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GC–MS Profiling of Essential Oil and Biological Activities of Methanol Extract of *Cymbopogon citratus*: Phytochemical, Antimicrobial, Antioxidant and Brine Shrimp Lethality Studies

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Abstract: ***Cymbopogon citratus*** **(lemongrass), a member of the Poaceae family, has long been used in traditional medicine throughout tropical regions to treat fever, infections, inflammation, and digestive issues, it has garnered substantial scientific attention. The chemical makeup of the essential oil and the biological activity of the *Cymbopogon citratus* methanol extract were examined in this study. GC-MS analysis of the hydrodistilled essential oil revealed 40 compounds, mostly monoterpenes and oxygenated derivatives like citral (neral and geranial), myrcene, limonene, geraniol, and β-caryophyllene. Flavonoids, tannins, saponins, phenolics, steroids/triterpenes, and alkaloids were found in the methanol extract through phytochemical screening; the most prevalent compounds were tannins (1.524 mg/g), flavonoids (1.413 mg/g), and phenolics (1.410 mg/g). With DPPH inhibition reaching 95.33% and metal-chelating activity up to 143.46% at the highest concentration, antioxidant evaluation demonstrated strong, dose-dependent activity across DPPH, ABTS, and metal-chelating assays. *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi* demonstrated the highest susceptibility to the extract's broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as fungi. The brine shrimp lethality assay produced an LC_{50} value of 81.27 µg/mL, indicating moderate cytotoxicity. Overall, the findings demonstrate *that C. citratus* essential oil and methanol extract possess significant antioxidant, antimicrobial, and cytotoxic properties, supporting their traditional medicinal use and highlighting their potential for natural therapeutic and pharmaceutical applications.**

Keywords: *Cymbopogon citratus, Essential Oil, Phytochemicals, Antioxidant Activity, Antimicrobial Activity, Cytotoxicity*.

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I. INTRODUCTION

For drug discovery and therapeutic development, natural products derived from medicinal plants continue to be a vital source of bioactive compounds (Newman 2020). In the past, a sizable percentage of contemporary medications have come from secondary metabolites found in plants, either directly or indirectly. These natural substances are significant leads in the creation of novel medications for the treatment of metabolic disorders, cancer, and infectious diseases because they frequently display remarkable structural diversity and biological specificity (Newman 2020). Additionally, traditional healthcare systems around the world continue to rely heavily on medicinal plants, especially in developing nations where plant-based remedies are frequently more accessible and less expensive than conventional medicines (Newman, 2020; Atanasov *et al.*, 2021). The wide range of phytochemicals found in medicinal plants, such as alkaloids, terpenoids, flavonoids, phenolic acids, tannins, and glycosides, are largely responsible for their pharmacological potential.

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Many of these compounds have antimicrobial, antioxidant, anti-inflammatory, and cytotoxic qualities.

Cymbopogon citratus (DC.) Stapf, or lemongrass, is one plant that has drawn a lot of attention because of its therapeutic value. Tropical and subtropical regions of Africa, Asia, and South America are home to *C. citratus*, a member of the Poaceae family (Tibenda *et al*., 2022). The plant is known for its distinctive lemon-like scent and is widely grown for culinary, fragrant, and medicinal uses. Fever, digestive issues, inflammation, hypertension, microbial infections, and nervous system disorders are just a few of the conditions that *C. citratus* has been used to treat in traditional medicine (Umar *et al*., 2016). This plant is widely used in ethnomedicine, which has prompted a great deal of scientific study to determine its phytochemical makeup and confirm its medicinal qualities.

The essential oil of *C. citratus*, which contains a complex mixture of volatile compounds, is largely responsible for its biological activities (Tibenda *et al*., 2022). Along with other components like β-myrcene, geraniol, limonene, and citronellal, the essential oil is primarily composed of monoterpenes, especially citral, a combination of two geometric isomers, geranial and neral (Ali *et al*., 2017; Luang-V *et al*., 2024). These volatile substances contribute to the plant's varied pharmacological actions as well as its unique scent. According to a number of studies, *C. citratus* essential oil has broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, indicating that it may be used as a natural antimicrobial agent (Ali *et al*., 2017; Shah *et al*., 2011). Furthermore, the essential oil has demonstrated antiviral and antifungal qualities (Tibenda *et al*., 2022), underscoring its potential to fight microbial infections.

