

Phytochemical Characterization and Antioxidant Analysis of Essential Oils Isolated from *Cymbopogon citratus*

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ABSTRACT

The drive for novel phytocompounds of distinct bioactivities for treating diverse disease conditions has necessitated research into various indigenous medicinal plants for promising drug candidates of immense therapeutic efficacies. This study therefore investigated the phytochemical composition and antioxidant activity of essential oils isolated from *Cymbopogon citratus* in contributing bioactive phytochemicals for drug discovery and development. Standard methods of Association of Official Analytical Chemists were employed to characterize phytochemicals while malonaldehyde (MDA) inhibition and reducing assays were used to assess antioxidant capacity. The results revealed that the oils contained phytochemicals in appreciable amounts: alkaloids (10.00%), terpenoids (3.00%), phenols (0.22 mg GAE/g), phytates (0.90%), tannins (0.20 mg TAE/g), flavonoids (0.02 mg CE/100g), and saponins (0.03 mg/g). Antioxidant assays demonstrated that the essential oils inhibited lipid peroxidation and increased glutathione levels in a non-dose-dependent manner. Maximum MDA inhibition and GSH elevation were observed at moderate concentrations (25–50 µg/ml), while activity declined at very low (10–20 µg/ml) and high doses (75–100 µg/ml), suggesting reduced efficiency of approximately 25% or possible pro-oxidant effects. Compared to standards, *C. citratus* essential oil exhibited lower potency but showed significant antioxidant capacity. These findings indicate that the plant essential oil contains appreciable amounts of phytochemicals and is a potential natural antioxidant source that may serve as a complementary therapeutic agent in oxidative stress-related conditions. Based on the study findings, *C. citratus* essential oils could find applications for developing novel drug candidates in pharmaceutical and cosmetics industries where natural antioxidants may augment chemical antioxidants for optimal preservation and safety.

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INTRODUCTION

The need for novel phytocompounds of diverse pharmacological and therapeutic efficacies have necessitated the push for research into indigenous medicinal plants for their contribution of promising drug candidates in treatment of various disease conditions and illnesses. Natural products including those from plants, animals and microorganisms have long been sourced to provide compounds that can be used to treat and manage diseases (Patwardhan *et al.*, 2008). Medicinal plants have historically been employed in traditional medicine and ayurvedism for their ability to ameliorate diverse ailments and illnesses such as malaria, colds, fever and wound-healing (Marrelli, 2021; Scott, 2005). This is primarily attributed to the presence of plant secondary metabolites also known as phytochemicals, which have been shown to possess pharmacological actions and have been applied as anti-bacterial, anti-fungal, anti-inflammatory, anti-malarial, anti-diabetic agents among others (Le Rhun *et al.*, 2019). However, even with the knowledge of the potency of plants in disease treatment and management, many of these medicinal plants remain inadequately underexplored, underexploited and underutilized basically because of a lack of scientific knowledge of their phytochemical profiles and antioxidant abilities. One of such plants of research interest in this present study is *Cymbopogon citratus*.

Cymbopogon citratus, popularly known as lemon grass, is a perennial medicinal oil-bearing grass that belongs to the

Poaceae family and is an example of an aromatic plant that produces essential oil. It originated from Southwest Asia, but grows spontaneously in tropical and subtropical regions such as Malaysia, India and Nigeria, and thus is a grass that basically has no strict requirements for propagation and growth (Aćimović *et al.*, 2019; Madi *et al.*, 2022). Lemon grass leaves and stems contain flavonoids, geranyl acetate, phenolic compounds, steroids and saponins that function as anti-microbial, anti-bacterial, anti-inflammatory agents (Isyaka *et al.*, 2024; Mukarram *et al.*, 2021; Lin *et al.*, 2020). They are used in ethnopharmacology for treating gastrointestinal and nervous disturbances and as anti-inflammatory, analgesic, sedative, and antipyretic (Sousa *et al.*, 2021).

