

Chemical composition, antioxidant, and anti-inflammatory activities of essential oils from *Cymbopogon citratus* (DC.) Stapf and *Chrysopogon zizanioides* (L.) Roberty cultivated in Kisangani, Northeastern Region of the Democratic Republic of the Congo

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ABSTRACT

Introduction

Essential oils are valuable natural products known for their bioactive properties. *Cymbopogon citratus* (lemongrass) and *Chrysopogon zizanioides* (vetiver) are aromatic plants cultivated in Kisangani, whose chemical composition and biological activities warrant investigation.

Purpose

This experimental study aimed to determine the chemical composition and to evaluate the *in vitro* antioxidant and anti-inflammatory properties of essential oils extracted from *Cymbopogon citratus* (DC.) Stapf and *Chrysopogon zizanioides* (L.) Roberty cultivated in Kisangani.

Methods

Leaves of lemongrass and roots of vetiver were collected from the Mavaolo concession and hydro-distilled to obtain essential oils (EOs). The extraction yields were $0.11 \pm 0.01\%$ for lemongrass and $1.40 \pm 0.67\%$ for vetiver, with densities of $0.88 \mu\text{g/mL}$ and $1.05 \mu\text{g/mL}$, respectively. Gas Chromatography–Mass Spectrometry (GC–MS) analysis revealed 17 and 24 peaks in the two species, of which 100% and 82.32% were identified, respectively.

Results

C. citratus oil consisted mainly of oxygenated monoterpenes (69.93%), primarily citral (36.64%) and nerol aldehyde (28.85%), while *C. zizanioides* oil contained predominantly oxygenated sesquiterpenes (87.67%), including khusimol (28.52%) and vetivenic acid (14.01%). Lemongrass essential oil exhibited strong antioxidant and anti-inflammatory activities, whereas vetiver oil showed weak antioxidant but strong anti-inflammatory properties compared with α -tocopherol used as a reference.

Conclusion

The findings indicate that *C. citratus* and *C. zizanioides* cultivated in Kisangani contain bioactive constituents of commercial potential. It is recommended that further cultivation studies be conducted to optimise essential oil yield.

INTRODUCTION

Cymbopogon citratus (DC.) Stapf, commonly known as lemongrass, is an herbaceous perennial that grows in clumps ranging from 30 to 60 cm in height and occasionally higher (Silou et al., 2017). *Chrysopogon zizanioides* (L.) Roberty, known as vetiver, is a robust, erect, perennial herbaceous plant with glabrous stems reaching heights of 1 to 2 metres (Vangu et al., 2023). Both plants are important sources of essential oils (EOs) used in the flavouring and fragrance industries, as well as in traditional and modern medicine.

Previous studies have shown that their EOs exhibit various pharmacological properties, including anti-amoebic, antibacterial, antidiarrhoeal, antifilarial, antifungal, and anti-inflammatory activities (Bansod et al., 2008; Wannissorn et al., 2005). These natural products may represent a promising alternative to synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which have been associated with potential long-term teratogenic, mutagenic, and carcinogenic effects in food, cosmetic, and pharmaceutical industries (Bourkhiss et al., 2010).

Additionally, plant-derived EOs offer an advantage over synthetic non-steroidal anti-inflammatory drugs (NSAIDs), which have been linked to increased risks of gastrointestinal, renal, and respiratory disorders, including asthma (Amoah et al., 2024; Dinarello, 2010).

Despite the commercial importance of synthetic citral, lemongrass remains one of the world's most important natural sources of citral. Historically, lemongrass oil was the principal natural source of citral used in vitamin A production (Attokaran, 2011). The commercial value of plant-based raw materials in the fragrance and pharmaceutical industries depends largely on the yield and composition of their EOs.

In the Democratic Republic of the Congo (DRC), both lemongrass and vetiver are cultivated mainly for domestic purposes. Expanding their cultivation could potentially enhance their contribution to the international EO market. However, limited research has been conducted on the chemical profiles and antioxidant and anti-inflammatory activities of these plants' essential oils.

