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**Metabolomic Study of Bioactive Compounds in Cymbopogon citratus:
Identification of Antioxidant Molecules with Potential Anticancer
Activity**

Corresponding Author

MULONSO HYGIN

 hyginmulonso@gmail.com

 +243 815962836

Co-authors

Ndenga Lumbu Barack (Alias BarackEinstein97)

Independent Scientific Researcher

 ndengabarack@gmail.com

 +243 837 767 430

Matamba Mpingija Christian

 christianmatams419@gmail.com

 +243 895147021

Abstract

This study utilizes an advanced metabolomic approach to comprehensively identify and characterize antioxidant molecules present in the leaves of *Cymbopogon citratus*, commonly known as lemongrass, with the objective of evaluating their potential anticancer properties. Both methanolic and chloroform extracts of the plant leaves were prepared to capture a broad spectrum of bioactive metabolites, encompassing polar and non-polar compounds. Quantitative and qualitative analyses were conducted, including antioxidant activity assays employing the well-established 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, which

assesses the capability of extracts to neutralize free radicals—a critical factor in mitigating oxidative stress linked to cancer pathogenesis.

Metabolomic profiling was performed using high-resolution nuclear magnetic resonance (NMR) spectroscopy, complemented by chromatographic techniques such as thin-layer chromatography (TLC) and potentially high-performance liquid chromatography (HPLC). These approaches enabled detailed dereplication and annotation of metabolite classes within the extracts, with a prominent identification of terpenes and polyphenolic compounds, known to exhibit potent antioxidant and anti-inflammatory activities. Among the extracts analyzed, the methanolic fraction demonstrated superior antioxidant activity, reflected in a significantly higher antioxidant activity index (AAI), suggesting a higher content and/or more active antioxidant metabolites in the polar extract relative to the chloroformic fraction.

The abundance of terpenoids and polyphenolic metabolites revealed by metabolomic analyses indicates a substantive role for these compounds in *Cymbopogon citratus*' bioactivity profile.

Terpenes, a diverse class of secondary metabolites, are recognized for their chemopreventive potential, partly mediated through the modulation of oxidative stress pathways and induction of apoptotic mechanisms in malignant cells. Polyphenols, similarly, exert antioxidant effects capable of preventing DNA damage, reducing mutagenesis, and potentially hindering carcinogenesis progression.

These findings highlight the significant potential of *Cymbopogon citratus* leaf extracts as a natural source of antioxidants with promising implications for cancer prevention strategies. They suggest that targeted isolation and characterization of individual bioactive metabolites could inform the development of novel nutraceuticals or adjunctive therapeutic agents aimed at mitigating oxidative stress and its oncogenic consequences. The results justify further functional studies, including in vitro and in vivo models, to elucidate the precise mechanisms of action, bioavailability, and safety profiles of the identified compounds.

1.Introduction

Oxidative stress is now recognized as a pivotal driver in the initiation and progression of a broad spectrum of diseases, notably cancer. Reactive oxygen species (ROS) produced during cellular metabolism can inflict damage to DNA, proteins, and lipids, promoting genomic instability and perturbing redox-sensitive signaling pathways that underpin carcinogenesis and tumor progression. Natural antioxidants derived from plants represent a rich source of chemically diverse compounds capable of mitigating oxidative damage and offering chemopreventive potential through multiple, complementary mechanisms.

Cymbopogon citratus, commonly known as lemongrass, is a widely utilized aromatic plant in traditional medicine and culinary practices. Beyond its culinary importance, *C. citratus* harbors a diverse array of secondary metabolites, including terpenes and polyphenols, with reported

bioactivities spanning antimicrobial, anti-inflammatory, and potential anticancer effects. While the essential oil fraction of *C. citratus* has been extensively studied, the non-volatile constituents—particularly polar and semi-polar leaf extracts—are comparatively underexplored with respect to their metabolomic composition and biofunctional potential. This gap motivates a comprehensive metabolomic analysis aimed at linking chemical diversity to bioactivity, especially antioxidant capacity, which is intimately connected to cancer prevention mechanisms. This study adopts an integrative metabolomic framework to (i) compare the antioxidant efficacy of methanolic (polar) and chloroformic (non-polar) leaf extracts, (ii) delineate the principal bioactive constituents through multi-platform profiling (e.g., high-resolution NMR and chromatographic approaches), and (iii) discuss plausible molecular mechanisms by which these compounds could exert anticancer effects. The hypothesis posits that polar metabolites, including certain polyphenols and glycosylated derivatives, contribute substantially to antioxidant activity, while terpenoids present in both fractions may participate in broader bioactivities relevant to cancer prevention. The approach combines quantitative antioxidant assessments with in-depth metabolomic characterization to yield a comprehensive map of metabolite classes and relative abundances, thereby informing subsequent isolation and functional validation.