The cytotoxic and anticancer properties of *C. citratus* essential oil have been studied in addition to its antimicrobial activity. According to recent research, the essential oil can stop cancer cells from proliferating by inducing apoptosis and modifying the expression of apoptotic genes. For example, the essential oil has been demonstrated to promote programmed cell death and inhibit the growth of HT-29 human colorectal adenocarcinoma cells, indicating potential therapeutic uses in the treatment of cancer (Luang-V *et al*., 2024). Additionally, the DPPH assay and other radical scavenging tests have shown that the essential oil has potent antioxidant qualities. Because oxidative stress is a major factor in the pathophysiology of many chronic diseases, such as cancer, cardiovascular disease, and neurodegenerative disorders, antioxidant activity is especially crucial (Atanasov *et al*., 2021).

The crude extracts of *C. citratus* leaves contain a range of non-volatile phytochemicals that may contribute to the pharmacological activities of the plant in addition to the volatile components found in the essential oil. These consist of phenolic compounds, flavonoids, tannins, saponins, and alkaloids, many of which are recognized for their anti-inflammatory, antimicrobial, and antioxidant properties (Unuigbe *et al*., 2019; Avoseh *et al*., 2015). Because of their capacity to scavenge free radicals, prevent lipid peroxidation, and shield biological systems from oxidative damage, phenolic compounds and flavonoids in particular have been extensively researched. These bioactive phytochemicals indicate that *C. citratus* crude extracts may have complementary biological effects to the essential oil.

Relatively few studies have combined thorough chemical profiling of the essential oil with systematic biological evaluation of solvent extracts derived from the same plant material, despite the large amount of research on either the essential oil or the crude extracts of *C. citratus*. While phytochemical screening and bioassays offer insights into the biological activities of plant extracts, advanced analytical techniques like gas chromatography–mass spectrometry (GC–MS) enable precise identification and characterization of volatile constituents within essential oils. Establishing correlations between particular phytochemical constituents and their observed pharmacological activities requires integrating chemical characterization with biological evaluation.

In order to provide a thorough assessment of *C. citratus*, the current study combines biological evaluation of its methanol leaf extract with chemical profiling of its essential oil using GC–MS analysis. Phytochemical screening, antioxidant assessment, antimicrobial testing, and cytotoxicity evaluation using the brine shrimp lethality assay were performed on the methanol extract. This study aims to identify potential bioactive compounds that could be leads for future pharmacological development and to contribute to the scientific validation of the traditional uses of *C. citratus* by combining chemical and biological analyses.

II. MATERIALS AND METHODS

- *Sample Collection*

With the identification number 1882ABU, the leaves of *Cymbopogon citratus* were gathered and verified at the Department of Biological Sciences' Botany division at Ahmadu Bello University in Zaria, Kaduna, Nigeria.

- *Extraction of Essential Oils*

Using a Clevenger-style device, fresh leaves and stems were hydrodistilled to extract essential oils. One kilogram of plant material was immersed in water and distilled at 100°C for three and a half hours. 1.5 mL of n-hexane was used to collect the condensed oil, and an extra 1 mL was used to rinse the device. The pure essential oil was extracted, weighed, and chilled until analysis after the combined extract was kept at -4 °C to remove any remaining water (Onocha *et al*., 2011).

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➢ *Gas Chromatography–Mass Spectrometry (GC–MS) Analysis*

Using an HP-5MS column and helium carrier gas, GC-MS analysis was carried out on an Agilent 7890A/5975 VL system. A 2 µL splitless injection was used, and the oven's temperature was set between 50 and 300 °C. Using Agilent ChemStation software, compounds were identified by comparing mass spectra and retention indices with the NIST library and published references.

➢ *Extraction of Crude Extracts*

The plant extracts were obtained through cold maceration. A rotary evaporator was used to filter and evaporate 285 g of C. citratus leaves at 78°C after they were submerged in 500 ml of methanol for 72 hours at room temperature.

➢ *Brine Shrimp Lethality Test (BSLT)*

The BSLT was first used to screen the extracts for cytotoxicity. Brine shrimp eggs (*Artemia salina*) were incubated in seawater for 48 hours to hatch into larvae. The concentrations of 1, 10, 100, and 1,000 µg/mL were used to prepare the extract. Each test vial was filled with ten larvae in triplicate, and after a day, mortality was noted. Finney's probit analysis was used to calculate the median lethal concentration (LC_{50}).