METHODS

Study Design

This experimental study investigated, *in vitro*, the antioxidant and anti-inflammatory potentials of EOs extracted from *C. citratus* leaves and *C. zizanioides* roots collected in Kisangani. The aim was to determine whether local environmental factors could influence their chemical composition and corresponding biological activities. The study was conducted from 15 July to 30 September 2024.

Collection and Identification of Plant Materials

Leaves of *C. citratus* and roots of *C. zizanioides* were collected in February 2024 from the Mavaolo concession in Bayangana, a locality approximately 10 km from Kisangani in the northeastern DRC (Figure 1). The plant specimens were identified and authenticated at the Herbarium of the Faculty of Science, University of Kisangani, under voucher numbers 050324 (*C. citratus*) and 060324 (*C. zizanioides*) (Figure 2).

Figure 1:

Map of the Mavaolo concession in the locality of Bayangana, city of Kisangani/DR Congo

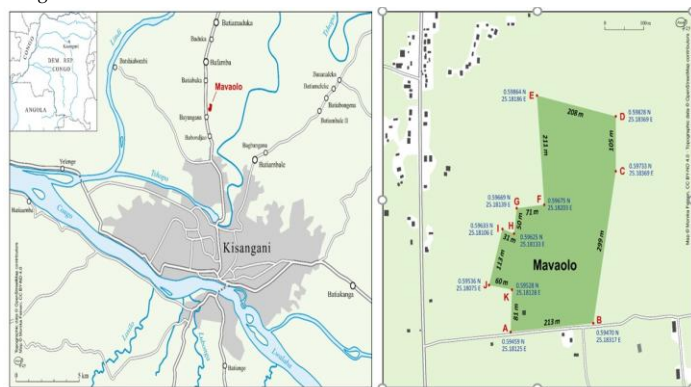


Figure 2:

C. citratus leaves (A) and herbarium specimen (B); *C. zizanioides* roots (C) and herbarium specimen (D)



Chemical Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS), Amplex Red (AR), sodium nitrite, sodium persulfate, and hydrogen peroxide were obtained from Sigma-Aldrich (Steinheim, Germany). Bovine serum albumin (BSA) was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Human myeloperoxidase (MPO) was procured from Calbiochem Millipore (Billerica, Madison, WI, USA). Ethanol and methanol (analytical grade) were supplied by Merck VWR (Leuven, Belgium).

Extraction of Essential Oils

Fresh leaves and roots were naturally air-dried for approximately 21 days, protected from light and humidity. The dried materials were then crushed and stored in black plastic bags to prevent photosensitisation of the EOs. Each plant sample underwent hydro-distillation for five hours using a Clevenger apparatus. Depending on the quantity of sample, the crushed material was placed in 2- or 5-litre glass flasks filled to two-thirds with water and heated to boiling using a heating mantle. The process was repeated three times per plant sample. The distilled EO was stored in amber glass bottles at 4°C, protected from light, until analysis.

Determination of Chemical Composition

EOs were diluted in hexane to a 1:10 (v/v) concentration. Chemical composition was determined using an Agilent Technologies 7890B Gas Chromatograph coupled with a 5977B Mass Spectrometer (Agilent, Santa Clara, CA, USA).

The GC system was equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm, 5% phenyl-95% methylpolysiloxane stationary phase). Helium served as the carrier gas at a flow rate of 1.2 mL/min. Injections were made in splitless mode, with 1 µL of EO injected.

Following Taguimjeu et al. (2025), the oven temperature was programmed from 50°C to 300°C (5 min hold) at a rate of 5°C/min. The MS detector operated at 70 eV ionisation energy, scanning a mass range of 40–400 amu, with the source temperature maintained at 230°C. Compound identification was based on comparisons with WILEY and NIST 17 libraries. Retention indices (RIs) were calculated using C7–C30 *n*-alkanes. Quantitative analysis was expressed as relative peak area means from three replicates.

Assessment of Antioxidant Activity

The antioxidant potential of EOs was assessed *in vitro* at various concentrations (59.5–23,800 µg/mL) using both ABTS and DPPH assays as described by Re et al. (1999), with minor modifications by Wahyu et al. (2017).