The lemon grass essential oil can be classified as West Indian lemon grass oil (*Cymbopogon citratus*), East Indian lemon grass oil (*Cymbopogon flexuosus*) and North Indian lemon grass oil (*Cymbopogon pendulus*). The essential oil can be obtained through various extractions methods including hydro-distillation and solvent-free microwave-based extraction (Chouhan *et al.*, 2023). Other effective extraction approaches can be the solvent extraction, supercritical fluid extraction, and solar energy extraction methods (Majewska *et al.*, 2019). It is a colorless or yellow liquid with an aroma of citrus, extracted from the dried leaves, with a yield of 0.5 to 2.0%, comprising 70% of citral, which consists of a mixture of two isomers: neral (Z-isomer) and geranial (E-isomer),

along with compounds such as geraniol, myrcene, and β -caryophyllene (Aćimović *et al.*, 2019; Avoseh *et al.*, 2023; Majewska *et al.*, 2019). The essential oils of the *Cymbopogon* species mainly comprise of monoterpenoids and sesquiterpenoids. The oil is used for flavoring and exhibits benefits such as antioxidant, anti-aging, and antimicrobial properties (Aćimović *et al.*, 2019).

Essential oils' applications have increased due to comprehensive requirements for pure natural ingredients, and they exhibit a wide range of biological activities, representing an actual potential product for the pharmaceutical industries. These bioactive components exhibit synergistic pharmacological effects, including antimicrobial, antioxidant, and anticancer properties (Islam *et al.*, 2024). Recent experimental studies have confirmed lemon grass essential oils' anti-proliferative and cytotoxic effects across various cancer cell lines (Maksimović *et al.*, 2025; Yin *et al.*, 2021). Proposed mechanisms underlying these effects include induction of apoptosis, cell cycle arrest, oxidative stress via ROS accumulation, mitochondrial impairment, and inhibition of metastasis. Nevertheless, literature on its phytochemical profile and antioxidant potentials are scarcely available and therefore the need to provide substantial information on these parameters.

In light of the above, we set out to characterize the phytochemicals and analyze antioxidant capacity of essential oils derived from *C. citratus* obtained from our locality to assess its potential in contributing novel phytocompounds in disease treatment.

MATERIALS AND METHODS

Chemicals and Reagents

Folin Ciocalteu's phenol reagent, sulphuric acid, ammonium hydroxide, phosphate buffer, saturated sodium carbonate solution, gallic acid, vanillin, standard iron (III) chloride solution and all other reagents used were of analytical grade.

Experimental Site

The experimental analyses were carried out at the Laboratory of Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria.

Sample Collection and Preparation

C. citratus plant was obtained from a residential premise in Amawbia, Awka South Local Government Area, Anambra State. The essential oils from fresh samples of *C. citratus* plant were extracted using the hydro-distillation method (Tajidin *et al.*, 2012; Cardoso *et al.*, 2013). Exactly 10.35 g of *C. citratus* leaves were washed, chopped into smaller pieces and placed in a modified Clevenger-type steam system round-bottomed flask. Distilled water was then added to the plant material and the oil was obtained by hydro-distillation. This process was repeated severally until sufficient amounts of oil required for the analysis was obtained. The hydro-distillation process yielded 0.83 g lemon grass essential oil from a 10.35 g lemon grass sample, giving approximately 8% yield of oil.

Quantitative Determination of Phytochemical Constituents

Total Alkaloids

Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 25°C. This was filtered with Whatman's filter paper No. 42 and the filtrate concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide (NH₄OH) was added drop-wise to the extract until the precipitate was

collected, washed with dilute NH₄OH (1% ammonia solution) and then filtered with pre-weighed filter paper. The residue on the filter paper was dried in a precision electrothermal oven (Model BNP 9052 England) at 800°C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample (Harborne, 1998).

Total Flavonoids

The flavonoid content was determined by the use of a slightly modified colorimetry method described previously by Barros *et al* (2007). A 0.5 ml aliquot of appropriately (2 mg/2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % NaNO₂ solution. After 6 min, 0.15 ml of 10 % AlCl₃ solution was added and allowed to stand for 6 min, and then 2 ml of 4 % NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank with reference standard prepared with catechin concentrations. The analyses were performed in triplicate. The result was expressed as mg Catechin equivalents per gram of sample (mg CE/100 g).