➢ *Antimicrobial Assay*

- *Preparation of the Medium:*

The medium is made by dissolving 34 g of Mueller Hilton Agar (MHA) powder in 1 liter of distilled water and heating the mixture until it dissolves completely. After being autoclaved for 15 minutes at 121°C, the mixture was cooled to 45–47°C before being transferred into sterile Petri dishes to solidify. A sterile cork borer was used to create holes in each agar plate, and a sterile cotton swab stick was used to evenly inoculate the plate surfaces with the microbial isolate. The holes were then filled with 0.5 cm^3 of the extract at various concentrations (1.0, 0.5, and 0.25 mg/cm^3), including the control. A transparent ruler was used to measure the diameters of the zones of inhibition after the plates had been incubated for 48 hours.

- *Preparation of Standard Drugs*

The standard drugs were prepared by dissolving 20 mg of each drug in 5 cm^3 of methanol to produce a stock solution of 4000 $\mu g/cm^3$. Particular volumes of the stock solution (0.4 cm^3, 0.3 cm^3, 0.2 cm^3, and 0.1 cm^3) were then transferred to vials and left to evaporate over the course of a day. Each vial was filled with two drops of DMSO and two centiliters of distilled water, yielding approximate concentrations of 800, 600, 400, and 200 $\mu g/cm^3$, respectively. Solvent alone was used to prepare a control.

- *Test of MIC & MBC*

The microdilution agar method was used to determine the MIC and MBC. The extract was serially diluted (1.25–160 mg/cm^3) and added to MHA plates that had been infected with the test microbe. The control was methanol. The lowest concentration that showed no discernible growth after 24 hours at 37°C was noted as the minimum inhibitory concentration (MIC). Samples from MIC wells were plated on MHA for MBC, and the lowest concentration that resulted in ≥99.9% bacterial killing was noted.

➢ *Antioxidant Assay Protocol*

- *DPPH free-radical Scavenging Activity*

To create sample solutions with concentrations of 0.25–1.5 mg/mL, 0.49, 0.98, 1.47, and 1.96 mg of extract were dissolved in 2 mL of solvent. DPPH was made by dissolving 39.4 mg in 100 mL of methanol, letting it stand for ten minutes, and then measuring its absorbance at 517 nm. For the assay, 0.5 mL of each test sample was combined with 2 mL of DPPH solution, shaken, and incubated for 10 minutes before absorbance at 517 nm was measured. The standard antioxidant BHA, which was made in distilled water, was prepared using the same method. The standard formula was used to determine the percentage inhibition of DPPH radicals.

$$\text{Inhibition} = \frac{A_{DPPH}-A_S}{A_{DPPH}} \times 100$$

Where A_{DPPH} and A_S are the respective absorbance of the neat DPPH and test solutions

- *ABTS: TEAC (Trolox equivalent antioxidant capacity) assay*

The ABTS assay measured antioxidant compounds' capacity to quench the $ABTS^+$ radical, a blue-green chromophore with a maximum absorbance at 734 nm. $ABTS^+$ was produced by reacting 7 mM ABTS with 2.45 mM potassium persulfate and letting the mixture sit in the dark for 12–16 hours. One milliliter of $ABTS^+$ solution (156 µM), one milliliter of NADH (468 µM), and one milliliter of sample solution (0.25–1.5 mg/mL) were combined for the assay, and the absorbance was measured at 734 nm. The standard was BHA made with distilled water. Using common antioxidant inhibition formulas, the percentage inhibition of the ABTS radical was determined.

$$\text{Inhibition} = \frac{A_{blank}-A_{Sample}}{A_{blank}} \times 100$$

Where A blank is the absorbance of the blank in absence of sample, and A sample is an absorbance in the presence of the sample.

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- *Metal Chelating Activity*

The extracts (0.25, 0.5, 1.0, and 1.5 mg/cm³), 1.5 cm³ of deionized water, and 0.5 cm³ of 1M $FeCl_2$ solution were all present in the reaction mixture. 1.0 cm³ of 5M ferrozine solution was added after 30 minutes. The absorbance at 562 nm was measured following a 10-minute incubation period at room temperature. The positive control was BHA. The following formula was used to determine the percentage inhibition of ferrozine-Fe^{2+} complex formation:

$$\% \text{ Activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Where A control is the absorbance of the blank in absence of sample, and A sample is an absorbance in the presence of the sample.