For the ABTS assay, ABTS (7 mM) was reacted with potassium persulfate (2.45 mM final concentration) and incubated in the dark at 25°C for 24 hours to generate the ABTS⁺ radical cation. The mixture was diluted with methanol to an absorbance of 0.85 ± 0.02 at 734 nm. A 2 µL aliquot of EO was added to 198 µL of ABTS⁺ solution in a microplate (Thermo Lab Systems, Finland). Control wells contained 2 µL of water and 198 µL of ABTS⁺ solution. After 10 minutes of incubation, absorbance was measured at 734 nm using a spectrophotometer (Ben et al., 2020).

For the DPPH assay, the radical scavenging activity was evaluated using the method by Blois (1958), modified by Bourkhiss et al. (2010). A 1 mM DPPH methanolic solution was stirred for 60 minutes and adjusted to an absorbance of 0.70 ± 0.02 at 517 nm. Antioxidant-induced discolouration (violet to yellow) was measured spectrophotometrically after 30 minutes using a Multiskan Ascent 96 plate reader (Thermo Lab Systems, Finland). Trolox® (vitamin E) served as the reference antioxidant, while the control contained 2 µL of water in 198 µL of DPPH solution.

Assessment of Anti-inflammatory Activity

The anti-inflammatory potential of *C. citratus* and *C. zizanioides* EOs was evaluated following the procedure described by Borive et al. (2025), with slight modifications. The assay relies on fluorescence emission at 584 nm induced by excitation at 571 nm. The MPO reaction cycle, similar to that of horseradish peroxidase (HRP), was coupled with the fluorescent probe Amplex Red and amplified by sodium nitrite.

After incubation at 37°C for 10 minutes, 100 µL of each test compound (five solutions of four concentrations) was added to a 96-well plate. Subsequently, 10 µL of NaNO₂ and 100 µL of a SIEFED/Amplex Red/H₂O₂ phosphate buffer solution were added. Positive and solvent controls (H₂O and DMSO) were included. The anti-inflammatory activity was calculated using the same equation applied to antioxidant analysis.

Statistical Data Analysis

Data analysis and graphical representation were performed using Microsoft Excel and STATA software. Results are presented as mean \pm standard deviation (SD). Statistical significance was set at $p < .05$.

Ethical Considerations

Ethical approval was not required as the study did not involve human or animal subjects. However, authorisation to conduct the research was obtained from the University of Kisangani authorities as part of the requirements for a master's degree in the Department of Pharmacy, Faculty of Medicine and Pharmacy. The approval also covered the transport of plant samples for analysis.

RESULTS

Chemical characteristics of lemongrass and vetiver essential oils

The essential oils (EOs) of the two plants exhibited almost similar organoleptic characteristics, including scent (pleasant, fresh, and fruity) and appearance (clear oily liquid with low mobility). However, *C. citratus* EO displayed a light-yellow hue, while *C. zizanioides* EO exhibited a dark-yellow hue.

As shown in **Table 1**, there were significant differences in the yield and major chemical groups between the two plants. The yields were $0.11 \pm 0.01\%$ and $1.40 \pm 0.67\%$ for lemongrass and vetiver EOs, respectively, and their densities were $0.88 \mu\text{g/mL}$ and $1.05 \mu\text{g/mL}$. Most of the chemical groups in *C. citratus* comprised oxygenated monoterpenes (69.93%), whereas those in *C. zizanioides* were predominantly oxygenated sesquiterpenes (87.67%). The difference may be explained by the type of raw plant material used—leaves for *C. citratus* and roots for *C. zizanioides*.

Tables 2 and 3 present the GC/MS analysis findings. Under the conditions used, 17 peaks were detected in *C. citratus* and 24 peaks in *C. zizanioides*. Of these, 100% of the 17 peaks and 82.32% of the 24 peaks were identified. The major compounds were citral ($36.64 \pm 3.18\%$) and neroli aldehyde ($28.85 \pm 1.94\%$) in *C. citratus*, and khusimol ($28.52 \pm 0.95\%$) and vetivenic acid ($14.01 \pm 1.83\%$) in *C. zizanioides*.