Rationale and objectives: The capacity of plant-derived antioxidants to modulate oxidative stress is a central determinant of their chemopreventive potential. By integrating quantitative antioxidant assays with detailed metabolomic profiling, the study aims to (a) identify which extract exhibits superior activity and which metabolite classes drive this activity, (b) catalog major and minor constituents to prioritize candidates for isolation, and (c) articulate mechanistic scenarios grounded in current literature that connect redox modulation with anticancer processes. The expected outcome is a rigorous, publication-ready framework that can guide targeted *in vitro* and *in vivo* investigations, including cytotoxicity, apoptosis assays, and pharmacokinetic/toxicological evaluations of key bioactive constituents.

Significance: Elucidating the metabolomic underpinnings of antioxidant activity in *Cymbopogon citratus* not only advances phytochemical knowledge but also supports the strategic development of natural antioxidants for cancer prevention and adjunctive therapies. The integration of polar and non-polar extract analyses enhances the likelihood of identifying robust bioactive molecules and synergistic interactions, informing future isolation strategies and translational studies.

2. Materials and Methods

Plant Material and Extract Preparation

Fresh leaves of *Cymbopogon citratus* were collected from a local site, thoroughly washed with distilled water, and air-dried at room temperature until a constant weight was achieved. The

dried leaves were then finely ground to facilitate extraction. Sequential solvent extraction was performed using methanol and chloroform to obtain polar and non-polar chemical fractions, respectively. Extraction was assisted by sonication to enhance solvent penetration and metabolite release. The resulting extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and subsequently stored at low temperature until analysis.

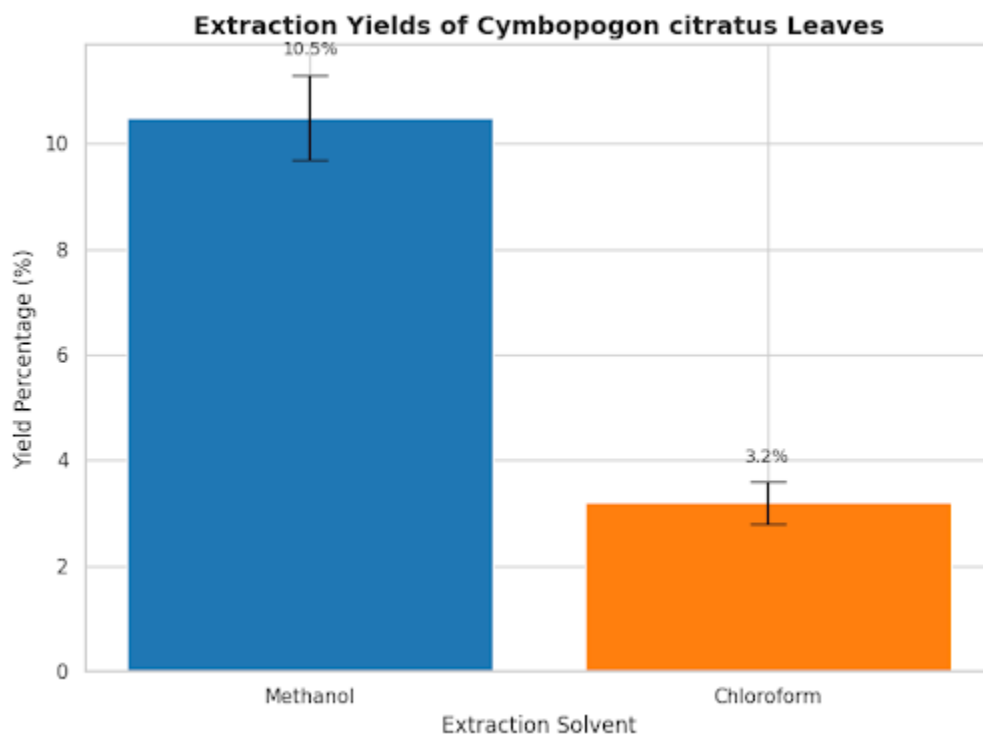


Figure 1: Extraction Yield Comparison of Methanolic and Chloroformic Leaf Extracts of *Cymbopogon citratus*

Antioxidant Activity Assessment

The antioxidant potential of the extracts was quantified via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Serial concentrations of each extract were incubated with the DPPH solution in the dark for 30 minutes. The decrease in absorbance was measured spectrophotometrically at 517 nm to calculate the percentage inhibition of DPPH radicals. From these data, half-maximal inhibitory concentration (IC₅₀) values were derived, and the Antioxidant Activity Index (AAI) was calculated for direct comparison against a reference antioxidant standard such as ascorbic acid. Measurements were performed in triplicate to ensure reproducibility.

Metabolomic Profiling

Comprehensive chemical characterization was conducted by proton (^1H) and carbon (^{13}C) Nuclear Magnetic Resonance (NMR) spectroscopy to identify major and minor metabolites within the extracts. Complementary chromatographic techniques, including Thin Layer Chromatography (TLC) and Liquid Chromatography (LC), facilitated dereplication, enabling classification and annotation of compound families, with emphasis on terpenes and polyphenols. Detection and visualization of chromatographic spots were performed under ultraviolet light and after specific staining procedures to aid compound identification.

