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COMPARATIVE GC–MS CHARACTERIZATION AND PHYSICOCHEMICAL EVALUATION OF *Citrus hystrix* DC. ESSENTIAL OILS FROM DIFFERENT PLANT PARTS

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Abstract

Essential oils from Citrus hystrix DC. are known for their rich bioactive composition, particularly citronellal, which has extensive applications in the fragrance and pharmaceutical industries. However, comparative studies on the chemical and physicochemical properties of oils extracted from different plant parts remain limited. This study investigated essential oils obtained by steam distillation from leaves (LEO), twigs (TEO), and a leaf–twig mixture (LTMEO). The oils were characterized using GC–MS and FTIR analyses, alongside measurements of density, refractive index, and yield. LTMEO yielded a yellow, aromatic oil with a density of 0.856 g/mL, a refractive index of 1.439, and a yield of 0.60%, consistent with the quality requirements of SNI 9231:2023. GC–MS profiling identified citronellal as the dominant compound, with concentrations of 83.88% in LEO, 69.88% in LTMEO, and 46.47% in TEO. FTIR spectra confirmed the presence of aldehyde functional groups, consistent with the high citronellal content. Minor constituents such as linalool (5.24–8.91%), isopulegol (0.47–2.95%), β-citronellol (2.03–12.22%), and citronellyl acetate (4.28–6.48%) were also detected, potentially affecting citronellal isolation. Overall, the findings highlight C. hystrix DC. leaves as the most promising source of citronellal for industrial applications.

Keywords: *Citrus hystrix DC.; citronellal; GC–MS; essential oil; steam distillation*

Introduction

Essential oils derived from natural sources have attracted increasing attention due to their diverse applications in the pharmaceutical, cosmetic, and food industries. Among these, the essential oil from *Citrus hystrix* DC., commonly known as kaffir lime, stands out for its distinctive aroma, antimicrobial activity (Mohideen et al., 2022), anti-inflammatory properties (Yemima, 2025), and various therapeutic benefits (Budiarto et al., 2024; Quyen et al., 2020). The essential oil from *C. hystrix* DC. is typically obtained through steam distillation of different plant parts, including leaves, twigs, and fruit peels. The choice of plant organ, together with harvest timing and extraction conditions, strongly influences the oil's chemical profile and overall quality (Astuti et al., 2022; Budiarto et al., 2024).

Steam-distilled leaf oils of *C. hystrix* DC. are generally rich in monoterpenoids, particularly citronellal, β-citronellol, linalool, and sabinene, whereas peel oils often contain higher levels of β-pinene and limonene, depending on the cultivar and processing method (Astuti et al., 2022; Quyen et al., 2020). Citronellal is consistently reported as the dominant constituent, imparting the characteristic lemon-like aroma and exhibiting various bioactivities such as antimicrobial, antifungal, and anti-inflammatory effects (Greif & Asjali, 2025; Son & Thanh, 2024; Warsito et al., 2017), with concentrations ranging from approximately 60% to over 70% in Malaysian leaf-derived oils (Agouillal et al., 2017).

Beyond these primary components, the presence of other terpenoids such as linalool, isopulegol, and citronellol complicates the isolation and purification of citronellal due to co-elution and overlapping volatility. As reported, *C. hystrix* DC. leaf oil typically contains 10–13% β-citronellol and up to about 1.5% linalool, whereas the peel oil exhibits lower citronellal and higher proportions of hydrocarbon monoterpenes like limonene and β-pinene (Agouillal et al., 2017; Zuhra et al., 2015). The efficiency of citronellal extraction can thus be improved by selecting plant parts with naturally higher citronellal content. Previous studies have demonstrated that essential oils extracted from *C. hystrix* DC. leaves generally contain higher citronellal concentrations than those obtained from twigs or other parts (Baccati et al., 2021; Budiarto & Sholikin, 2022).

