

Research Article

Zonal Effect on the Essential Oil Profile from *Cymbopogon citratus* (Lemongrass) Leaves and *Citrus sinensis* (Orange) Peels harvesting in Mali

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Abstract

Nowadays, *Cymbopogon citratus* (DC.) Stapf (Poaceae), (Lemongrass) and *Citrus sinensis* (L.) Osbeck (Rutaceae), (orange) are very coveted for aromatic, food and medicinal purposes. Lemongrass leaves and orange zests are reputed to be rich in essential oils, which are highly prized by the food industry. The aim of this study was to determine the essential oil (EOs) extraction yields and their profile of these species collected in three agro-ecological zones of Mali. EOs were extracted by steam distillation and their profile was determined by thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS). All registered data revealed zonal variation of extraction yields as well as in EO components (p -value < 0.05). Extraction yields varied from 0.32 ± 0.02 to $0.44 \pm 0.03\%$ for lemongrass and 0.32 ± 0.01 to 0.50 ± 0.03 for orange. These yields were higher in Sahelian zones for both species. Moreover, chromatography technique highlighted an important diversity of lemongrass and orange peel in individual EO components. Thus, Retention factors values recorded from TLC allowed to detect a maximum of different EO individuals: 15 for lemongrass and 13 for orange, mainly in Sahelian. Similarly with GC technique, more EO elements were found: a total of 23 for orange zests samples and 17 for lemongrass. Samples collected in Sudano-guinean sites presented the highest number of total individual EO constituents, 16 for orange and 12 for lemongrass. The major individual EO components were Limonene ($43.14 \pm 0.20\%$), Neral ($36.00 \pm 0.14\%$), and M-Camphorene ($19.88 \pm 0.17\%$) for leaves of lemongrass, and carvone ($19.59 \pm 0.58\%$), Citral ($17.99 \pm 0.01\%$), and Neral ($17.48 \pm 0.10\%$) for orange zests. This richness and diversity could be useful to better valorization of lemongrass leaves and orange peel in Mali.

Keywords

Cymbopogon citratus, *Citrus sinensis*, Essential Oils, Yield, Composition, Zones, Mali

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1. Introduction

Cymbopogon citratus (DC.) Stapf (Poaceae), (Lemongrass) commonly known as Indian verbena or lemongrass, is a member of the Poaceae (grass) family. As a herbaceous plant with long, blue-green leaves, it is found in tropical and semi-tropical regions of Asia, South and Central America and Africa, as well as in all tropical countries [1, 2]. *C. citratus* leaves are the most coveted part [1] used as an infusion, decoction or maceration due their nutritional and therapeutics needs. Recipes made from Lemongrass leaves are reputed to manage number diseases such as coughs, elephantiasis, flu, gingivitis, headaches, stomachaches, leprosy, malaria, ophthalmic, pneumonia, and metabolic disorders [3]. In addition, species is also used as a natural cleaner that aids in many organs' detoxification of human being (liver, pancreas, kidneys, bladder, and digestive tract). Its ability to decrease the levels of uric acid, cholesterol, blood pressure, excess lipids, and other toxins in the human body was reported [3].

Citrus sinensis (L.) Osbeck (Rutaceae) or sweet orange belonging to the Rutaceae family, is widely cultivated in tropical and subtropical zones [4]. The fruit of citrus of very prized in addition to its juice, while peels removed from the fruit are discarded in the environment. The literature estimates that each year, about 50% of orange residues (peel) are being generated from the citrus-processing industries [5], which are either used as animal feed. Most part of these peels (zests) are improperly disposed in the nature, and consequently may cause environmental pollution [5].

Studies reported the richness of Lemongrass leaves and orange zests in essential oils (EOs), which can be a major pool of different food-value added products. Because EOs are highly coveted due their involvement in the treatment of many diseases and to their aromatic and nutritional purposes. For instance, EOs are endowed with antibacterial, antiparasitic, antifungal, anti-insecticidal properties [2, 3, 6].