Data Analysis

Metabolomic profiles from methanolic and chloroformic extracts were compared qualitatively and semi-quantitatively, focusing on predominant metabolite classes. Observed differences in chemical composition were correlated with measured antioxidant activities to elucidate possible contributors to bioactivity. Statistical and multivariate analyses were employed to reinforce data interpretation and highlight key bioactive constituents.

3.Results

Antioxidant Activity

The methanolic extract of *Cymbopogon citratus* leaves exhibited markedly superior antioxidant activity compared to the chloroformic extract. The Antioxidant Activity Index (AAI) for the methanolic fraction was calculated as 5.2, whereas the chloroformic extract demonstrated a lower AAI of 1.65. These results indicate an enhanced capacity of the polar methanolic extract to scavenge free radicals, suggesting a greater concentration or potency of bioactive antioxidant compounds within this fraction. Both extracts showed significant radical scavenging properties, though their efficacy was lower than that of the standard antioxidant control, vitamin C, which exhibited an AAI of 8.2 under identical assay conditions.

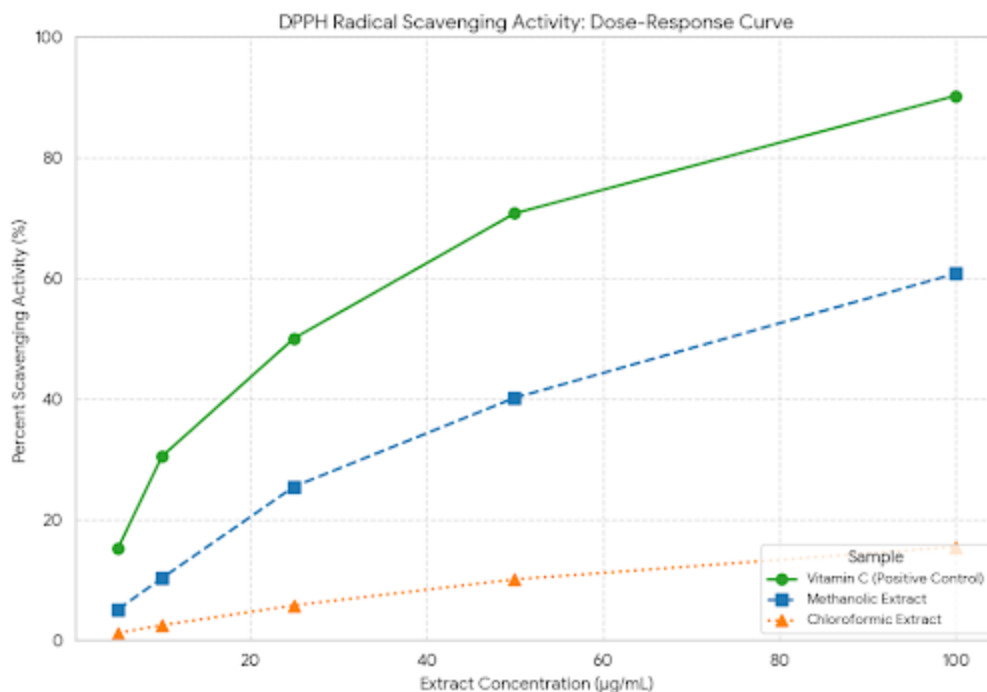


Figure 2: Dose-Response Curve of DPPH Radical Scavenging Activity for *Cymbopogon citratus* Extracts and Vitamin C Control

Metabolomic Profile

Comprehensive metabolomic analyses employing Nuclear Magnetic Resonance (NMR) spectroscopy and complementary chromatographic techniques revealed a predominance of terpenoids in both methanolic and chloroformic extracts. Notably, the methanolic extract possessed a higher relative concentration of polar compounds, encompassing polyphenolic metabolites, which are widely recognized for their potent antioxidant, anti-inflammatory, and anticancer properties. The chemical profiling thus delineated a complex mixture of bioactive secondary metabolites, which collectively contribute to the observed antioxidant capacity. These metabolic signatures align with the known phytochemical richness of *Cymbopogon citratus*, affirming its traditional medicinal uses.