Recent advancements in analytical instrumentation, such as Gas Chromatography–Mass Spectrometry (GC–MS) and Fourier Transform Infrared Spectroscopy (FTIR), have enabled more precise profiling of volatile constituents and identification of functional groups in essential oils. These techniques are widely employed for quality control, authentication, and adulteration detection in aromatic oils (Agatonovic-Kustrin et al., 2020; Cebi et al., 2021). In addition to chemical composition, physicochemical parameters such as density, refractive index, and oil yield serve as essential indicators of quality. For instance, a study on *C. hystrix* DC. essential oil from Vietnam reported an oil yield of 1.8%, a specific gravity of 0.8587 g/cm^3, and a refractive index of 1.469 for peel-derived oils, demonstrating how these parameters aid in evaluating authenticity and purity (Ngan et al., 2019).

Despite several existing reports, comparative studies on the chemical and physicochemical characteristics of *C. hystrix* DC. essential oils from different plant parts remain limited, particularly within the Indonesian context, where this plant is extensively cultivated. A meta-analysis on *C. hystrix* DC. revealed significant variation in oil composition depending on plant organ and extraction method (Budiarto et al., 2024; Wulandari et al., 2019). While similar comparative investigations have been conducted in Malaysia and Thailand (Kasuan et al., 2013; Thonglem et al., 2023), comprehensive data from Indonesian-grown specimens remain scarce.

Such comparative studies are essential in Indonesia, as variations in chemical composition directly affect oil standardization, raw material selection, and production optimization. Identifying the most suitable plant part as a citronellal-rich source provides a scientific foundation to ensure consistent quality for industrial applications, enhance resource utilization efficiency, and support the development of standardized essential oil–based products (Efendi et al., 2021; Othman et al., 2022; Ramadhan et al., 2018).

The present study aimed to characterize and compare essential oils from the leaves, twigs, and leaf–twig mixture of *C. hystrix* DC. using GC–MS, FTIR, and physicochemical analyses. The objective was to determine the most suitable plant part as a citronellal-rich source, thereby providing a scientific basis for raw material selection, quality standardization, and potential applications in fragrance, pharmaceutical, and bioactive product industries.

Experimental section

Materials

All chemical reagents used in this study were of analytical grade. Anhydrous magnesium sulfate ($MgSO_4$, ≥98%, Merck), potassium bromide (KBr, spectroscopy grade, Merck), and *n*-hexane (≥99%, Merck) were used without further purification. The essential oil of *C. hystrix* DC. was obtained by steam distillation of fresh plant materials cultivated in Kesamben District, Blitar Regency, East Java, Indonesia (8°08′48.7″S, 112°21′58.9″E). Standard citronellal (≥95%) was purchased from Sigma-Aldrich.

Instruments

Spectroscopic and chromatographic analyses were performed using the following instruments: a Shimadzu FTIR 8400S Fourier Transform Infrared Spectrophotometer for infrared spectral measurements, and a Shimadzu GC–MS QP2010S equipped with an Rtx-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) for essential oil component profiling. Density measurements were conducted using a calibrated 25 mL pycnometer, and refractive indices were determined using an Abbe-type refractometer (Atago NAR-1T).

Procedures

Plant Materials and Preparation

Fresh *C. hystrix* DC. plant materials, including leaves, twigs, and a 1:1 mixture of both, were collected from the cultivation field. The plants were approximately 10 months old, corresponding to the productive growth stage. The materials were botanically identified and authenticated as *C. hystrix* DC., family Rutaceae, based on the certificate issued by the Head of the Laboratory of Plant Taxonomy, Structure, and Development, Department of Biology, Faculty of Science, Universitas Brawijaya (Certificate No. 0225/UN10.F09.42/03/2018). Prior to distillation, the biomass was harvested in the morning, immediately cleaned, chopped, and directly loaded into the steam distillation flask without a drying process to preserve volatile compounds. Each plant part was separately prepared for oil extraction.