EOs with insecticidal properties have been used to kill insects all over the world since ancient times. Due to their low

toxicity to warm-blooded mammals and to their high volatility, these plant components are considered to be an alternative to conventional pesticides [6]. In addition, they are found to be safer for the environment [7] than synthetic insecticides with many side effects [8, 9]. Furthermore, Thanks to their safety category and exemption from mammalian toxicity by the United States Food and Drug Administration, EOs constituents open a novel eco-friendly approach toward food protection [7, 10]. Despite these advantages of the EOs contained in lemongrass leaves and orange zests, these two species remain under-investigated in Mali. This study was undertaken to fill this gap through the determination of EO profiling according to the ecological area in Mali. Results could enable to select the best ecological zones for harvesting and in order to select the EOs of the best quality for production of natural preservatives in Mali.

2. Materials and Methods

2.1. Zones of Sampling

Mali, a West African country with a surface area of 1,246,814 km², lies between the 10th and 25th parallels of northern latitude and between longitudes 4° East and 12° West.

Orange peel and lemongrass leaf samples were collected from three agro-ecological zones in Mali, located between parallels 10° and 15° north latitude and meridians 5° and 10° west longitude. The Sahelian, Sudanian, and Sudano-Guinean zones were represented by the communes of Banamba, the district of Bamako, and the communes of Bougouni, respectively (Figure 1). These sites were chosen for their high consumption of orange and lemongrass, as well as their abundance, availability and accessibility.

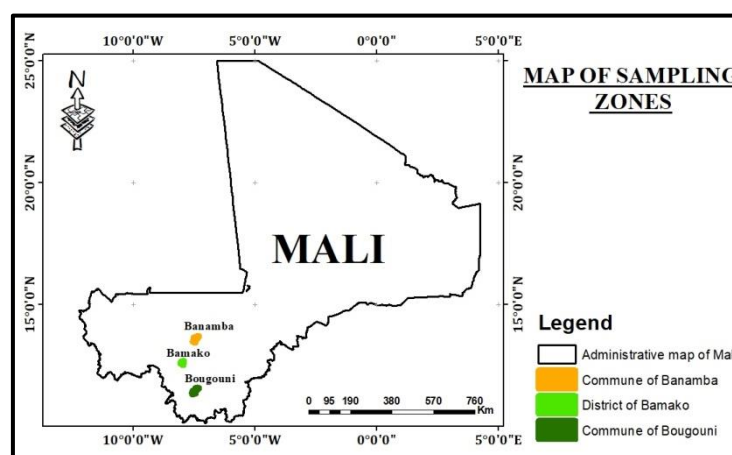


Figure 1. Map of Mali showing the zones of sample collection.

2.2. Sampling Collection

Samples of orange peels and lemongrass leaves were collected from gardens and orchards in three agro-ecological zones in Mali. The Sahelian zone was represented by the Commune of Banamba, the Sudano-Guinean zone by Bougouni and the Sudanian zone by the district of Bamako (Figure 1). Freshly collected samples were packed in perforated plastic bags. After identification and authentication by “Laboratory of Tropical Ecology (LET)” at the University of Sciences, Techniques and Technologies of Bamako (USTTB), samples were carefully washed. Lastly, they were cut into smaller pieces using a stainless-steel knife to facilitate the diffusion of the essential oil through water vapor during extraction process.

2.3. Methods

2.3.1. Extraction of Essential Oils

Steam distillation (SD) has been regarded as the most common method applied for the extraction of essential oils, due to its simplicity and low investment cost [11].

A quantity of 1 kg of samples, freshly cut, were introduced into a flask of the “Alambic” extractor (Aura, Inox 304 - SPR-B, Troyes, France) containing 4 L of distilled water (report 1:4; m/v). The flask was heated using a gas canister to a temperature of 95 °C. When boiling, water vapors carried the essential oils through a stainless-steel coil immersed in a refrigerant. After 90 minutes, the essential oil-water vapor mixture (distillate) was collected. After condensation, the distillate was transferred into a decanting ampoule to separate essential oil from water.