Total Tannins

The tannin content of the *C. citratus* essential oil was determined using the method according to AOAC (1995). One gram of the sample was extracted with 10 ml of 70 % ethanol and centrifuged at 2500 rpm for 5 min to remove the residue. The supernatant (0.5 ml) was diluted with 4.5 ml of distilled water. This was followed by the addition of 0.5 ml of 0.1 M FeCl₃ and 0.3 ml of 0.1 M potassium ferrocyanate. Distilled water (6 ml) was added to dilute the whole mixture and the absorbance read at 720 nm using a UV-visible spectrophotometer. Tannic acid was used as standard and the result recorded as mg Tannic Acid Equivalent (mg TAE) per gram of the sample.

Total Phenols

The total phenol content of the samples was determined using the method of Barros *et al* (2007). One ml of the extract solution was mixed with Folin and Ciocalteu's phenol reagent (1 ml). After 3 min, saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (UV-Visible spectrophotometer). Gallic acid was used as standard and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Spectrophotometric Determination of Saponins

The method of Hiai *et al.* (1976) was used for determining saponin content. This method is based on the principle that saponins react with vanillin in the presence of concentrated sulphuric acid to produce a pink to reddish colored complex. This complex absorbs maximally at 544 – 560 nm (depending on the saponin type). The absorbance is proportional to the saponin concentration. One gram of sample (extract) was dissolved in 10 ml of 80 % methanol. This was mixed thoroughly and allowed to stand for about 2 – 3 hrs. The samples were filtered or centrifuged and the supernatant used for the assay. Exactly 0.25 ml of the supernatant was mixed with 0.25 ml of 5% vanillin in methanol and subsequently 2.5 ml of 72% sulphuric acid (v/v). This was mixed thoroughly and incubated in a water bath at 60°C for 10 min. This was immediately cooled in an ice bath for 5 min and the absorbance recorded at 544 – 560 nm (usually 544 or 550 nm)

using methanol as blank. Diosgenin prepared in methanol was used as standard. Saponin content in mg/g was calculated thus:

$$\text{Saponin (mg/g)} = \frac{C \times V}{M}$$

Where C = concentration from standard curve (mg/ml)

V = volume of extract (ml)

M = mass of plant material (g)

Phytate Content Determination

The phytate content was determined using the method of Young and Greaves (1999). Aliquots (2 g) of the ground samples were weighed into different 250 ml conical flasks which was soaked in 100 ml of 2 % concentrated HCl for 3 hrs. The sample was then filtered using Whatman filter paper (No. 4). The filtrate (25 ml) was placed in 250 ml beaker and 50 ml distilled water added to it. Exactly 5 ml of 0.3 % ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195g iron per ml. The percentage phytic acid was calculated using the formula:

$$\text{Phytic acid (\%)} = \frac{\text{Titre Value} \times 0.00195 \times 1.195}{2} \times 100$$

Total Terpenoids

Dried plant extract 100 mg (W_i) was taken and soaked in 9 mL of ethanol for 24 hours (Indumathi *et al.*, 2014). The extract after filtration was extracted with 10 mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (W_f). Ether was evaporated and the yield (%) of total terpenoids content was measured by the formula:

$$\frac{W_i - W_f}{W_i} \times 100$$

In vitro Lipid Peroxidation and Antioxidant Enzymes Assays

Malondialdehyde Assay

The principle of this assay is based on lipid peroxidation occurring when free radicals attack polyunsaturated fatty acids in biological membranes, leading to the formation of malondialdehyde (MDA), a reactive aldehyde and biomarker of oxidative stress. MDA reacts with thiobarbituric acid (TBA) under high temperature and acidic conditions to form a pink-colored MDA-TBA adduct (TBARS), which absorbs strongly at 532 nm. The inhibition of MDA formation by an extract indicates its antioxidant potential. This was carried out by adopting the methods of Ohkawa *et al.* (1979), Halliwell and Gutteridge (2007) and Buege and Aust (1978). Firstly, liver tissue of goat was homogenized and then one gram of it was dissolved in 10 ml of cold phosphate buffer 4°C (0.1 M; pH 7.4). (10 % liver homogenate). This was centrifuged at 3500 rpm for 10 min and the supernatant used for the assay. The first reaction medium contained 0.5 ml of the supernatant of the liver homogenate, 0.5 ml of the test sample/buffer/control and 0.1 ml of 0.1 mM FeCl_3 . The volume was subsequently made up to 2 ml with phosphate buffer (1.1 ml). This was now incubated for 1 hr at 37°C. After incubation, 1.0 ml of 15 % TCA and 1.0 ml of 0.67 % TBA were added for precipitation. This was mixed using a vortex