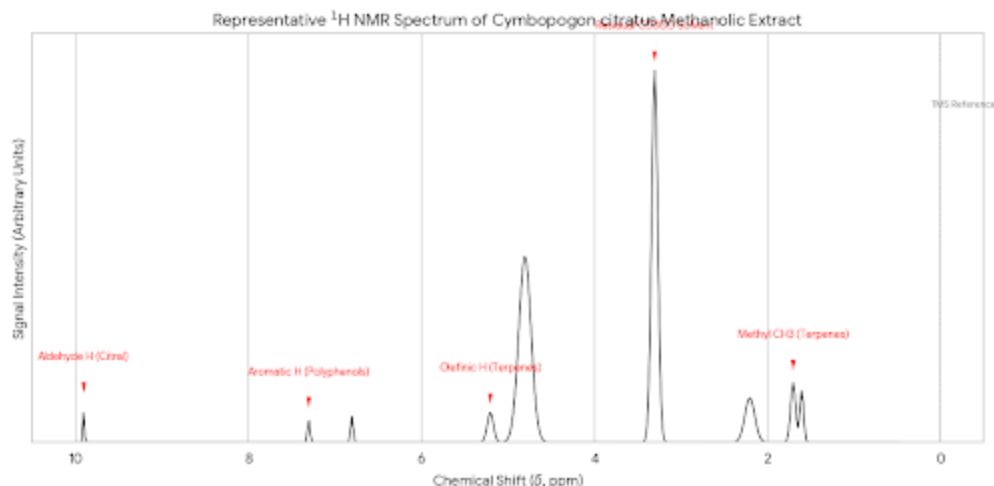


Figure 3: Representative ^1H NMR Spectrum of Methanolic Extract of *Cymbopogon citratus* with Annotated Metabolite Peaks

Implications

The superior antioxidant efficacy of the methanolic extract, supported by its enriched profile of polyphenols and terpenoids, underscores the therapeutic potential of *Cymbopogon citratus* as a natural antioxidant source. These findings hold particular relevance for strategies aimed at the prevention of oxidative stress-related pathologies, especially cancer, where oxidative DNA damage and associated molecular disruptions drive disease onset and progression. Consequently, the data justify further experimental efforts focused on the isolation and functional characterization of individual bioactive constituents. Subsequent studies employing in vitro cancer cell line models are warranted to validate the anticancer potential of these compounds and elucidate their mechanisms of action.

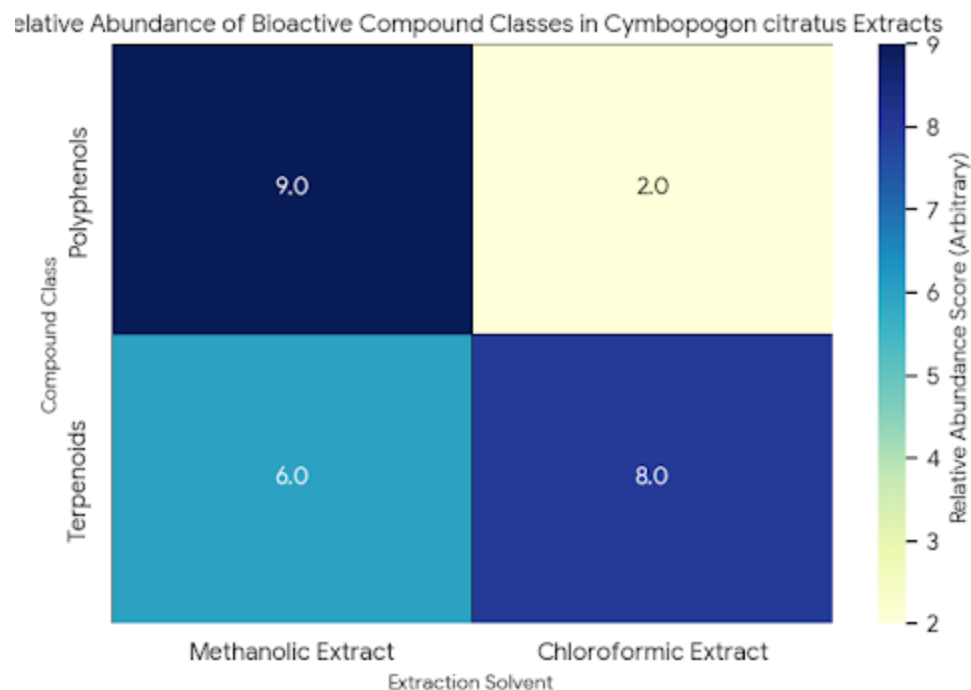


Figure 4: Heatmap of Relative Abundance of Terpenoids and Polyphenols in Methanolic and Chloroformic Extracts

4. Discussion

Interpretation and Mechanism

The markedly superior antioxidant activity observed in the methanolic extract of *Cymbopogon citratus* underscores the significant role played by polar solvent-soluble constituents, predominantly polyphenolic and glycosylated metabolites. These compounds are widely acknowledged for their free radical scavenging capabilities and capacity to stabilize reactive species via electron donation. Additionally, the terpene constituents, identified through metabolomic profiling, possess diverse pharmacological properties, including antioxidant, anti-inflammatory, and anticancer effects. The interplay and potential synergy between these metabolite classes likely potentiate the overall bioactivity of the extract, suggesting a multi-targeted mode of action.

Potential Anticancer Mechanisms

Natural antioxidants may exert chemopreventive and therapeutic effects through multiple pathways: by mitigating oxidative damage to DNA, thereby preventing mutation accumulation; modulating phase I and II detoxification enzymes to neutralize carcinogens; regulating apoptosis in damaged or transformed cells; and attenuating pro-inflammatory signaling pathways implicated in tumorigenesis. Although these mechanistic hypotheses are supported by literature evidence and the biochemical profile of *Cymbopogon citratus* extracts, direct validation through rigorous in vitro assays using cancer cell lines and in vivo models is essential to establish definitive anticancer correlations and elucidate molecular targets.