Extraction yield was calculated using the following equation.

$$\text{Yield (\%)} = \frac{\text{Essential oil extracted (g)}}{\text{Plant material (g)}} \times 100$$

2.3.2. Screening of Essential Oils by TLM

Essential oils (EO) were characterized by thin-layer chromatography (TLC). A 0.25 mm thick of aluminum-backed silica gel plate (dimension 5 x 10 cm) was used. The EO solution was prepared by dissolving 100 µL in 500 µL of absolute ethanol. Ten (10) µL of EO diluted was dispensed onto the TLC plate using a micropipette. After drying the samples on plate at room temperature (25 - 30 °C), the plate was carefully placed in a migration glass tank containing the eluent, a mixture of Toluene - ethyl acetate (95-5; v/v). After approximately 2 h of elution, the plate removed from the glass tank was dried at 90 °C for 10 min to completely remove the solvent. Revelation was carried out using Vanillin Reagent (1 g vanillin dissolved in 100 mL of 90% ethanol, then acidified with 2 mL of concentrated sulfuric acid). After heating at 90 °C for 5 min, separated compounds (spots) were visualized using a UV light

(254 nm and 365 nm). The retention factor or frontal ratio (Rf) of each spot was calculated using the following equation. Following, the Retention Factor (Rf) for each spot was calculated as the ratio of the eluted distance (mm) for a spot on TLC plate relative to the total distance (mm) that the solvent front travelled [12].

$$\text{Retention Factor} = \frac{\text{Distance travelled (cm)}}{\text{Total distance (cm)}}$$

2.3.3. Essential Oils Identification and Dosage by GC-MS

Essential oils (EOs) from orange and lemongrass were identified and quantified by Gas Chromatography-Mass Spectrometry (GC-MS) method described by Chegini & Abbasipou [6] and Owen et al. [12]. An Agilent 7890A series G-C MS chromatograph, equipped with an automatic injector and an apolar (HP-5) column (60 m x 0.22 mm i.d. film thickness: 0.25 µm), coupled to an Agilent Turbo Mass detector. A volume of 1 µL of EO sample (EO diluted in hexane; v/v) was injected into the GC in splitless mode with an injection temperature of 280 °C. The carrier gas was helium (1 mL/min) with a column head pressure of 25 psi. The injector temperature was 250 °C. The furnace temperature was programmed as follows: Initial temperature of 70 °C followed by an increase of 25 °C per minute to 150 °C, then an increase of 5 °C per minute to 200 °C and a final increase of 10 °C per minute until 280 °C was reached. This temperature was maintained for 15 minutes. The ionization energy was 70 eV with a mass range of 35–350 Da, while the source temperature was 150 °C.

Resulting EOs from analyzed samples were identified by comparing their GC retention times to those obtained from known standard reference samples of EO components, either with known compounds or published spectra in the literature. After identification, the EO components (Sigma Aldrich) were quantified by external calibration curves based on the peak area.

2.4. Statistical Analysis

All analyses were performed in triplicate and the values were represented as the means ± standard deviation (SD). One-way ANOVA was preceded by checking the normality followed by the Fischer test ($p < 0.05$) to compare the means using Minitab 18.1 software. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Extraction Yields

After extracting essentials by steam distillation technique, the registered yields are depicted in the Table 1.

Table 1. Extraction yields of essentials oils (%).

Zones of harvesting	Leaves of lemongrass	Peels of orange
Sahelian	0.44±0.03 ^a	0.50±0.03 ^a
Sudanian	0.33±0.03 ^b	0.32±0.01 ^c
Sudano-Guinean	0.32±0.02 ^b	0.43±0.01 ^b
p-value	0.002	0.00004
F-value	21.00	82.33

* For each sample, the averages that do not share any letters are significantly different.

For each sample, EO extraction yields varied from one site

to another (p-value < 0.05). The highest amount of EO were registered in Sahelian zones for both species, i.e., 0.44±0.03% for leaves of lemongrass and 0.50±0.03% for orange zests.

3.2. EO Components Profile with TLC Method

Characterization of essential oils profiling from lemongrass leaves and orange peels were assessed by TLC and GC-MS. The values of Retention Factor values are summarized in Table 2.