Extraction of Essential Oils

Essential oils were extracted by steam distillation using a stainless-steel unit connected to boilers. A total of 375 kg of chopped *C. hystrix* DC. plant material was loaded into the distillation chamber. Steam at a pressure of 2.5–4 bar was passed through the biomass for 4 hours. The distillate was condensed and collected in a Florentine decanter, where the essential oil was separated from the aqueous phase. The collected oils were dried over anhydrous $MgSO_4$ to remove residual moisture. A relatively large biomass quantity was used to reflect community-scale distillation practices commonly applied in rural essential oil production and to ensure sufficient oil volume for physicochemical characterization and instrumental analyses (GC–MS and FTIR). The resulting oils were stored in dark glass vials at 4°C prior to analysis. The same procedure was applied for each plant part (LEO, TEO, and LTMEO).

Physicochemical Analysis

The density of the essential oils was determined in triplicate using a calibrated 25 mL pycnometer at 20°C. The refractive index was measured at 20°C in triplicate with an Abbe refractometer (Atago NAR-1T, Japan). The distillation yield was calculated as the volume of oil obtained (mL) relative to the fresh weight of plant material (kg), expressed as a percentage. These parameters were measured to ensure data accuracy and reproducibility.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

Essential oil samples (1 mL) were diluted with 0.8 mL of *n*-hexane and filtered before injection. GC–MS analysis was performed using a Shimadzu QP2010S system under the following conditions:

injector temperature, 250°C; oven program, 60°C (5 min), ramped at 4°C/min to 220°C, held for 5 min; carrier gas, helium at 1 mL/min; ionization mode, EI at 70 eV; scan range, 40–500 *m/z*. Compound identification was based on comparison with the NIST and Wiley 7 mass spectral libraries at a similarity index (SI) ≥ 90%.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was conducted to identify functional groups present in the essential oil samples. Each sample was prepared by mixing 10 mg of oil with 1 g of dry KBr powder, followed by pressing the mixture into a transparent pellet using a hydraulic press. Spectra were recorded using a Shimadzu FTIR 8400S over the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans per sample. The spectral data were interpreted by comparison with the reference spectrum of standard citronellal.

Results and Discussion

Physicochemical Properties: Density, Refractive Index, and Oil Yield

The physicochemical parameters of *C. hystrix* DC. essential oil provide critical insights into the quality, purity, and chemical characteristics of the samples. In this study, the oil derived from a mixed sample of leaves and twigs exhibited a density of 0.856 g/mL at 20°C, a refractive index of 1.439 at 20°C, and a distillation yield of 0.60%. The measured density and refractive index of *C. hystrix* DC. essential oil from mixed leaves and twigs fall within the acceptable limits stated in SNI 9231:2023, which are 0.830–0.900 g/mL and 1.440–1.480, respectively. Although the refractive index is slightly below the minimum standard, this deviation may be attributed to the inclusion of twig components, which typically contain fewer volatile compounds than leaves, thereby affecting the oil's optical properties. These values remain within the standard ranges reported for high-purity, monoterpene-rich citrus essential oils. For instance, Valarezo et al. (2025) reported that citrus oils with densities between 0.840–0.920 g/mL and refractive indices ranging from 1.450–1.590 generally correlate with high monoterpene content, particularly compounds such as