Table 1:

Yield and major chemical groups of *C. citratus* and *C. zizanioides* essential oils

Characteristics	<i>C. citratus</i>	<i>C. zizanioides</i>	p-value
Quantity extracted (g)	500.00	75.00	< 0.001
Quantity of EO (g)	0.55	1.05	
EO yield % (mean \pm SD)	0.11 ± 0.01	1.40 ± 0.67	
Density ($\mu\text{g/mL}$)	0.88	1.05	
Oxygenated monoterpenes	69.93	0	
Oxygenated sesquiterpenes	6.72	87.67	
Oxygenated diterpenes	2.37	0.60	
Hydrocarbon sesquiterpenes	8.87	4.15	
Ketones	6.60	0	
Hydrocarbon monoterpenes	5.51	0	
Carboxylic esters	0	0.78	
Other compounds	0	6.80	

Table 2:

Composition of essential oils hydrodistilled from the leaves of *Cymbopogon citratus* (n = 3)

N°	Component name	CAS No.	RI (Exp.)	RI (Lit.)	Area % (Mean \pm SD)
1	Sulcatone	110-93-0	988	988	6.60 ± 1.64
2	Linalool	78-70-6	1102	1102	5.51 ± 0.92
3	Rose furan oxide	92356-06-4	1180	1177	2.25 ± 1.41
4	Neroli aldehyde	106-26-3	1245	1246	28.85 ± 1.94
5	Citral	5392-40-5	1276	1277	36.64 ± 3.18
6	Eugenol	97-53-0	1369	1370	2.19 ± 1.96
7	α -Copaene	3856-25-5	1377	1377	0.65 ± 0.28
8	α -Gurjenene	489-40-7	1412	1412	0.71 ± 0.24
9	Caryophyllene	87-44-5	1422	1422	3.18 ± 0.85
10	β -cis-Bergamotene	13474-59-4	1436	1436	0.47 ± 0.04
11	α -Humulene	6753-98-6	1455	1455	0.73 ± 0.27
12	α -Curcumene	644-30-4	1489	1488	1.86 ± 1.14
13	Valencene	4630-07-3	1497	1496	0.67 ± 0.21
14	γ -Cadinene	39029-41-9	1516	1516	2.46 ± 0.52
15	Caryophyllene oxide	1139-30-6	1586	1586	4.86 ± 0.60
16	Neophytadiene	0-00-0	1840	1840	1.06 ± 0.40
17	1,3-Cyclohexadiene-1-carboxaldehyde	61447-89-0	2111	2113	1.31 ± 0.67

Note. Database retention indices (RIs) for the non-polar column are the closest values to experimental data (a). Relative peak area $> 0.5\%$ (b). Data from NIST, 2023 (c). Data from Pherobase (d). CAS number for each compound (e).

Table 3:

Chemical composition of essential oil from *Chrysopogon zizanioides* roots (n=3)

N°	Component name	CAS No.	RIa (Exp.)	RIa (Lit.)c,d	Area (%)b
1	Unknown compound	106988-87-8	-	-	5.05 ± 1.52
2	trans- β -Ionone	1139-30-6	1486	1486	1.66 ± 0.21
3	Viridiflorene	6-46-21747	1513	1504	0.60 ± 0.17
4	β -Calacorene	50277-34-4	1545	1547	0.63 ± 0.35
5	9,10-dehydroisolongifolene	0-00-0	1555	1558	1.44 ± 0.79