Retention factors values recorded from TLC allowed to detect several different EO individuals in both samples: from 10 to 15 for lemongrass and from 12 to 13 for orange. Leaves of lemongrass samples from Sahelian zone show higher EO constituents (15). As for orange peels samples, both Sahelian and Sudano-guinean presented the maximum EO components (13).

Table 2. Retention factor values from Thin Layer Chromatography (TLC).

N°spot	Essential oils from lemongrass leaves			Essential oils from orange zests		
	Sahelian	Sudanian	Sudano-guinean	Sahelian	Sudanian	Sudano-guinean
1	0.04	0.03	0.03	0.03	0.03	0.04
2	0.11	0.11	0.21	0.04	0.05	0.16
3	0.13	0.24	0.25	0.16	0.13	0.18
4	0.25	0.25	0.31	0.21	0.19	0.19
5	0.38	0.31	0.41	0.31	0.31	0.33
6	0.50	0.50	0.50	0.43	0.44	0.48
7	0.56	0.53	0.69	0.50	0.45	0.54
8	0.60	0.69	0.73	0.64	0.50	0.60
9	0.69	0.75	0.79	0.65	0.53	0.66
10	0.75	0.93	0.94	0.69	0.60	0.69
11	0.79	0.94		0.79	0.63	0.73
12	0.85			0.85	0.88	0.85
13	0.91			0.88		0.91
14	0.93					
15	0.98					
Total of components	15	11	10	13	12	13

3.3. EO Components Profile with GC-MS Method

Individual EO compounds identified by GC were counted and summarized in the Figures 2 & 3. Their concentrations expressed in percentages (%) are showed in Tables 3 & 4 for lemongrass leaves and orange zests, respectively.

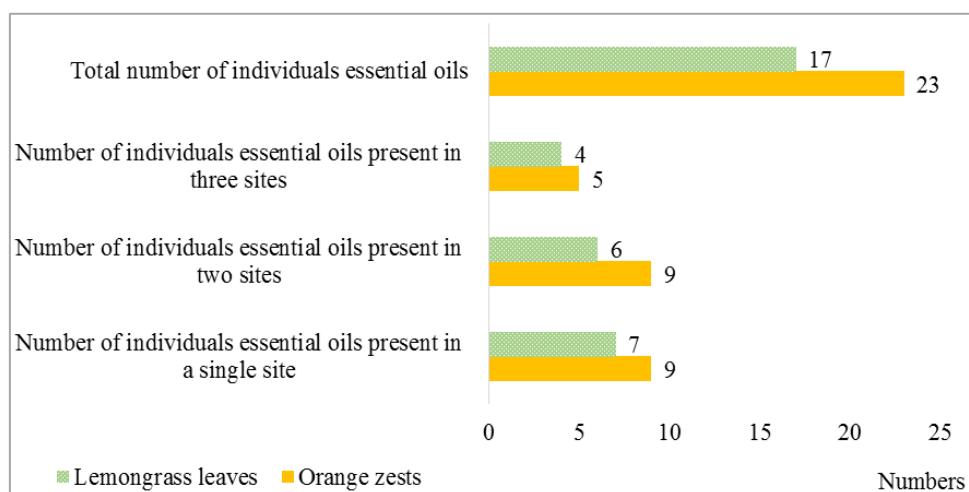


Figure 2. Number of individuals essential oils per site and samples.