mixer and boiled in a water bath at 95°C for 15 - 20 min. This was then centrifuged at 3500 rpm for 10 min and the absorbance of the supernatant was read at 532 nm. The negative control contained buffer only (no pro-oxidant), while the positive control was butylated hydroxyl toluene or vitamin C. The blank contained all reagents minus the liver homogenate to account for sample absorbance (sample blank). Graded concentrations of both samples and positive controls were prepared (0, 10, 25, 50, 75, 100 $\mu\text{g/ml}$). Lipid peroxidation inhibition was calculated thus:

$$\text{Inhibition (\%)} = \left(1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right) \times 100$$

Where: A sample = Absorbance of test sample

A control = Absorbance of control (no test compound).

$$\text{MDA concentration (nmol/ml)} = \frac{A \times Vt \times 1000000}{\epsilon \times l \times Vs}$$

A = Absorbance at 532 nm

Vt = Total reaction volume (ml)

Vs = Sample volume used for absorbance reading (ml)

ϵ = molar extinction coefficient for MDA-TBA complex ($1.56 \times 10^5 \text{ M}^{-1} \text{cm}^{-1}$)

l = Path length of cuvette (cm, usually 1 cm).

10^6 (1000000) = conversion factor from mol to nmol per ml.

Reduced Glutathione Assay

Reduced glutathione was estimated by the method of Ellman (1959). The principle of the method is based on reduced glutathione on reaction with 5, 5'-dithiobis nitro benzoic acid (DTNB) producing a yellow-colored product with absorption maximum at 412 nm. To one ml of the supernatant, three ml of phosphate buffer and 0.5 ml of Ellman's reagent was added and incubated for 15 min at room temperature. The intensity of yellow color formed was then measured at 420nm. A series of standards (10 – 50 μg) were treated in a similar manner along with blank containing 1.0 mL distilled water. The amount of GSH was expressed as nmol/mg protein.

Data Analysis

Data analysis was performed using IBM Statistical Package for Social Sciences (SPSS) version 25. Results were presented in tables as Mean $\pm$ Standard Deviation of triplicate measurements while statistical significance between groups were established using One-way Analysis of Variance at 95% confidence interval ($p < 0.05$).

RESULTS AND DISCUSSION

Phytochemical Composition of Essential Oils from *C. citratus*

The phytochemical analysis of *C. citratus* essential oil showed a diverse range of phytochemicals, with alkaloids (10.0 ± 0.01 %) and terpenoids (3.0 ± 0.05 %). Other phytochemicals were contained in considerable amounts including phenols (0.2 ± 0.01 mg GAE/g), tannins (0.2 ± 0.04 mg TAE/g), phytates (0.9 ± 0.01 %), flavonoids (0.1 ± 0.01 mg CE/100 g) and saponins (0.1 ± 0.01 mg/g). The relatively high level of alkaloids suggests a strong pharmacological potential while phenols and flavonoids provide insights into its antioxidant potential.

Table 1: Phytochemical Composition of Essentials Oils of *C. citratus*

Phytochemicals	Composition
Tannins	0.2 ± 0.04 mg TAE/g
Total phenols	0.2 ± 0.01 mg GAE/g
Flavonoids	0.1 ± 0.01 mg CE/100 g
Alkaloids	10.0 ± 0.01 %

Phytochemicals	Composition
Phytates	0.9 ± 0.01 %
Terpenoids	3.0 ± 0.05 %
Saponins	0.1 ± 0.01 mg/g

Values are presented as Mean ± Standard deviation of triplicate determinations

Percentage Inhibition of MDA and GSH Concentration of *C. citratus* Essential Oil

Table 2 below shows that lemon grass essential oil exhibits strong antioxidant activity, demonstrated by inhibition of MDA and elevation of GSH. Its activity is non-dose-

dependent, with 25–50 µg/ml being the most effective range. Compared to controls (BHT and ascorbic acid), *C. citratus* oil was less potent but still demonstrated significant antioxidant activity, indicating an optimal effect at moderate concentrations.