N°	Component name	CAS No.	RIa (Exp.)	RIa (Lit.)c,d	Area (%)b
6	Unknown compound	194607-96-0	-	-	3.58 ± 0.24
7	Spatulenol	6750-60-3	1580	1580	0.57 ± 0.07
8	Caryophyllene oxide	1139-30-6	1585	1586	1.27 ± 0.22
9	Unknown compound	30557-76-7	-	-	3.44 ± 0.10
10	Junenol	472-07-1	1621	1627	1.94 ± 0.73
11	Kongol	5945-72-2	1649	1652	1.63 ± 0.37
12	Velerenol	101628-22-2	1656	1655	7.35 ± 0.47
13	Longiverbenone	64180-68-3	1669	1670	2.13 ± 0.82
14	Khusinol	24268-34-6	1674	1674	2.51 ± 0.33
15	Unknown compound	66512-56-9	-	-	5.59 ± 0.35
16	IPDON	1000189-10-2	1696	1690	2.44 ± 0.17
17	Vetiselinenol	28102-68-3	1724	1723	3.34 ± 0.12
18	Khusimol	16223-63-5	1744	1740	28.52 ± 0.95
19	Isovalencenol	22387-74-2	1788	1793	2.67 ± 0.10
20	Vetivenic acid	16203-25-1	1813	1811	14.01 ± 1.83
21	Nootkatone	4674-50-4	1828	1833	0.83 ± 0.04
22	Vetiverone	15764-04-2	1842	1842	4.28 ± 0.15
23	Palmitic acid	57-10-3	1967	1968	2.40 ± 2.13
24	Phytol	150-86-7	2113	2114	2.10 ± 1.73

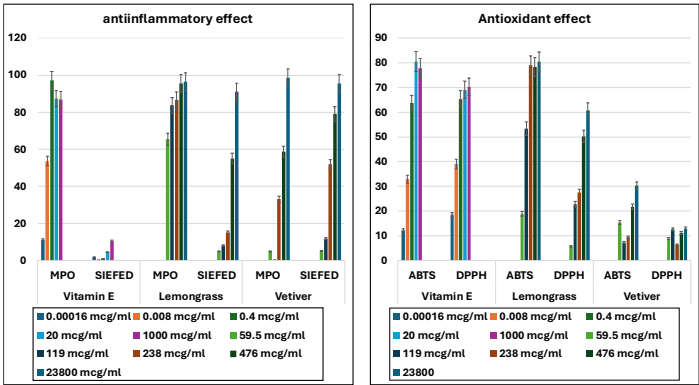
Note. aDatabase RIs shown in this table for the non-polar column are the closest values to experimental data. bRelative peak area greater than 0.5%. cData from NIST 2023. dData from Pherobase. eCAS number for each compound. IPDON: 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-ol.

Biological activities

The percentages of relative activities at different concentrations, as determined by the ABTS and DPPH tests, are shown in Figure 3. The EO of lemongrass exhibited higher antioxidant activity than Vitamin E, which was used as the standard. The antioxidant effect was more pronounced with the ABTS test than with the DPPH test. However, the antioxidant activity of vetiver EO was negligible compared with that of lemongrass and Vitamin E in both tests.

The percentage of relative activities at different concentrations indicated that the MPO assay was more sensitive than the SIEFFED test. At higher concentrations, both lemongrass and vetiver EOs exhibited anti-inflammatory effects comparable to Vitamin E.

Figure 3: Anti-inflammatory and antioxidant activities of lemongrass and vetiver essential oils and Vitamin E.



DISCUSSION

Hydrodistillation of local lemongrass leaves and vetiver roots produced EOs with specific and known characteristics, differing slightly in hue and density, and yielding 0.11% and 1.40%, respectively. These results align with findings from Pandey et al. (2024), Kadarohman et al. (2014), and Yanto et al. (2016). Tchoumboungang et al. (2009) reported a 0.67% EO yield in lemongrass collected in Douala, Cameroon, whereas Miora (2018) recorded a 0.30% yield in vetiver cultivated in Réunion Island. Kanko et al. (2004) found that lemongrass leaves contain between 1.36% and 2.01% EO, indicating that yield is not constant.

According to Djousse et al. (2022), variations in EO yields can be attributed to several factors, including plant origin, drying time, water content before extraction, environmental interactions (climate, soil, isolation duration), fertiliser type, harvest time, and extraction method. Ram et al. (2003) demonstrated that mineral amendments enhance vegetative growth and yield. In Kisangani, the absence of mineral fertilisers may explain the low lemongrass EO yield (0.11%). Additionally, the two plants are genetically distinct.