III. RESULTS

➢ *GC–MS Analysis of C. citratus Essential Oil*

Forty chromatographic peaks were found in the GC-MS analysis of *C. citratus* essential oil. By comparing with the NIST 17 library, compound identification was accomplished. Using Agilent ChemStation software, compounds were identified by comparing mass spectra and retention indices with the NIST library and published references. The retention times (RT) and percentage area of each identified compound are listed in the table below.

Table 1: GC–MS result of *C. citratus* Essential Oil

Peak No.	RT (min)	Area %	Identified Compound (Library Match)
1	32.247	0.42	Citral (trans-citral / geranial)
			Neral (cis-citral)
2	11.499	0.32	Piperazine derivative
3	32.602	0.29	Neral (cis-citral)
4	23.922	0.57	Limonene
5	11.985	0.31	Geranyl acetate
6	12.054	0.25	β-Caryophyllene
7	12.111	0.27	Citronellal (methyl ester)
8	12.162	0.50	Myrcene (α-myrcene)
9	12.225	0.61	Aromatic amine derivative
10	12.271	0.34	Geraniol (methyl derivative)
11	12.323	0.70	Dehydroabietol benzoate
12	12.420	0.72	Carbazole derivative
13	12.466	0.20	Methandriol derivative
14	12.511	0.89	Benz(a)anthracene-7-carbonitrile
15	12.569	0.26	Trimethylaniline derivative
16	12.666	0.29	Bromo-thiophene derivative
17	12.740	0.20	Oxacycloheptadecan-2-one
18	12.797	0.38	Tetraoxa-trisilaheptane derivative
19	12.843	0.28	α-Ketostearic acid
20	12.883	0.36	Oxalic acid, hexylpentadecyl ester
21	12.912	0.32	Syringic acid (TMS derivative)
22	12.946	0.38	Tricosanoic acid
23	13.061	1.08	Steroid derivative
24	13.118	0.47	Undecynoic acid dodecyl ester
25	13.164	0.62	Triamterene derivative
26	13.215	0.69	Oxacycloheptadecan-2-one
27	13.267	0.59	Benzaldehydehydrazone derivative
28	13.312	0.85	Oleic acid / cis-vaccenic acid
29	13.358	0.35	Benzenesulfonamide derivative
30	13.398	0.54	Tetradecenoic acid (E-9)
31	13.433	0.55	Oleic acid / 9-octadecenoic acid
32	13.513	0.85	Octadecenoic acid isomers
33	13.553	0.36	Epoxypentacosane derivative
34	13.662	0.85	9-Octadecenoic acid (E)
35	13.770	0.29	2-Hydroxyethyl oleate
36	13.868	0.61	Hexadecenoic acid (Z-11)

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37	13.919	0.35	Oleic acid
38	14.045	0.90	Octadecenoic acid trifluoroethyl ester
39	14.102	0.53	Palmitoleic acid / Oleic acid
40	14.154	0.72	Oleic acid isomer

➢ *Phytochemical Screening*

The different phytochemical components of the *C. citratus* methanol extract were examined both qualitatively and quantitatively. Tables 2a and 2b display the outcomes of both tests.

Table 2a: Qualitative Phytochemical result of the methanol extract of *C. citratus*

Secondary metabolite	**Result**
Carbohydrate	+
Saponins	+
Flavonoid	+
Anthraquinones	_
Cardiac glycosides	+
Tannins	+
Steriod/Triterpenes	+

Keys: (+) → Present,(-) → Absent

Table 2b: Quantitative Phytochemical result of the methanol extract of *C. citratus*

Metabolites	**Result**
Saponins	0.812 ± 0.51
Flavonoid	1.413 ± 0.17
Tannins	1.524 ± 0.10
Terpenoid	0.251 ± 0.92
Phenol	1.410 ± 0.61
Alkaloid	0.632 ± 0.22
Oxalate	0.681 ± 0.20
Phytate	0.251 ± 0.30

➢ *Antimicrobial zone Inhibition of the crude extract*

Analysis of methanol extract of *C. citratus* antimicrobial zone inhibition is presented in table 3 below.