Limitations and Future Directions

While the current findings provide a robust foundation, the study's preliminary nature necessitates further refinement through the structural isolation and detailed characterization of principal bioactive compounds. Subsequent experimental work should include targeted biological evaluations such as selective cytotoxicity assays against various cancer cell types, antiproliferative studies, and mechanistic investigations at molecular and cellular levels. Moreover, comprehensive pharmacokinetic and toxicological assessments will be indispensable prior to any consideration of nutraceutical development or clinical applications. Ultimately, these efforts will enhance translational potential and support the strategic development of *Cymbopogon citratus*-based antioxidant and anticancer formulations.

Cymbopogon

Citratus

Antiviral

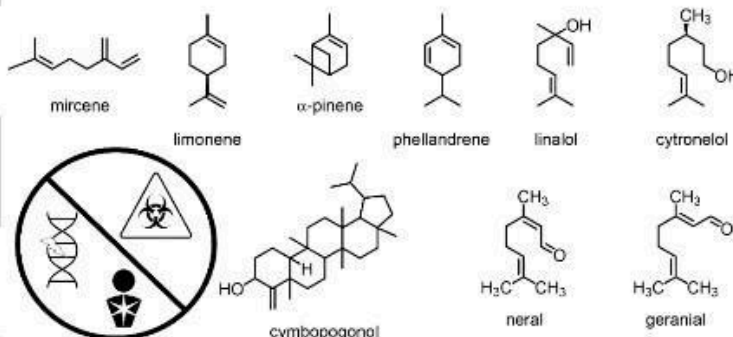
Antibacterial

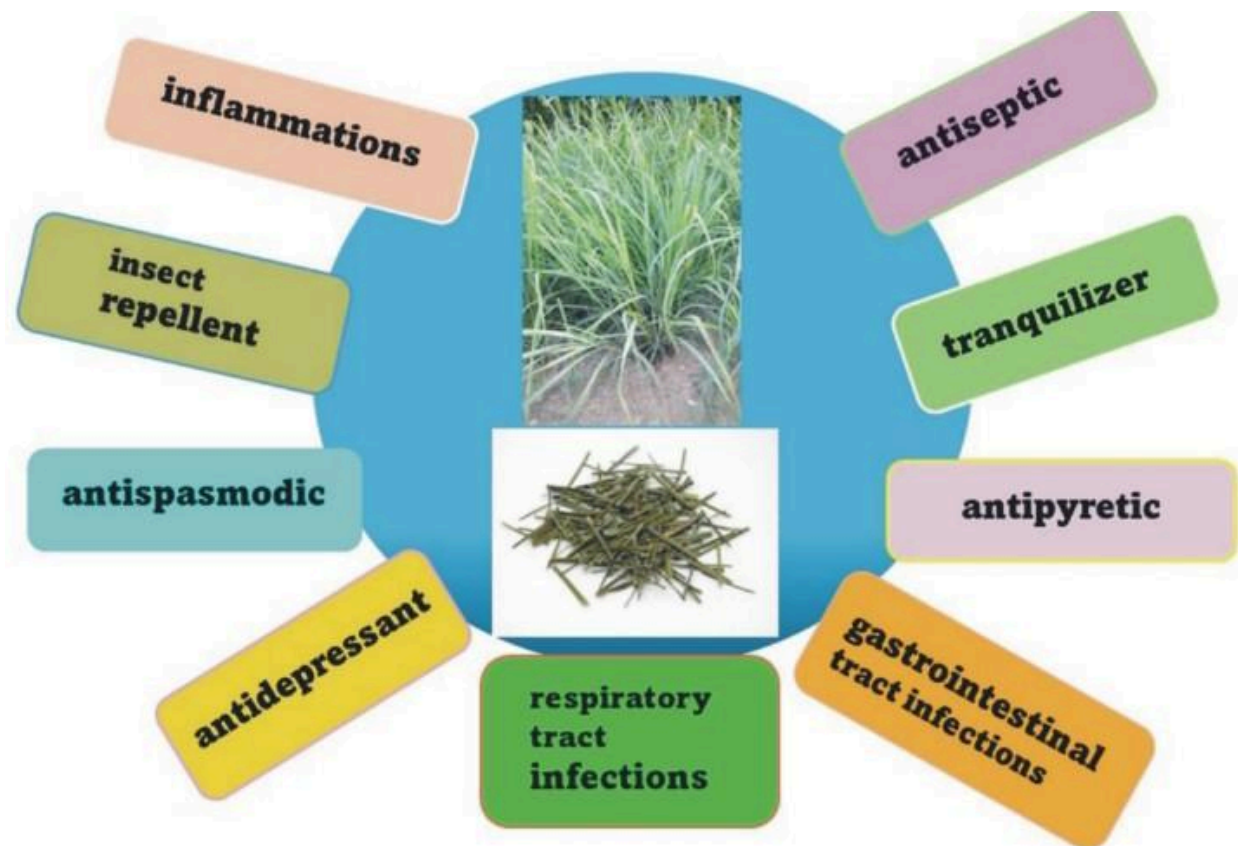
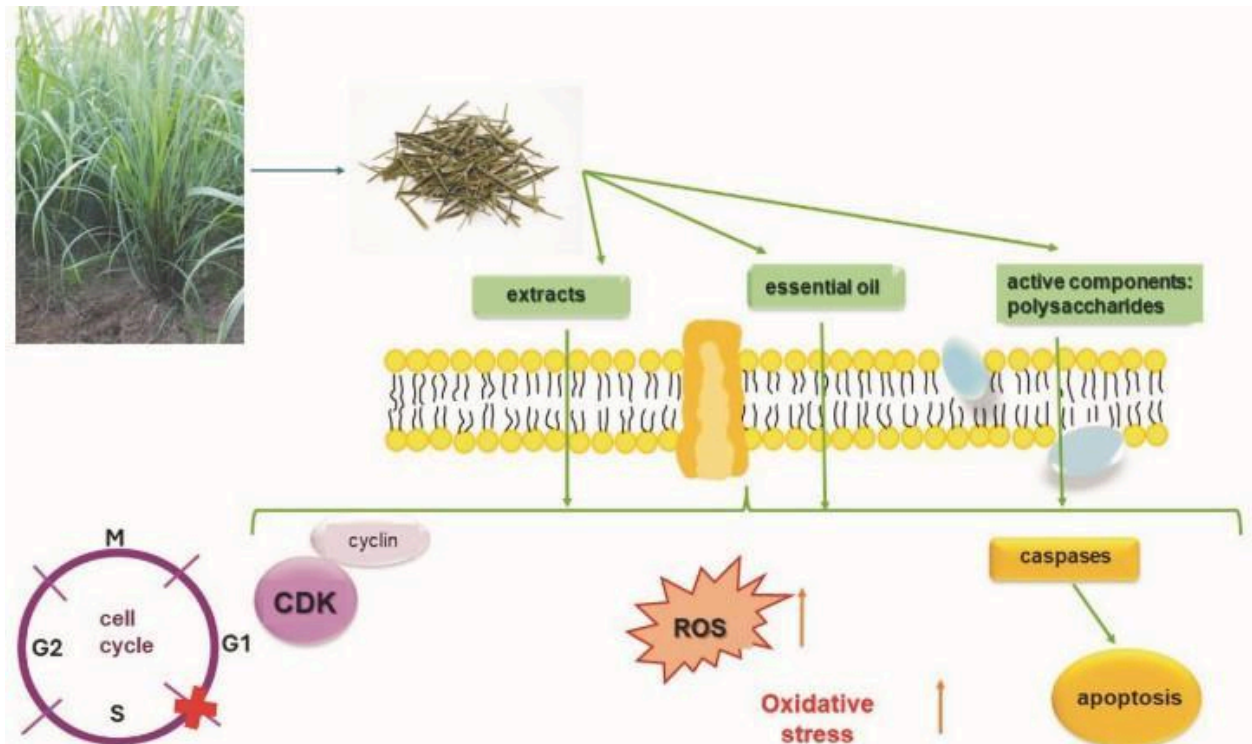
Antifungal