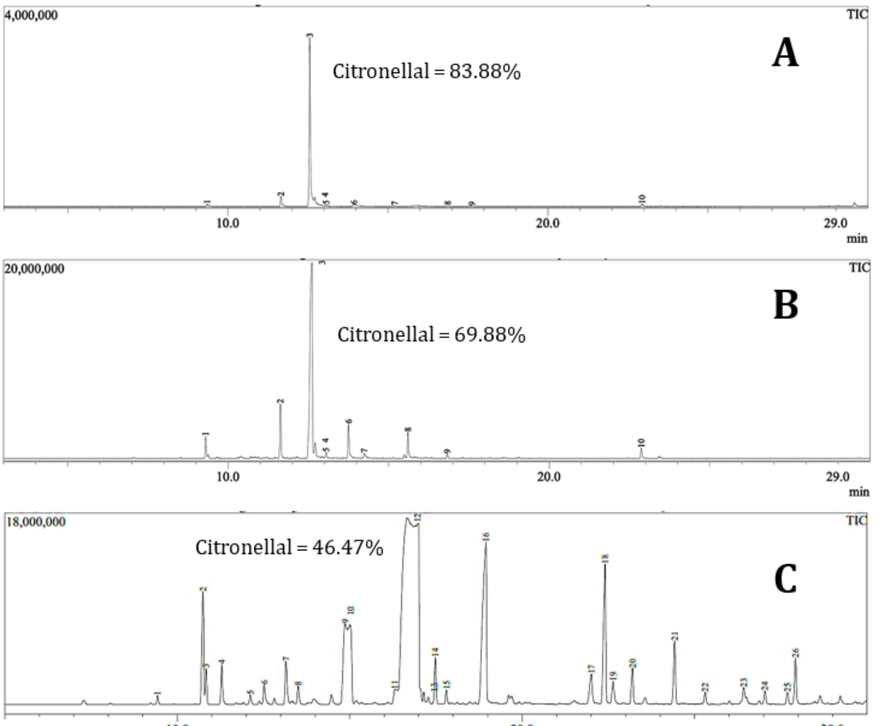

Figure 1. GC–MS chromatogram of essential oil extracted from *C. hystrix* DC: (a) leaves; (b) leaf–twig mixture; and (c) *twig.*

900 g/mL and 1.440–1.480, respectively. Although the refractive index is slightly below the minimum standard, this deviation may be attributed to the inclusion of twig components, which typically contain fewer volatile compounds than leaves, thereby affecting the oil's optical properties. These values remain within the standard ranges reported for high-purity, monoterpene-rich citrus essential oils. For instance, Valarezo et al. (2025) reported that citrus oils with densities between 0.840–0.920 g/mL and refractive indices ranging from 1.450–1.590 generally correlate with high monoterpene content, particularly compounds such as citronellal and limonene.

Similarly, a study on lemon (*Citrus limon*) essential oil showed a density of 0.855 ± 0.005 g/mL and a refractive index of 1.470 ± 0.005 in authentic samples (Boughendjioua & Djeddi, 2017). Thus, the observed values in the LTMEO sample support its classification as an authentic, monoterpene-rich essential oil, consistent with the high citronellal content determined via GC–MS analysis.

From 375 kg of fresh *C. hystrix* DC. biomass subjected to steam distillation, the essential oil yield was calculated at 0.6%, corresponding to approximately 2.25 kg (≈ 2.63 L) of oil, based on a measured density of 0.856 g/mL. The relatively low yield of 0.6% observed for mixed leaves and twigs is considered typical, especially when distilling mature plant parts using conventional steam distillation. Quyen et al. (2020) reported essential oil yields of 1.24% from *C. hystrix* DC. leaves under steam distillation, highlighting that leaf-derived oils generally yield higher percentages than mixtures containing twigs. Moreover, a recent investigation across four agroclimatic regions of Indonesia by Efendi et al. (2021) confirmed that oil yields from *C. hystrix* DC. leaves ranged from 0.78% to 1.50%, depending significantly on soil pH, rainfall, and nutrient status. Their study demonstrated a strong positive correlation between yield and agroenvironmental variables, helping to explain why yields below 1% are common when using mature biomass or under suboptimal conditions. The present yield is therefore comparable to reported ranges for *C. hystrix* DC. essential oils, confirming the reliability of the distillation process at the pilot-plant scale.

GC–MS Analysis of Essential Oils from Different Plant Parts

Citronellal, a monoterpenoid aldehyde, is a key bioactive compound widely utilized in the flavor, fragrance, pharmaceutical, and agrochemical industries due to its distinctive lemon-like aroma and broad-spectrum biological activities, including antimicrobial, antifungal, anti-inflammatory, and insect-repellent properties (Venancio et al., 2025). Given its wide-ranging applications, optimizing the content and purity of citronellal in essential oils is of paramount importance for industrial manufacturing and therapeutic formulations.