Table 3: Result of the Methanol Extract of *C. citratus* Antimicrobial Zone Inhibition

Microbial strain	**Concentration (x10^2 µg/ml)**			
	4	5	6	7
Staphylococcus aureus	3.3	9	11.7	23.3
Streptococcus pneumonia	2.3	4.7	9	16.3
Salmonella typhi	6.3	10.7	15.3	23.7
Corynbacteriumulcerans	5	9.7	16.3	28
Escherichia coli	5	9.7	16.3	29.7
Pseudomonas aeruginosa	7.3	14.7	22.3	30
B.cereus	8.7	12.3	17	24.7
Trichophytonrulorrum	4	8.3	11.7	16
Aspergillusniger	8	12.3	17.3	27.7
Candida ablican	4.7	8.7	14	22.7
Control	-	-	-	-

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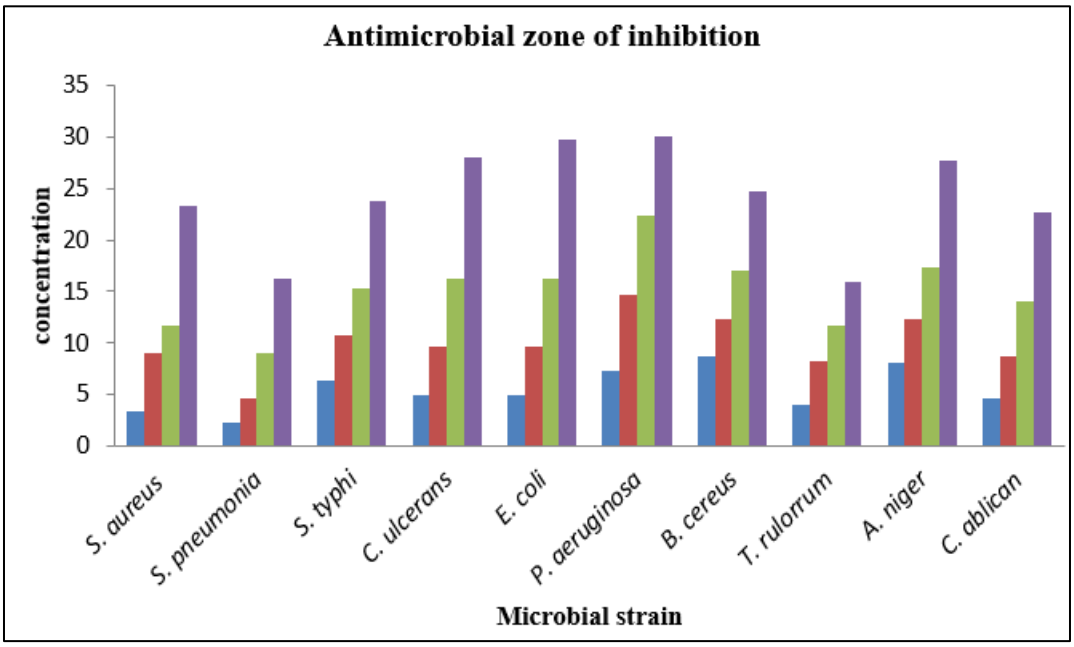

Fig 1: Antimicrobial Zone Inhibition of the Methanol Crude Extract Against Several Microbial Strains

- *Minimum Inhibitory Concentration (MIC) of the crude extract*

The result of the minimum inhibitory concentration of the methanol extract of *C. citratus* is shown in Table 4.

Table 4: MIC Result of *C. citratus* Methanol Extract

Bacterial strains	Ciprofloxacin	Methanol
Staphylococcus aureus	$0{:}648\times10^{-3}$	1.69
Streptococcus pneumonia	$0{:}648\times10^{-3}$	6.49
Salmonella typhi	$0{:}648\times10^{-3}$	6.49
B. cerues	$0{:}648\times10^{-3}$	3.37
Pseudomonas aeruginosa	$0{:}041\times10^{-3}$	6.75
Escherichia coli	$0{:}041\times10^{-3}$	6.75
Trichophytonrulorrum	$0{:}648\times10^{-3}$	5.75
Aspergillusniger	$0{:}648\times10^{-3}$	5.75
Candida ablican	$0{:}648\times10^{-3}$	6.75