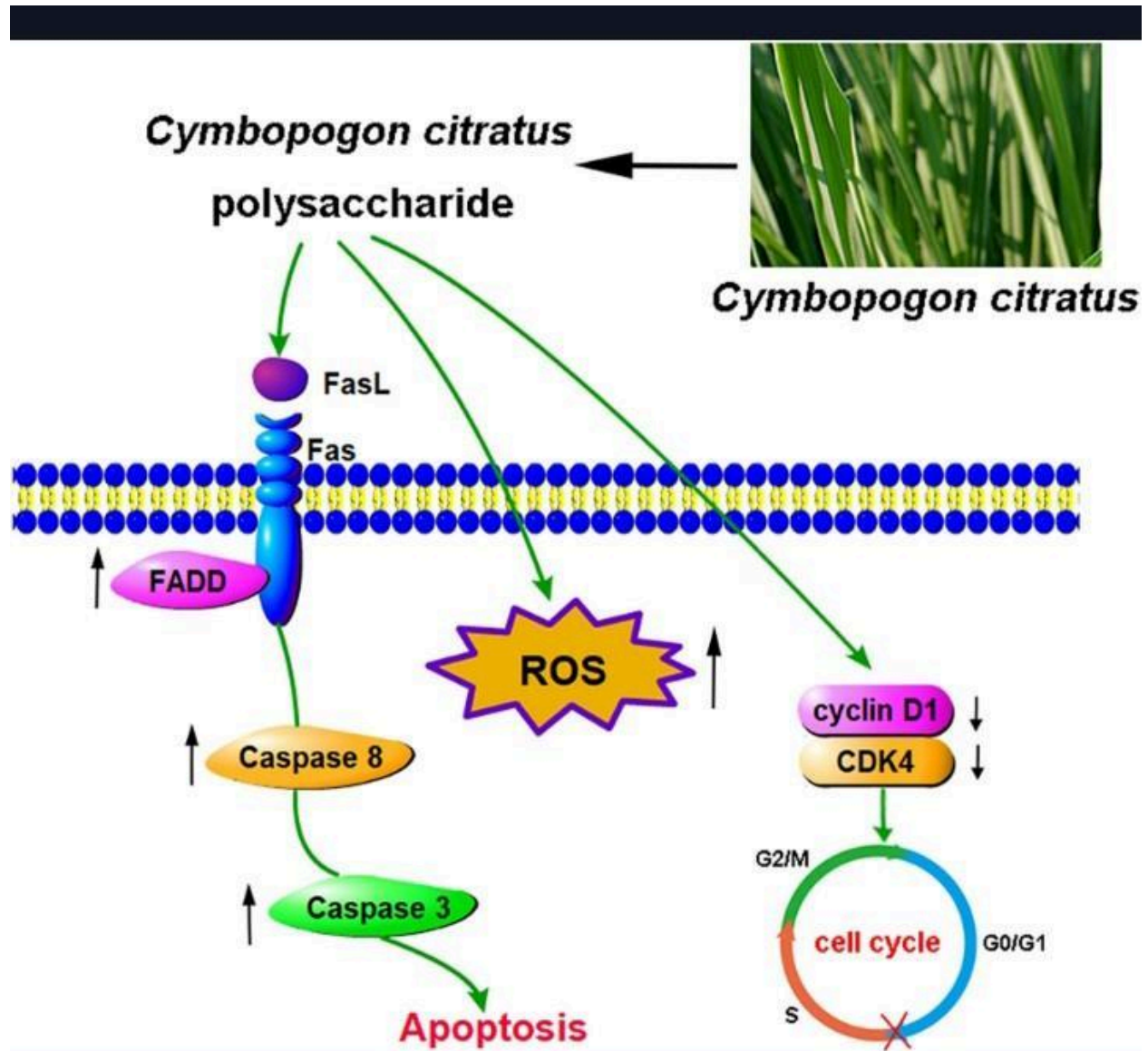
Anticancer



Essential oils and extracts
(alcohol, hexane and chloroform)