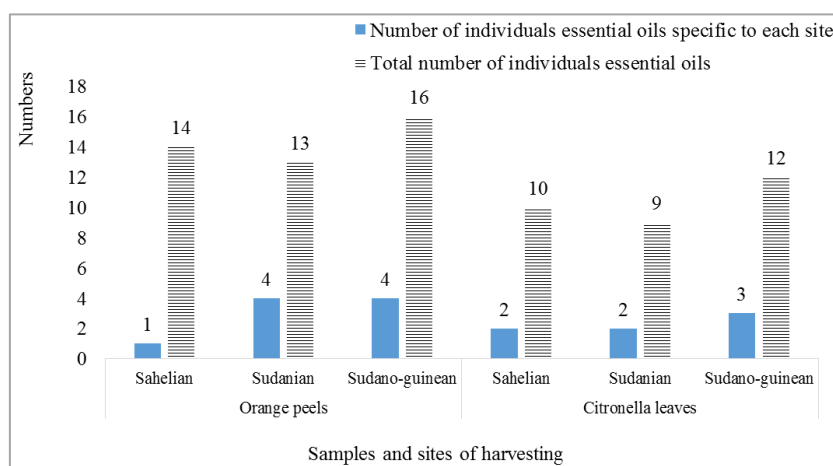


Figure 3. Repartition of individuals essential oils per site and samples.

Table 3. Levels of individual essential oils from lemongrass leaves (%).

EO components	Zones of sample collection		
	Sahelian	Sudanian	Sudano-guinean
Ageratriol	1.31 ±0.01		
B bisabolene		9.26 ±0.06	10.46 ±0.06
Caryophyllene	12.43 ±0.07		7.58 ±0.17
Caryophyllene oxide		7.52 ±0.31	8.88 ±0.17
Citral			2.78 ±0.03
Citronellol			3.74 ±0.08
Farnesol	11.26 ±0.06		5.56 ±0.08
Geraniol	12.15 ±0.07		2.77 ±0.04
Geranyle geraniol	1.59 ±0.06	0.91 ±0.088	1.26 ±0.06
Humulenol	10.03 ±0.04	2.09 ±0.01	2.49 ±0.01
Ledol		10.19 ±0.04	

EO components	Zones of sample collection		
	Sahelian	Sudanian	Sudano-guinean
Levomenol	11.32±0.11	1.94±0.08	6.34±0.08
Limonene			43.14±0.20
M-camphorene	19.88±0.17	17.05±0.07	
Neral	17.12±0.11	36.00±0.14	4.94±0.06
Nerolidol	2.85±0.07		
Trans farnesol		8.27±0.04	
Total of components	10	9	12

Table 4. Levels of individual essential oils from orange peels (%).

EO components	Zones of sample collection		
	Sahelian	Sudanian	Sudano-guinean
Aromandendrene		10.86±0.17	
A-terpineol		3.52±0.03	11.17±0.10
Carene		4.90±0.07	
Carveol		5.18±0.03	
Carvone	5.04±0.08		19.59±0.58
Caryophyllene	4.21±0.07		3.47±0.04
Caryophyllene oxide	2.46±0.03		2.02±0.03
Citral	16.50±0.00	17.99±0.01	12.65±0.21
Citronellal	9.94±0.34	11.58±0.10	6.08±0.03
Citronellol	7.96±0.06	4.01±0.03	3.03±0.04
Copaene	6.78±0.31		5.34±0.20
Farnesene	3.04±0.06	3.29±0.04	2.58±0.03
Farnesol			2.72±0.03
Geraniol		5.18±0.03	
Humulenol	0.71±0.01	1.21±0.03	
Ledol			1.40±0.07
Limonene	9.16±0.23	6.71±0.13	3.10±0.07
Limonenol	5.69±0.16		0.75±0.01
Linalol			9.50±0.04
Neral	3.24±0.23	17.48±0.10	
Nerolidol	5.31±0.13		
Terpineol	12.22±0.06	8.03±0.03	5.75±0.07
Verbenol			10.76±0.34
Total of components	14	13	16