GC–MS analysis of essential oils extracted from *C. hystrix* DC. leaves, twigs, and their mixture revealed marked variations in citronellal concentration. All samples were dominated by citronellal; however, the highest content was detected in LEO (83.88%), followed by LTMEO (69.88%) and TEO (46.47%), as shown in **Figure 1 (a–c)**. These results underscore the importance of selecting the most suitable plant part to maximize citronellal yield. For instance, leaf-derived oils generally contain higher citronellal levels than those from twigs or peels, making leaves the preferred source for commercial extraction (Warsito et al., 2017).

Citronellal has consistently been reported as the predominant constituent of *C. hystrix* DC. leaf essential oil. Astuti et al. (2022) identified citronellal content as high as 85.07% in fresh leaf oil using GC–MS analysis. More recent findings by Naibaho et al. (2024) reported slightly lower yet comparable levels of 77.3% in fresh leaves and 74.1% in dried leaves, highlighting that high citronellal concentrations correlate strongly with antibacterial activity. These consistent results across multiple studies reinforce citronellal's role as a chemotaxonomic marker and the principal bioactive component of *C. hystrix* DC. essential oil.

Other major constituents identified in *C. hystrix* DC. essential oils included linalool, isopulegol, β-citronellol, citronellyl acetate, and sabinene, as shown in **Table 1**. Their relative concentrations varied depending on the plant part used. Notably, leaf-derived oils tended to exhibit higher levels of β-citronellol and citronellyl acetate than peel oils, while sabinene and linalool occurred in trace amounts. These compositional differences were likely governed by organ-specific physiology and enzymatic activity, particularly those involving terpene synthases and alcohol dehydrogenases regulating monoterpenoid biosynthesis.

Table 1. Major Compounds in *C. hystrix* DC. Essential Oils by Plant Part

Compound	LEO (%)	TEO (%)	LTMEO (%)
Citronellal	83.88	46.47	69.88
Linalool	6.98	5.24	8.91
Isopulegol	1.69	0.47	2.95
β-Citronellol	2.03	12.22	6.07
Citronellyl acetate	-	6.48	4.28
Sabinene	2.04	4.42	3.29

Note: *C. hystrix* DC. essential oil from leaves (LEO), twigs (TEO), and a leaf–twig mixture (LTMEO).

The higher citronellal content observed in *C. hystrix* DC. leaves compared to twigs can be attributed to physiological and biosynthetic differences between plant organs. Leaves are metabolically more active sites for secondary metabolite production, supported by greater activity of monoterpene synthase enzymes, including citronellal synthase, which directly enhances citronellal accumulation (Wang et al., 2023; Zhang et al., 2020; Zhang et al., 2023). Integrated metabolomic and transcriptomic studies on aromatic plants have demonstrated that differential expression of terpene synthases and upstream methylerythritol phosphate (MEP) pathway genes correlates with higher oxygenated monoterpene levels in leaves than in woody tissues. Moreover, environmental factors such as light intensity and photosynthetic activity, which primarily affect leaf tissues, upregulate key enzymes in the MEP pathway and promote monoterpenoid biosynthesis (Jiang et al., 2016). These physiological and molecular insights explain why leaf-derived oils consistently exhibit enriched citronellal levels and underscore the importance of leaf selection for producing high-citronellal essential oils for industrial applications.

The major constituents identified in *C. hystrix* DC. essential oils (i.e., citronellal, citronellol, linalool, isopulegol, and citronellyl acetate) are all classified as oxygenated monoterpenes. The molecular structures of these compounds, presented in **Figure 2**, illustrate their characteristic functional groups that contribute to the distinct aroma and bioactivity of the essential oil.