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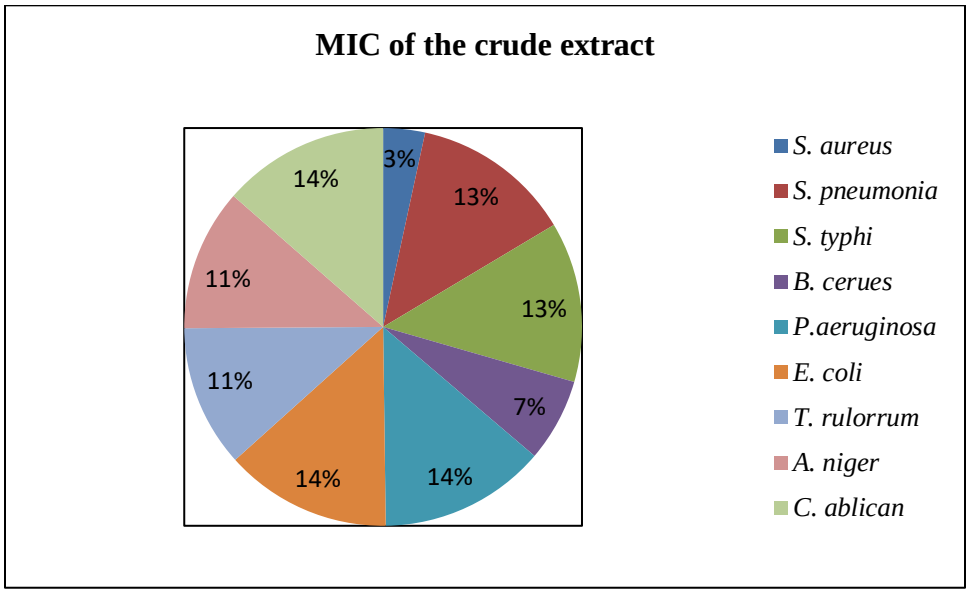

Fig 2: Minimum Inhibitory Concentration (MIC) of the methanol crude extract against several bacterial strains.

➢ *Antioxidant Assay Result*

The methanol extract of*C.citratus*was analyzed for its antioxidant activities using 3 types of Assay; DPPH, ABTS and Metal Chelation. Results are shown in table 5

Table 5: Antioxidant Activities of Methanol Extract of *C.citratus* and Standard (BHA)

Assay	Concentration (mg/mL)	% Inhibition (Mean ± SD)
DPPH (Extract)	0.25	50.70 ± 0.29
	0.50	77.67 ± 0.49
	1.00	88.90 ± 0.34
	1.50	95.33 ± 0.82
ABTS (Extract)	0.25	54.37 ± 0.87
	0.50	74.73 ± 11.75
	1.00	78.33 ± 5.94
	1.50	86.93 ± 0.57
Metal Chelation (Extract)	0.25	42.90 ± 0.72
	0.50	114.43 ± 1.36
	1.00	128.95 ± 1.81
	1.50	143.46 ± 2.26
BHA (Standard)	0.25	47.90 ± 1.21
	0.50	58.63 ± 0.32
	1.00	77.23 ± 1.11
	1.50	81.27 ± 1.00

➢ *Brine shrimp lethality test (BSLT) result*

The BSLT result of the methanol extract of *C. citratus* is shown in Table 6

Table 6: BSLT result of the crude extract *C. citratus*

Plant extract	LC_{50} U/L limit
Methanol extract	81.27 (134.51/455.55)

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IV. DISCUSSION

Cymbopogon citratus essential oil was found to have a complex chemical profile with 40 constituents, mostly monoterpenes and oxygenated derivatives, according to GC–MS analysis. Citral (a combination of geranial and neral), myrcene, limonene, β-caryophyllene, geraniol, and geranyl acetate were the main compounds found. The plant's distinctive lemon scent and many of its pharmacological characteristics are mostly attributed to citral, which is widely acknowledged as the primary bioactive marker of *C. citratus* essential oil (Oladeji *et al*., 2019). Citral is a crucial substance in pharmaceutical and food preservation applications due to its potent antimicrobial, anti-inflammatory, and anticancer properties, according to several studies (Luang-V *et al*., 2024; Ali *et al*., 2017). Citral's predominance in this study is therefore in line with earlier reports of citral-rich chemotypes of *C. citratus* grown in tropical and subtropical climates.