Regardless the sampling site, the gas chromatography shows a total of 23 individual EO constituents for orange zests samples and 17 for lemongrass leaves samples (Figure 2). Figure 3 reveals that samples from sudano-guinean area presented the highest number of total individual EO constituents, 16 for orange and 12 for lemongrass. Four EO molecules for lemongrass Geranyle geraniol, Humulenol, Levomenol, and Neral (Table 3) were commonly found in all three collection sites against five EO molecules for orange (Citral, Citronellal, Citronellol, Farnesene, and Limonene) (Table 4). The number of site-specific EO molecules was higher in the Sudano-guinean site with 3 EO for lemongrass and in both Sudanian and Sudano-guinean with 4 EO (Figure 3). Lesser number of site-specific EO was registered from Sahelian areas for both lemongrass samples (Ageratriol at $1.31 \pm 0.01\%$ and Nerolidol at $2.85 \pm 0.07\%$) (Table 3) and orange samples (Nerolidol with a concentration of $5.31 \pm 0.13\%$) (Table 4). Analyses show that the Sahelian areas had the highest levels of individual EO common to all three sites. For instance, the highest contents of Humulenol ($10.03 \pm 0.04\%$), Levomenol ($11.32 \pm 0.11\%$) and M-camphorene ($19.88 \pm 0.17\%$) were registered from leaves of lemongrass (Table 3). As for peels of orange, these EO components were Terpineol with $12.22 \pm 0.06\%$, Citronellol with $7.96 \pm 0.06\%$, and Limonene with $5.69 \pm 0.16\%$ (Table 4).

4. Discussion

This current work performed the EO profile of lemongrass leaves and orange peels harvested from different agroecological areas in Mali. It is a part of a project focused on the valorization of local and neglected resources in Mali.

Data obtained from EO extraction demonstrated the abundance of these components in lemongrass and orange. Statistical test revealed that extraction yields were zone-dependent (p -value < 0.05) and varied from 0.32 to 0.50% for analyzed samples. Sahelian areas presented the highest extraction yields, which were $0.44 \pm 0.03\%$ and $0.50 \pm 0.03\%$ for lemongrass leaves and orange peels, respectively.

Higher EO extraction yields of lemongrass leaves were reported by Ameh et al. [13] using effleurage method (1.96%) and the hydro-distillation method (0.95%). As for orange peel, the yields obtained in this study were higher than those reported in literature: 4.23% with an improved distillation method [14]; from 0.92 to 2.73% using hydro-distillation technique [15]; 1.16% using microwave assisted hydrodistillation. Similar extraction yields were obtained by Kamal et al. [16] who reported that *C. sinensis* had the highest oil value yield of 0.24–1.07%.

All these values recorded are lower to the expected yield mentioned in the literature, which found steam distillation yields about 3–4% aromatic compounds [17]. It is well known that EO extraction yields can vary according to harvesting periods, state of ripeness, climate factors, extraction method and the quality of the equipment used [11, 17, 18].

Chromatography methods enabled to identify many categories of EO components in the investigated samples. Gas chromatography (GC) technique detected more total number of different individual EO constituents in lemongrass (17) and orange (23) comparatively to TCL technique with 15 and 13 EO, respectively for lemongrass and orange. This difference could be linked to the high performance of GC technique, which is more accurate. Globally, it appears that Sahelian areas were richer in total number of individual EO constituents, regardless the samples. Lemongrass zests recorded individual EO constituents' total number of 16 and 12 for orange peels and lemongrass leaves, respectively. In terms of individual EO diversity, Sudanian zones led with 16 and 12 for orange peels and lemongrass leaves, respectively.

GC quantification revealed that for most of the EO molecules common to the three collection zones, the highest concentrations were recorded in the Sahelian zone. These EO components were Humulenol with $10.03 \pm 0.04\%$, Levomenol with $11.32 \pm 0.11\%$ and M-camphorene with $19.88 \pm 0.17\%$ from leaves of lemongrass. Orange peels highlighted were $12.22 \pm 0.06\%$ of Terpineol, $7.96 \pm 0.06\%$ of Citronellol, and $5.69 \pm 0.16\%$ of Limonene. This richness could be explained by the low moisture content in Sahelian zones, where rainfall is low (250 to 550 mm) compared to the other two zones (550 to 1100 mm) [19]. These levels are in line with the high EO extraction yields registered in the samples collected in Sahelian areas for both species. They also corroborate literature findings which estimated that compound concentrations are generally influenced by moisture content in plant material [11]. For a better exploitation of EO of these species, samples from this area could be very profitable.

