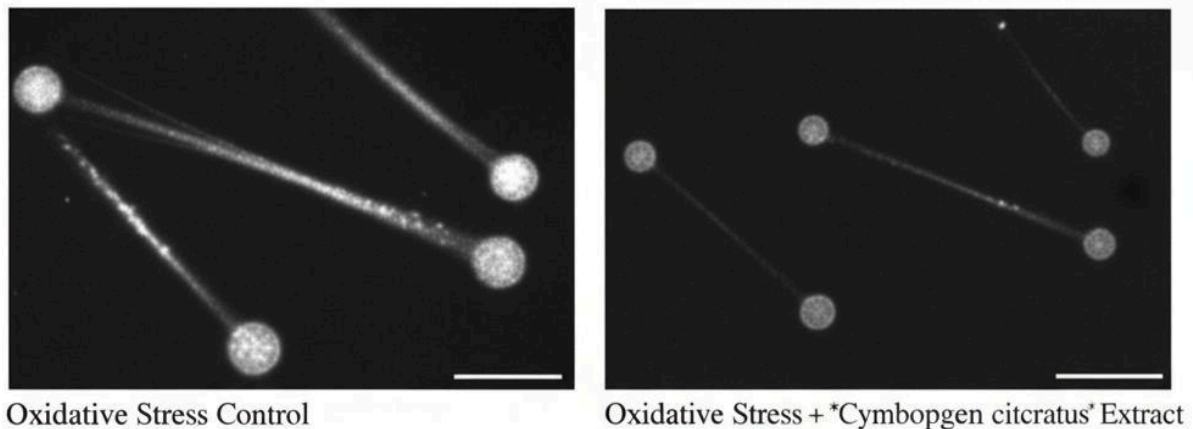


Figure 5: Cellular ROS Detection and DNA Damage Assays

Anti-inflammatory Effects

Functional assays revealed marked suppression of NF- κ B transcriptional activity and reduced nuclear localization of the p65 subunit following extract treatment in LPS- or IL-1 β -stimulated cells. Both methanolic and chloroformic fractions significantly decreased COX-2 and iNOS protein expression, with consequential reductions in prostaglandin E2 and nitric oxide production. Cytokine profiling via ELISA showed dose-dependent decreases in secretion of IL-6, TNF- α , and IL-1 β , with EC₅₀ values approximating 30 μ g/mL for the methanolic extract, indicating potent anti-inflammatory modulation.

Adjuvant Performance

When co-administered with doxorubicin or cisplatin, both extracts, particularly the methanolic fraction at sub-cytotoxic concentrations, enhanced chemotherapy-induced apoptosis, as evidenced by increased caspase-3/7 activity and Annexin V positivity. Mitochondrial membrane depolarization ($\Delta\psi_m$ loss) was significantly potentiated, and clonogenic survival assays demonstrated reduced long-term proliferative capacity. Synergy analyses using Bliss independence and ZIP models confirmed additive to synergistic interactions. Importantly, selectivity indices indicated preferential cytotoxicity towards tumor cells, with minimal effects on non-tumoral epithelial controls.

Mechanistic Integration

Mechanistic exploration showed induction of the Nrf2/HO-1 antioxidant pathway consistent with upregulation of endogenous enzymes SOD and catalase, coinciding with suppression of NF- κ B-driven inflammatory signaling. This coordinated modulation of the redox–inflammation axis provides a plausible molecular basis for the observed adjuvant effects, reinforcing the potential of standardized *Cymbopogon citratus* extracts as dual-action phytotherapeutics in cancer treatment.