The chemical profiles of *C. citratus* essential oils that have been previously reported are further supported by the presence of additional monoterpenes like limonene, geraniol, and myrcene (Tibenda *et al*., 2022). Numerous biological activities, such as antimicrobial, antioxidant, and anti-inflammatory effects, have been demonstrated for these compounds (Onawunmi 1989; Ekpenyong *et al*., 2015). Among these, limonene is a compound of significant pharmacological interest due to its numerous reports of antimicrobial, antioxidant, and chemopreventive properties (Bakkali *et al*., 2008; Araújo-Filho *et al*., 2021). Similarly, studies conducted both in vitro and in vivo have linked geraniol, an acyclic monoterpene alcohol, to a wide range of biological activities, such as antifungal, antioxidant, anti-inflammatory, and anticancer effects (Ben Ammar, 2023). Because β-caryophyllene interacts with cannabinoid receptor type 2 (CB2) pathways to exhibit significant anti-inflammatory, analgesic, and cytoprotective properties, its detection is also noteworthy (Klauke *et al*., 2014). Additionally, the extraction of less volatile components during hydrodistillation may be reflected in the presence of fatty acid derivatives like oleic acid. Hydrodistillation can enable the co-extraction of higher-molecular-weight and semi-volatile compounds, adding to the oil's overall chemical complexity and potential bioactivity, even though volatile terpenoids usually predominate in essential oils (Ekpenyong *et al*., 2015). Through potential synergistic interactions between various phytochemicals, the coexistence of these compounds enhances the oil's overall biological activity (Shah *et al*., 2019).

Qualitative phytochemical screening of the methanol extract revealed the presence of several classes of secondary metabolites, including flavonoids, tannins, saponins, phenolics, steroids/triterpenes, and alkaloids. The biological activities of these groups of phytochemicals are well known, and they are frequently linked to the therapeutic benefits of medicinal plants (Atanasov *et al*., 2021). The most prevalent components, according to quantitative analysis, were tannins (1.524 mg/g), flavonoids (1.413 mg/g), and phenolics (1.410 mg/g). Because these metabolites are known to have potent antioxidant qualities, the high concentrations of phenolic and flavonoid compounds are especially important (Tibenda *et al*., 2022). In order to prevent oxidative damage to biomolecules like lipids, proteins, and nucleic acids, phenolic compounds can donate hydrogen atoms or electrons to neutralize free radicals (Rekha *et al*., 2012). Therefore, the study's findings support previous findings that *C. citratus* leaves contain significant levels of bioactive secondary metabolites with significant therapeutic implications (Unuigbe *et al*., 2019).

The antioxidant activity of the methanol extract was evaluated using three complementary assays, namely DPPH radical scavenging, ABTS radical scavenging, and metal-chelating assays. The extract demonstrated strong antioxidant activity across all assays, with a clear dose-dependent increase in inhibition. In the DPPH assay, the extract exhibited an inhibition value of 95.33% at the highest concentration (1.5 mg/mL), surpassing the activity of the standard antioxidant BHA (81.27%). This high radical-scavenging activity suggests the presence of potent hydrogen-donating compounds within the extract, most likely phenolics and flavonoids. The strong activity seen in this study suggests a high capacity of the extract to neutralize reactive oxygen species. The DPPH assay is commonly used to assess antioxidants' ability to donate hydrogen atoms to stabilize free radicals (Brand-Williams *et al*., 1995).

Strong radical-scavenging activity was also demonstrated by the ABTS assay, with inhibition values rising from 54.37% at lower concentrations to 86.93% at the highest concentration examined. The strong activity seen further supports the antioxidant potential of *C. citratus* extracts, and the ABTS assay is especially helpful for assessing both hydrophilic and lipophilic antioxidant compounds. Similar findings have been documented in earlier research, where the presence of phenolic and flavonoid compounds in C. citratus extracts demonstrated notable ABTS radical-scavenging activity (Anwar *et al*., 2024).