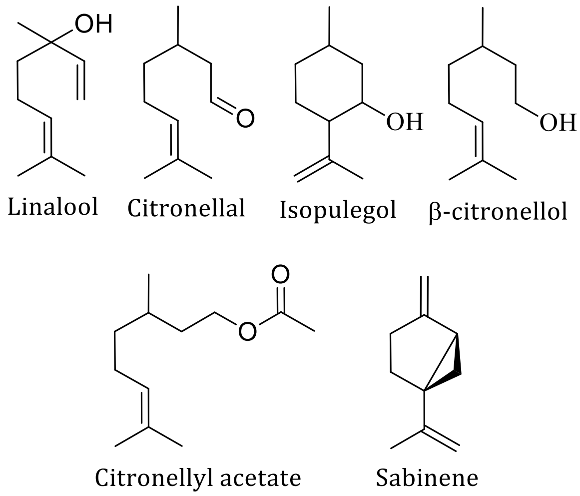

Figure 2. Chemical structures of major compounds in *C. hystrix* DC. essential oil

Recent chemical profiling studies strongly support this observation. Baccati et al. (2021), in their comprehensive comparative analysis of *C. hystrix* DC. leaf essential oil across multiple germplasms and geographic regions, reported citronellal concentrations ranging from 58.9 % to 81.5 %, β-citronellol levels between 3.4 % and 8.2 %, and citronellyl acetate contents ranging from 0.7 % to 5.1 % in the analyzed leaf oil samples, indicating a consistent chemotype dominated by citronellal.

Minor compounds such as linalool, β-citronellol, citronellyl acetate, and sabinene, although present in lower abundance compared to citronellal, play important roles in shaping the aroma profile and bioactivity of *C. hystrix* DC. essential oil. Linalool has been reported to mediate plant–insect and plant–pathogen interactions, contributing floral and citrus nuances while enhancing antimicrobial activity through its volatile properties (Zhang et al., 2023). β-Citronellol provides additional anti-inflammatory and gastroprotective effects, acting through COX-II, 5-LOX, and eNOS pathways, as recent mechanistic studies indicate (Iqbal et al., 2024). Meanwhile, citronellyl acetate is valued in flavor, perfumery, and cosmetic applications; its synthesis via enzymatic esterification of citronellol, as demonstrated in recent work, underscores its potential importance as a minor component contributing to aroma complexity (Haq et al., 2024). These findings indicate that minor constituents should not be regarded merely as supplementary compounds but as bioactive and economically significant components that enhance both the functionality and value of the essential oil.

The high abundance of citronellal in *C. hystrix* DC. leaf oil, reaching up to 83.88 %, strongly supports its use as the preferred source for citronellal-based synthesis, particularly for applications requiring high-purity monoterpenoid aldehydes, such as antimicrobial agents, insect repellents, and pharmaceutical intermediates.

Moreover, the distinct chemical profiles observed among LEO, TEO, and LTMEO underscore the metabolic specificity of different plant organs and suggest potential for tailored applications. For example, study results show that TEO exhibited a lower proportion of citronellal (~46.4 %) but a relatively higher content of linalool (~13.1 %) and isopulegol, indicating its suitability for fragrance or aromatherapeutic applications rather than antimicrobial use, where citronellal predominates. In contrast, LEO and LTMEO maintained higher citronellal contents (~61–78 %), making them more relevant for antimicrobial or anti-inflammatory purposes. Such compositional variations are attributable to enzymatic regulation and developmental physiology within plant tissues and can be strategically utilized to align essential oil applications with the target compound profile. Fractional distillation profiling data of mixed leaf-twig oils revealed 46.4 % β-citronellal, 13.11 % linalool, 11.03 % β-citronellol, 6.76 % citronellyl acetate, and 5.91 % sabinene (Habsari et al., 2018).

Leaf-derived oils of *C. hystrix* DC., characterized by their high citronellal content, hold promising potential across various applied domains. Citronellal is well known for its insect-repellent activity and is utilized in formulations to reduce mosquito bites (Iovinella et al., 2022). In antimicrobial studies, citronellal has shown inhibitory effects on Gram-positive bacteria, contributing to the antibacterial potency of essential oils (Venancio et al., 2025). Moreover, citronellol, a related monoterpenoid, has demonstrated even stronger microbicidal potential and membrane-permeabilizing activity, underscoring the value of oils rich in these components (Mielczarek et al., 2025).