Table 2: Percentage Inhibition of MDA and GSH Concentrations by *C. citratus* Essential Oil against Standards

Concentration of extracts (µg/ml)	% Inhibition of MDA by <i>C. citratus</i>	% Inhibition by BHT	% Inhibition by Ascorbic acid	GSH concentration of <i>C. citratus</i> (µg/ml)
10	57.19 ± 8.99	72.88 ± 5.57	70.92 ± 0.07	12.51 ± 0.19
25	66.93 ± 3.71	77.25 ± 4.11	76.01 ± 1.68	14.60 ± 0.71
50	71.63 ± 2.01	96.54 ± 6.77	78.76 ± 4.29	16.09 ± 0.26
75	62.48 ± 4.31	65.95 ± 2.79	74.38 ± 2.11	16.75 ± 1.29
100	41.18 ± 6.04	62.45 ± 3.40	69.48 ± 2.56	14.82 ± 0.47

Values are presented as Mean ± Standard deviation of triplicate determinations

Malondialdehyde Assay of Essential Oils from *C. citratus*

The scatter plot as shown in Figure 1 further illustrates the antioxidant activity of *C. citratus* oil, BHT, and ascorbic acid at different concentrations. BHT had the highest percentage inhibition, peaking above 90 % at 50 µg/ml. Ascorbic acid

showed stable inhibition (approximately 75 %) across concentrations. The oil showed moderate inhibition (~65 – 70 %), which declined at higher concentrations. BHT is the strongest antioxidant, ascorbic acid is consistently effective, while *C. citratus* oil shows weaker and less stable activity.

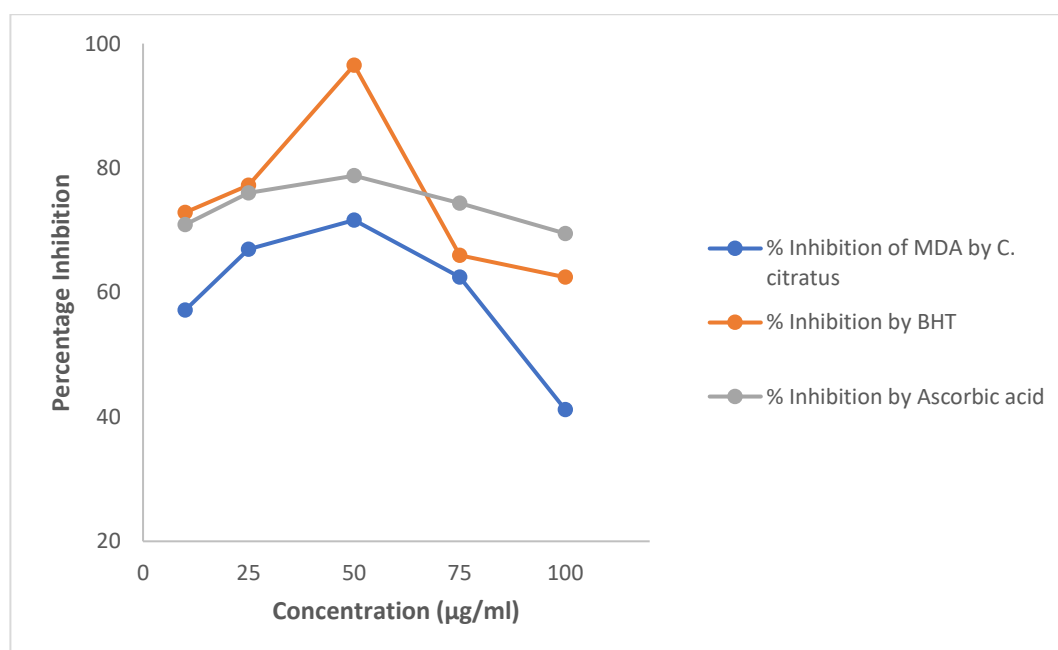


Figure 1: Scatter Plot showing Percentage Inhibition Levels of Essential Oils from *C. citratus*

Reduced Glutathione Concentration by Essential Oils from *C. citratus*

Figure 2 below further shows that *C. citratus* oil affects GSH levels in a non-dose-dependent manner. Low (10 µg/ml) and high doses (100 µg/ml) result in lower GSH. Moderate doses

(25–75 µg/ml) increased GSH levels with the highest level observed at 75 µg/ml. Lemon grass oil enhances antioxidant (GSH) levels at moderate concentrations, but very low or very high doses reduce the effect.