In addition, the three most important individual EO components with higher concentrations regardless the zones were Limonene ($43.14 \pm 0.20\%$), Neral ($36.00 \pm 0.14\%$), and M-camphorene ($19.88 \pm 0.17\%$) for lemongrass; carvone ($19.59 \pm 0.58\%$), Citral ($17.99 \pm 0.01\%$), and Neral ($17.48 \pm 0.10\%$) for orange zests. The composition of the essential oils of *C. citratus* determined by GC and GC/MS were found to contain 19 and 27 constituents for samples collected in Mali and Ivory Coast, respectively [20]. The same authors mentioned that leaves of *C. citratus* oil harvested in Mali presented a high proportion of citral (75%), (geraniol/neral ca 2/1), some myrcene (6.2–9.1%) and geraniol (3.0–5.6%). For orange peel, the same EO constituents were observed by Olabinjo & Oliveira [17] using GC-MS who found limonene as the predominant individual components. A study working on several EO antimicrobial sensitivity test revealed that limonene showed the most significant antibacterial effect in the growth and reproduction of *S. aureus* [21].

The composition of essential oils is generally influenced by numerous parameters. These are either natural, of internal origin (genetics, location, maturity), external (soil, climate conditions, etc.) or technological process [11, 20]. Likewise, ecological factors have been incriminated to influence the EO composition: geographical factors (altitude, latitude, etc.), soil

type, climate (sunshine, temperature, rainfall, etc.) are all parameters responsible for variations [13, 17, 20]. Other factors such as the use of fertilizer and the presence of rhizosphere fungi in the cultivation soil have also been reported to enhance the EO levels, especially citral content [22].

Nowadays, food industries are facing a great challenge linked to the contamination of food products with different microbes such as bacteria, fungi, viruses, and other parasites. In several developing countries, 25–30% loss of foods and foodborne diseases caused by microbial contamination have been a major concern [10]. This scourge pushes scientific community to find natural effective and affordable agents to manage it. In this sense, plant-based preservatives, especially EOs and their active components extracted from lemongrass and orange, are gaining cumulative attention in the food industries having wide-spectrum antibacterial, antifungal, antimycotoxicogenic, and antioxidants potentials [2, 10, 21]. Work also reported the utilization of EOs as the best alternatives to chemical pesticides because of their lower toxicity on the non-target and low persistence in the environment [6].

Based on these benefits of EOs, the leaves of lemongrass and peels of orange could be helpful in the contribution of food security and fighting foodborne diseases due their richness in various bioactive EO agents.

5. Conclusion

This work established the composition of essential oils (EOs) in lemongrass leaves and orange zests samples harvested from three agroecological areas in Mali.

Higher extraction yields were obtained from samples collected in Sahelian zones, while those harvested from Sudano-guinean sites showed the highest number of total individual EO constituents for each species. An important profile diversity of EO components were observed in all samples. Therefore, extraction yields and EO profile were agroecological site-dependents. These data showed that lemongrass leaves and orange zests are potential sources EO constituents.

This richness and diversity are important for choosing the area for collecting samples that will be used for the production of natural preservatives from lemongrass leaves and orange peel in Mali.

Abbreviations

CNRST	National Center for Scientific and Technological Research
EOs	Essential Oils
EO	Essential Oil
FAPH	Faculty of Pharmacy
FCRIT	Competitive Fund for Research and Technological Innovation
FST	Faculty of Sciences and Techniques
GC-MS	Gaz Chromatography-Mass Spectrometry

INRMPT	National Research Institute of Traditional Medicine and Pharmacopoeia
ISA	Institute of Applied Sciences
LET	Laboratory of Tropical Ecology
SD	Steam Distillation
TLC	Thin Layer Chromatography
U.PRO.CO.HE	Essential Oil Production and Marketing Unit
USTTB	University of Sciences Techniques and Technologies
UV	Ultra-Violet

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Author Contributions

Brahima Coulibaly: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing

Cheickna Daou: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing

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Fassé Samaké: Investigation, Supervision, Writing – original draft

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Conflicts of Interest

The authors declare no conflicts of interest.

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