Overall, these data demonstrate that the composition of *C. hystrix* DC. essential oils varies notably among plant parts, highlighting the importance of source selection for research and industrial purposes. The correlation between high citronellal content and leaf-derived oil aligns with previous reports emphasizing the leaf's biosynthetic capability for monoterpenes (Baccati et al., 2021; Efendi et al., 2021). Such findings underscore not only the chemotaxonomic value of citronellal but also its potential as a targeted biomarker to guide raw-material selection in essential oil standardization and application.

FTIR Spectroscopic Characterization

FTIR spectra of the essential oils and standard citronellal (see **Figure 3**) provided insights into the functional groups present. The LTMEO oil sample exhibited strong absorption bands at 1726 cm^{-1}, corresponding to the C=O stretching of aldehydes, and at 2922 and 2874 cm^{-1}, attributed to C–H stretching vibrations of alkyl chains. Additional bands observed at 1454 and 1379 cm^{-1} were assigned to C=C stretching modes, while a weak band near 973 cm^{-1} was associated with out-of-plane C–H bending of terminal alkenes.

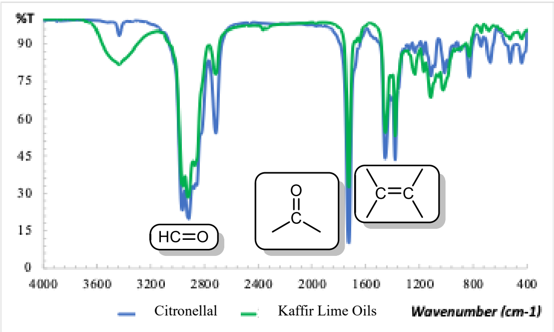

Figure 3. FTIR spectra of *C. hystrix* DC. essential oils and standard citronellal.

These absorptions closely matched those of the standard citronellal spectrum, reinforcing its role as the dominant compound in the oil. Comparable findings have been reported in citrus oil authentication studies, where FTIR reliably identified aldehyde carbonyl and terpene-specific vibrations as diagnostic markers. Salamah et al. (2024) demonstrated that FTIR can effectively differentiate citrus peel oils from different species by emphasizing carbonyl and alkene bands as discriminating features. Similar approaches in *Citrus aurantium* (Riswanto et al., 2023) and Moroccan *Eucalyptus* oil (El Orche et al., 2024) also highlighted the diagnostic power of aldehyde C=O stretching and alkene-associated bands for species and origin differentiation.

Furthermore, Agatonović-Kustrin et al. (2020) confirmed that ATR-FTIR combined with chemometric techniques such as PCA and HCA could reliably detect functional group vibrations and distinguish authentic from adulterated essential oils. These comparisons strengthen the interpretation that the observed FTIR profile reflects a citronellal-rich chemotype and support the application of FTIR as a rapid and nondestructive method for essential oil authentication and quality control.

The GC–MS and FTIR results provide comprehensive insights into the chemical and physicochemical characteristics of *C. hystrix* DC. essential oils, supporting their utilization in flavor, fragrance, and bioactivity-driven applications. The data highlight the significant correlation between physicochemical parameters and chemical profiles, emphasizing the importance of integrated quality evaluation in essential oil research and industrial practice.

Conclusion

This study revealed that the essential oils obtained from *C. hystrix* DC. leaves contained the highest citronellal content compared to those from twigs and mixed samples, as supported by GC–MS, FTIR, and physicochemical analyses. The novelty of this work lies in providing comparative data on Indonesian-grown plants, contributing to the standardization of raw materials for citronellal-rich essential oils. Despite its strengths, this study has certain limitations, including the relatively low yield and the absence of statistical analyses across replicates, which should be addressed in future investigations. Further research is recommended to include citronellal purification, detailed bioactivity assays, and comparative analyses with peel-derived oils to enhance industrial applicability. Overall, the findings highlight the potential of *C. hystrix* DC. leaves as the most promising source of citronellal for fragrance, pharmaceutical, and bioactive product development.

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