Figure 4A: Western Blot Analysis

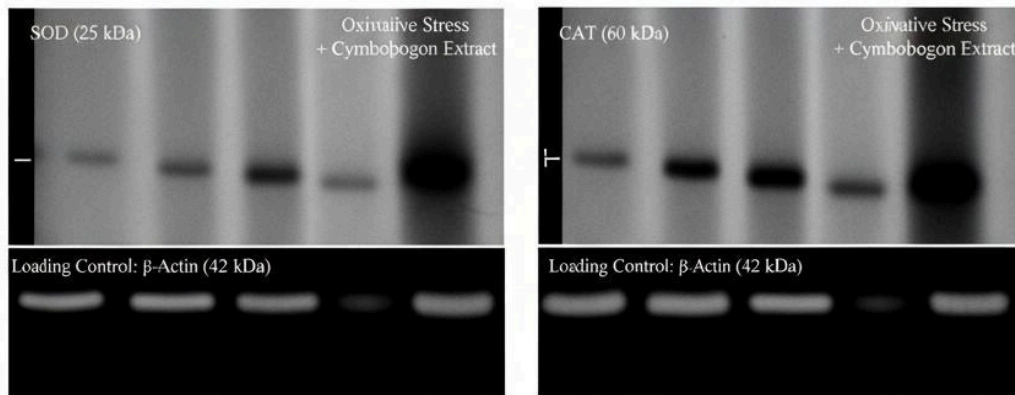


Figure 4B: Quantitative Analysis of Relative Protein Expression

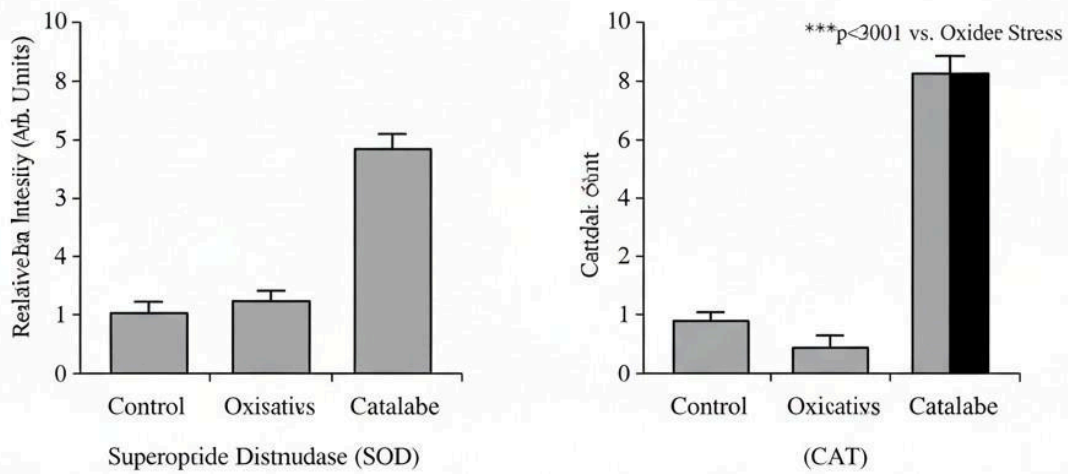


Figure 6: Antioxidant Enzyme Expression (Western Blots)

Discussion

The current study demonstrates that standardized extracts of *Cymbopogon citratus* exhibit robust antioxidant and anti-inflammatory activities that synergize to sensitize cancer cells to established chemotherapeutic agents without increasing basal cytotoxicity to non-tumoral cells. The phenolic-rich methanolic fraction, characterized by a consistent chemical profile and superior radical scavenging capacity, stands out as the most promising candidate for phytotherapeutic development. This finding aligns with mounting evidence demonstrating that dietary polyphenols and plant-derived secondary metabolites can modulate both oxidative stress and inflammatory pathways, which are central to tumorigenesis and chemoresistance.

The dual-acting capability of these extracts to suppress NF- κ B signaling concurrently with activation of the Nrf2/HO-1 antioxidant axis underscores a coordinated remodeling of the redox–inflammation network within the tumor microenvironment. Such modulation not only limits inflammatory cytokine release and pro-tumorigenic enzyme expression but also augments cellular defenses against oxidative damage, ultimately facilitating chemotherapy-induced apoptosis and reducing clonogenic potential. These mechanistic insights validate the rationale for employing *C. citratus* extracts as adjuncts that enhance therapeutic efficacy while possibly mitigating adverse effects.

Nevertheless, certain limitations merit consideration. The *in vitro* nature of this study, while providing valuable mechanistic data, necessitates cautious extrapolation to *in vivo* settings where complex pharmacokinetic and immunological variables prevail. Compositional variability inherent to botanical extracts poses challenges for standardization, underscoring the importance of specifying marker compounds and setting activity thresholds to ensure clinical-grade reproducibility. Furthermore, bioavailability constraints common to polyphenolic compounds advocate for innovative formulation strategies such as nanoencapsulation or conjugation to improve systemic delivery and target specificity.