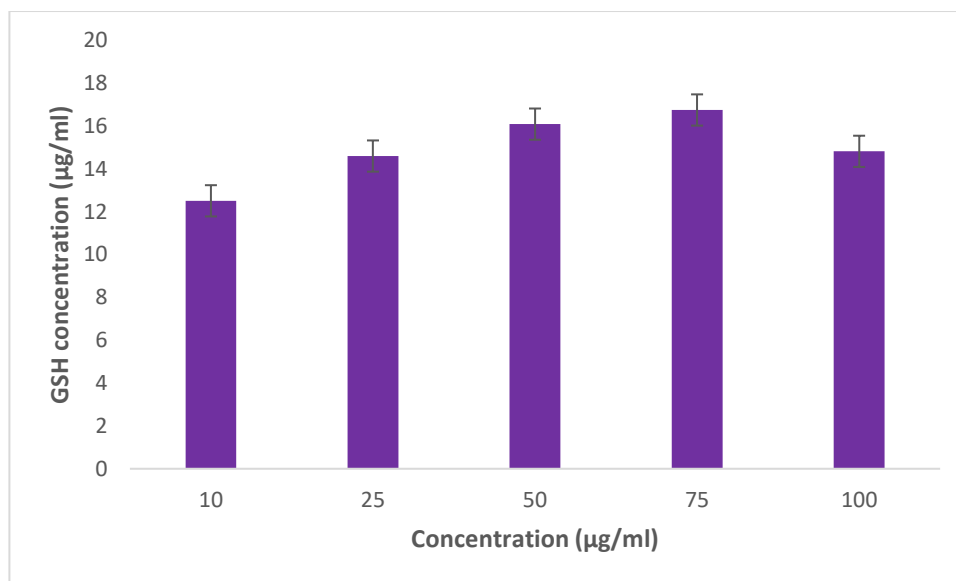


Figure 2: GSH Levels of Essential Oils from *C. citratus*

Discussion

This study investigated the phytochemical constituents and antioxidant activity of essential oils derived from *C. citratus* in order to assess its potential role in offering novel phytochemicals. The phytochemical analysis showed that the essential oil from *C. citratus* contained a mixture of secondary metabolites with particularly high measured amounts of alkaloids and appreciable amounts of terpenoids. Although methods and units differ between phytochemical screens and GC–MS compositional studies, literature consistently identifies citral (a blend of geranial and neral) as the dominant volatile constituent of *C. citratus* oil and as the principal driver of many biological activities attributed to the plant's essential oil. Recent GC–MS studies reported citral contents in the 60–80% range and identify accompanying monoterpenes such as myrcene, geraniol and β -caryophyllene (Luang-In *et al.*, 2024). These compositional studies help explain why terpenoid-derived activities (antioxidant/pro-oxidant and cytotoxic) are regularly observed even when total terpenoid quantitation by other assays appears modest. The results confirmed the presence of other bioactive compounds such as phenols, tannins, flavonoids, phytates and saponins. These compounds are known for their pharmacological importance, including antimicrobial, anti-inflammatory, antioxidant, and anticancer activities (Avoseh *et al.*, 2023; Islam *et al.*, 2024). The predominance of alkaloids and terpenoids suggests that they may largely account for the plant's essential oil varied biological effects.

The antioxidant assays demonstrated that *C. citratus* oil inhibited lipid peroxidation (MDA formation) and elevated GSH levels in a concentration-dependent manner. Maximum antioxidant activity was observed at 25 – 50 µg/ml, where both MDA inhibition and GSH concentration peaked. These findings support earlier reports that *C. citratus* essential oil and its major component, citral, possess strong antioxidant potential through free radical scavenging and modulation of oxidative stress (Hartatie *et al.*, 2019; Kusumawati and Indrayanto, 2013). Interestingly, at very high concentrations (100 µg/ml), the antioxidant efficiency of *C. citratus* oil markedly declined, as reflected by reduced MDA inhibition and GSH levels. This suggests a biphasic effect, where optimal antioxidant protection is achieved at moderate doses, while excessive concentrations may exert pro-oxidant activity. Similar observations have been reported in studies of