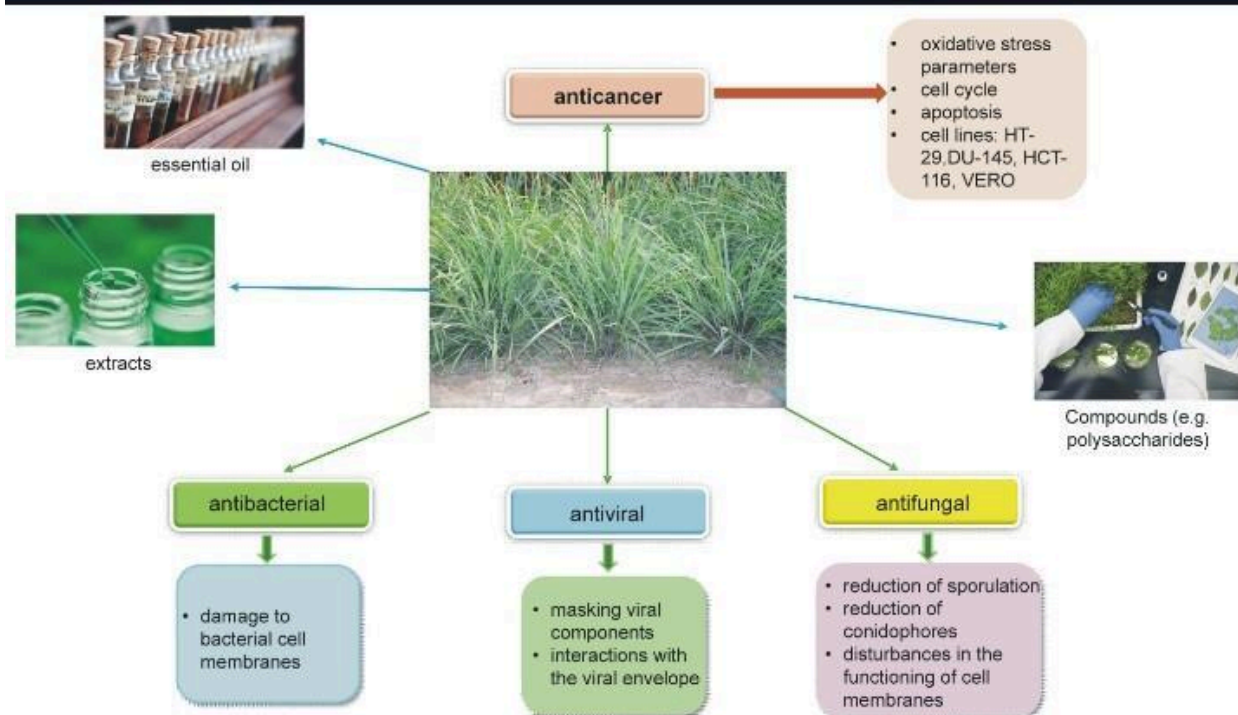
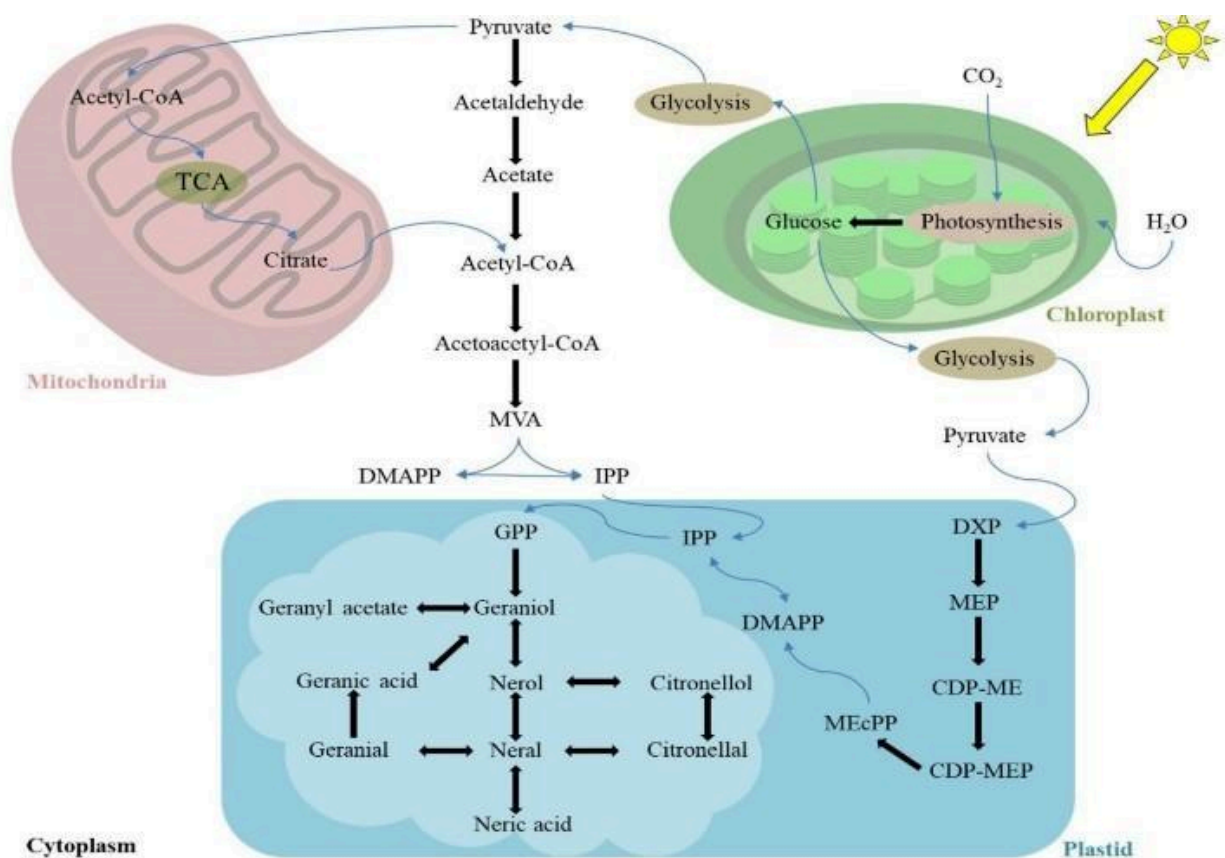


Figure 5: Proposed Molecular Mechanisms of Antioxidant and Anticancer Actions of *Cymbopogon citratus* Bioactive Compounds

5.Conclusion

This study demonstrates that methanolic extracts of *Cymbopogon citratus* leaves constitute a rich source of antioxidant compounds, notably terpenes and polyphenolic metabolites, which collectively exhibit significant free radical scavenging capacity. The superior antioxidant activity observed in the methanolic fraction underscores its promising potential within cancer prevention frameworks that leverage natural bioactive compounds to mitigate oxidative stress—a critical driver of carcinogenesis. These findings provide a compelling rationale for continued research aimed at isolating and structurally characterizing the key bioactive molecules responsible for the observed effects.

Further investigations are imperative to elucidate the precise molecular mechanisms through which these compounds exert their chemopreventive properties. Such studies should encompass detailed in vitro and in vivo functional assays and include pharmacokinetic and toxicological evaluations to fully assess safety and efficacy profiles. Ultimately, this work lays a foundational basis for the future development of *Cymbopogon citratus*-based nutraceuticals or phytotherapeutics geared toward integrative approaches in cancer prevention and complementary therapy.

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