GC/MS analysis identified 17 components in lemongrass and detected 24 peaks in vetiver, of which four (18%) could not be identified from the available databases. The dominant compounds in lemongrass were citral (36.64%) and neroli aldehyde (28.85%), both belonging to the monoterpene hydrocarbon group. This agrees with Khasanah (2025) and Hartatie et al. (2019), who reported E-citral (synonym of citral), Z-citral (synonym of neroli aldehyde), and β-myrcene as the main constituents of C.

citratus EO. In vetiver roots, khusimol (28.52%) and vetivenic acid (14.01%) predominated among oxygenated sesquiterpenes and ketones, consistent with previous studies.

Bourgou et al. (2012), Golmakani et al. (2015), and Himed et al. (2019) noted that EO composition varies according to extraction method, plant variety, and maturity stage. Among all *C. zizanioides* EOs analysed, khusimol has been consistently identified as the compound with the highest concentration (11–30%) (Oliveira et al., 2022; Pandey et al., 2024; Lunz et al., 2021; Hammam et al., 2019). Champagnat et al. (2006) further observed that the EO compositions of *C. zizanioides* from nine countries (Brazil, China, Haiti, India, Java, Madagascar, Mexico, Réunion, and Salvador) are distinct, with alcohols and acids being particularly significant.

From a pharmacological perspective, both plants were expected to demonstrate antioxidant and anti-inflammatory effects, as illustrated in Figure 3. Compared with the standard (Vitamin E), the potency of EOs from lemongrass and vetiver varied across the five dilutions and inhibitory procedures. Lemongrass EO displayed a strong, dose-dependent antioxidant potential (10–80%) and exhibited antioxidant activity comparable to α -tocopherol. Its effect was more pronounced with the ABTS assay than with DPPH.

Vetiver EO, however, showed negligible antioxidant activity compared with lemongrass and Vitamin E in both assays. The percentage of relative anti-inflammatory potential differed by assay type—MPO or SIEFFED. The activity of α -tocopherol was detectable in the MPO test but almost absent in the SIEFFED assay. At high concentrations, both lemongrass and vetiver EOs exhibited anti-inflammatory effects greater than Vitamin E. Lemongrass EO demonstrated both strong antioxidant and anti-inflammatory properties, while vetiver EO was predominantly anti-inflammatory. The trial was conducted on two separate occasions.

Avosch et al. (2015) demonstrated that citral content is a key determinant of citronella EO quality. Several studies have also confirmed the antioxidant properties of these oils. Kim et al. (2005) showed that EOs possess free radical scavenging activity comparable to standard antioxidants

such as butylated hydroxytoluene (BHT) and α -tocopherol. At 10 $\mu\text{g/mL}$, the EO dissolved in methanol exhibited significant DPPH free radical scavenging activity. Zahoor et al. (2018) reported that ethanolic extracts of *C. zizanioides* root and *C. citratus* leaf exhibit a variety of antioxidant activities, including oxidative reduction and superoxide radical scavenging.

Subhadradevi et al. (2020) found that both species scavenge free radicals and mitigate oxidative stress, acting as natural antioxidant sources. Samaan et al. (2022) observed overall antioxidant capacities of 75% at 0.1 mg/mL. Figueirinha et al. (2010) confirmed that *C. citratus* and *C. zizanioides* are globally recognised for such properties, though their precise anti-inflammatory mechanisms remain unclear. Vera et al. (2013) demonstrated that these EOs possess nitric oxide (NO) scavenging activity—important since NO, produced by activated inflammatory cells, plays a key role in acute and chronic inflammation.

Citral, one of the active compounds, has been identified as a potential anti-obesity and antidiabetic agent (Hasani-Ranjbar et al., 2009). However, despite its low acute toxicity, citral is an irritant and known skin sensitiser. Studies have shown that it can induce DNA damage in human cell cultures and developmental toxicity in animals at high doses (Anna Souza et al., 2020). Therefore, citral use should be approached cautiously due to its potential genotoxicity, particularly after metabolism by hepatic enzymes.

CONCLUSION

This study confirmed that lemongrass and vetiver species cultivated in Kisangani contain bioactive components in sufficient quantities and possess distinct chemical profiles that confer commercial value, comparable to those of species marketed globally. Further cultivation and extraction studies are recommended to enhance EO yield and to evaluate their therapeutic potential in inflammatory diseases, with particular attention to toxicity patterns.

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Conflicts of Interest: None declared.

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