natural phytochemicals, where high doses can paradoxically enhance oxidative stress (Bailly, 2020). The substantial inhibition of MDA formation and increase in reduced glutathione (GSH) at moderate concentrations (25 – 50 µg/mL), with diminished activity at very low and high doses match the dose-dependent and sometimes biphasic behavior that has been reported for plant-derived antioxidants. Multiple reviews and experimental reports have documented that natural phenolics and volatile terpenes can act as antioxidants at moderate concentrations but may show reduced efficacy or pro-oxidant effects at higher doses, a phenomenon with important implications for cancer therapy because induction of reactive oxygen species (ROS) in tumor cells is one route to trigger apoptosis. Thus, the decline in MDA inhibition and GSH at 100 µg/mL from the assay is inconsistent with the literature on dose-dependent redox behavior of botanical extracts (Sotler *et al.*, 2019).

When compared to the standard antioxidants BHT and ascorbic acid, *C. citratus* oil demonstrated weaker potency but significant antioxidant activity. While BHT achieved very high inhibition values (> 100 %) and ascorbic acid maintained stable inhibition (70 – 80 %) across concentrations, *C. citratus* oil showed moderate inhibition that declined at higher concentrations. This suggests that while *C. citratus* essential oil cannot fully replace synthetic antioxidants, it implies it could serve as a safer, natural complementary source of antioxidant protection. Crucially with respect to anti-cancer relevance, a substantial body of *in vitro* work attributed direct cytotoxicity against cancer cells to citral and to whole lemon grass oil (Nagata *et al.*, 2024; Maksimović *et al.*, 2025).

Mechanistic studies show that lemon grass essential oil can decrease intracellular ROS, promote mitochondrial dysfunction, activate caspase cascades (cleaved caspase-3 and related effectors), modulate Bcl-2/Bax family proteins in favor of apoptosis and induce cell-cycle arrest (often G2/M) (Maksimović *et al.*, 2025; Nagata *et al.*, 2024). Several reports demonstrate these effects across multiple human cancer cell lines (including lung A549, prostate and breast lines) and at concentrations overlapping with the range where the assay observed antioxidant/pro-oxidant activity (Balusamy *et al.*, 2020). These mechanistic observations provide a plausible link between the redox changes the result measured (MDA, GSH) and cytotoxic endpoints reported by other groups in which moderate redox modulation can protect cells, while

stronger ROS induction by citral/lemon grass essential oil can overwhelm tumor cell antioxidant defenses and trigger apoptosis (Balusamy *et al.*, 2020). Some studies also report additional anti-tumor mechanisms that broaden lemon grass essential oil's therapeutic rationale: inhibition of enzymes tied to chemoresistance (e.g., ALDH isoforms), interference with microtubule/tubulin dynamics and suppression of invasion in breast and other cancer models (Nagata *et al.*, 2024; Viktorová *et al.*, 2020). These observations suggest lemon grass essential oil components may act both as cytotoxics and as chemosensitizers, potentially enhancing the efficacy of conventional agents in combination therapies. However, such multi-targeted activity depends strongly on oil composition and dose and so requires careful standardization (Maksimović *et al.*, 2025).

Overall, these findings highlight that *C. citratus* essential oil may possess promising candidates for natural antioxidant therapy with implications for preventing oxidative damage and offering phytocompounds that may be crucial in managing diseases such as cancer. Furthermore, they could find applications as alternatives to artificial chemical antioxidants used in food industries for preservation of packaged foods and in cosmetics industries for skin care products to enhance their antioxidant attributes (Isyaka *et al.*, 2024).

CONCLUSION

This study demonstrated that *C. citratus* essential oil contains diverse phytochemicals, predominantly alkaloids and terpenoids, which contribute to its biological activities. The oil exhibited significant antioxidant effects, shown by MDA inhibition and GSH elevation. The finding that antioxidant effects peak at moderate doses and decline at high doses echoes the dual antioxidant/pro-oxidant behavior reported for many plant-derived agents and highlights the narrow therapeutic window that must be mapped for oxidative damage control especially in diseases as cancer and diabetes. Going forward, this study requires further research using high-throughput instrumentation techniques in quantifying and determining its chemical profiles while recommending that future studies should employ computational approaches to analyze individual phytochemical components in interacting with key molecular protein targets in notable disease conditions.

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COMPETING INTERESTS

The authors declare that no competing interests exist

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