At the highest concentration tested, the extract's metal-chelating activity was especially strong, reaching 143.46%. By sequestering transition metal ions, especially iron and copper, metal-chelating antioxidants reduce their involvement in Fenton and Fenton-like reactions that produce extremely reactive hydroxyl radicals. Chelating agents help prevent the production of reactive oxygen species and reduce oxidative damage to cellular macromolecules, such as proteins, lipids, and DNA, by decreasing the availability of these pro-oxidant metal ions (Halliwell & Gutteridge, 1992). The presence of tannins and phenolic compounds found during phytochemical analysis may be responsible for the strong metal-chelating activity seen in this investigation. According to Jan Pokorny *et al* (2001), these phytochemicals have several hydroxyl and carbonyl groups that can efficiently bind metal ions and form stable complexes, preventing metal-catalyzed oxidative

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reactions. The high chelating activity observed is in line with the known antioxidant qualities of plant polyphenols and indicates that these components play a major role in the extract's overall antioxidant potential (Rice-Evans *et al.*, 1997; Prior *et al*., 2005). As a result, the extract's ability to chelate metals may be one of the main ways it protects against oxidative stress. All things considered, the antioxidant assay results show that *C. citratus* extracts are a promising natural source of antioxidant compounds, supporting their possible use in food preservation systems and pharmaceutical formulations (Sohail *et al*., 2024).

Different levels of inhibitory activity against bacterial and fungal strains were found in the methanol extract's antimicrobial evaluation. Larger zones of inhibition were seen at higher concentrations of the extract, which demonstrated broad-spectrum antimicrobial activity. The organisms most susceptible to the extract were *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These results are in line with previous studies showing that essential oils and *C. citratus* extracts have strong antibacterial activity against a variety of pathogenic microbes (Subramaniam *et al*., 2020; Vazirian *et al*., 2012). The antimicrobial activity of the extract may be attributed to the synergistic effects of several bioactive compounds, including citral, geraniol, limonene, flavonoids, and tannins. These compounds are known to disrupt microbial cell membranes, interfere with enzyme activity, and inhibit microbial growth (Olorunnisola *et al*., 2020).

The extract showed significant antifungal effects against *Aspergillus niger*, *Candida albicans*, and *Trichophyton rubrum* in addition to its antibacterial activity. This study's antifungal activity is consistent with earlier research showing that *C. citratus* has potent inhibitory effects against a number of fungal pathogens (Ekpenyong *et al*., 2015). The presence of phenolic compounds and terpenoids, which can damage fungal cell membranes and prevent ergosterol synthesis, thereby affecting cell integrity and viability, may be linked to the extract's capacity to prevent fungal growth.

The brine shrimp lethality assay, a preliminary screening method for identifying bioactive compounds with potential anticancer properties, was used to assess the cytotoxic potential of the methanol extract. This study's LC_{50} value of 81.27 µg/mL shows moderate cytotoxicity. Plant extracts with LC_{50} values less than 100 µg/mL are deemed biologically active and deserving of additional research, according to the classification put forth by Meyer *et al* (1982). Citral, phenolic compounds, and flavonoids which have been shown to have antiproliferative effects against a variety of cancer cell lines may be responsible for the observed cytotoxicity (Luang-V *et al*., 2024; Al Weshahi *et al*., 2025). These findings, therefore, suggest that the methanol extract of *C. citratus* may contain compounds with potential applications in anticancer drug development.

Overall, the study's findings demonstrate *C. citratus's* varied biological activities and rich phytochemical composition. The observed antioxidant, antimicrobial, and cytotoxic activities are probably caused by the combination of volatile terpenoids found by GC-MS analysis and non-volatile phenolic compounds found in the methanol extract. The results highlight *C. citratus's* potential as a source of bioactive compounds for pharmaceutical and nutraceutical applications in addition to supporting its traditional medicinal uses. To completely understand the mechanisms underlying these biological activities, more research involving the isolation, purification, and structural characterization of the active constituents as well as in vivo pharmacological assessments would be required.

V. CONCLUSION

This study offers a thorough assessment of the chemical and biological characteristics of Cymbopogon citratus. The GC–MS analysis showed a varied profile of bioactive constituents dominated by citral, myrcene, limonene, geraniol, and other oxygenated monoterpenes; the methanol extract showed strong antioxidant activity, significant antimicrobial properties, and moderate cytotoxicity; these bioactivities are probably supported by a variety of phytochemicals, such as flavonoids, phenolics, tannins, and terpenoids.

Overall, the results demonstrate the potential uses of C. citratus in natural medicine, pharmaceuticals, and food preservation while also supporting its traditional medicinal use.

CONFLICTS OF INTEREST

The published version of the manuscript has been read and approved by all authors. No conflicts of interest are disclosed by the writers.

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