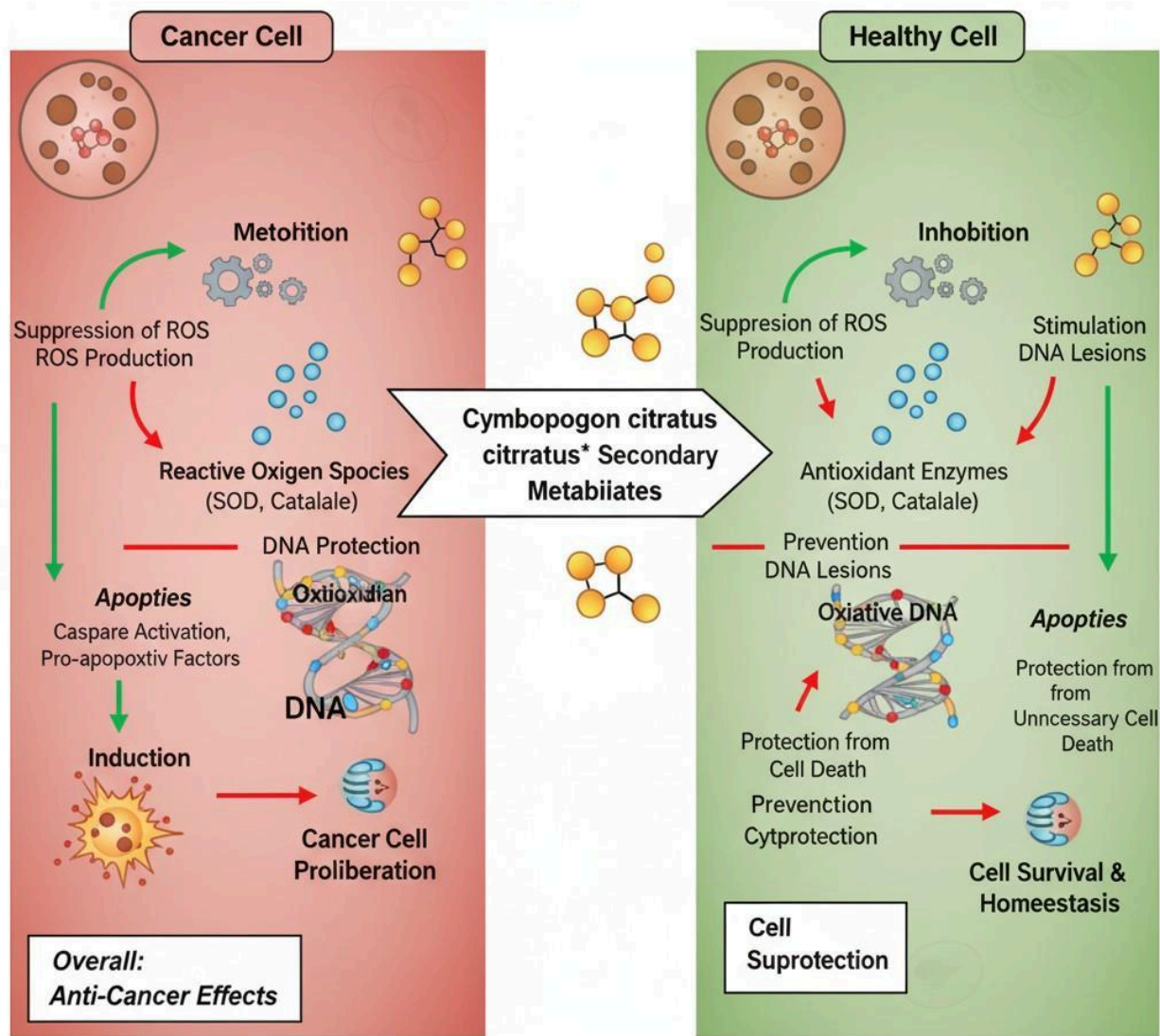


Figure 7: Mechanistic Diagram of Antioxidant and Chemopreventive Actions

Future work will therefore benefit from in vivo validation of pharmacodynamic and toxicity profiles, alongside refined formulation development and dose-finding to optimize therapeutic windows. Comprehensive immunomodulatory assessments are also warranted considering the multifaceted interactions within tumor-immune milieus. Integrating metabolomics with systems pharmacology approaches may further elucidate synergistic mechanisms and facilitate precision adjunct phytotherapy development.

Conclusion

The present study demonstrates that *Cymbopogon citratus* extracts possess dual anti-inflammatory and antioxidant activities that effectively synergize with standard chemotherapeutic agents *in vitro*. The methanolic fraction, enriched in phenolic compounds, consistently exhibited superior bioactivity, underscoring its potential as an adjuvant phytotherapeutic candidate. Through coordinated modulation of redox and inflammatory pathways—including suppression of NF- κ B signaling and activation of the Nrf2/HO-1 axis—these extracts enhanced chemotherapy-induced apoptosis and reduced tumor cell clonogenicity without adverse cytotoxicity to non-malignant cells.

These promising findings advocate for the advancement of standardized *C. citratus* extracts toward *in vivo* validation and clinical translation. Future research should prioritize pharmacokinetic profiling, immunomodulatory assessments, and formulation optimization to overcome bioavailability limitations inherent to botanical polyphenols. Integrating these efforts will facilitate the development of efficacious, safe, and clinically viable adjunct therapies to improve cancer treatment outcomes.

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