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# Chemical Composition Analysis of *Eucalyptus citriodora* Essential Oil Using GC-MS and NMR Spectroscopy

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## ABSTRACT

**Background and Objective:** The simultaneous utilization of GC-MS and NMR offers valuable and complementary insights into the molecular weight and chemical structure of compounds, respectively. This research aimed to employ GC-MS and NMR spectroscopy to analyze the essential oil extracted from dried leaves of *Eucalyptus citriodora* sourced from Kaduna, Nigeria. **Materials and Methods:** The leaves were collected from various trees in a farm site in Kaduna metropolis, Kaduna state and processed into an extract. Hydrodistillation extraction (HDE) and GC-MS (HDE-GC-MS) techniques were employed to extract and analyze the volatile compounds, or VOCs, from *Eucalyptus citriodora* leaf, with each component's percentage reported as raw area percentage based on the total ion current. **Results:** The extraction method employed to obtain the essential oil was hydrodistillation, resulting in a yield of 3.5% (v/w). Examination of the oil composition identified twenty-nine distinct components, collectively constituting 100% of the volatile oils. Among these constituents, citronellal stood out as the primary monoterpene, with isopulegol, citronellol, 3-tetradecanol and citronellic acid following suit. Further validation through 2D NMR analysis conclusively identified citronellal as the principal component. Additionally, minor quantities of other compounds such as citronellyl acetate, p-methane-1,8-diol, cyclohexylacetone, 5-caranol, caryophyllene, caryophyllene oxide, β-pinene, eucalyptol, myrcenol, menthone, citronellol epoxide, linalool, citronellyl formate, α-pinene, methyleugenol, geraniol and geranyl acetate were identified. **Conclusion:** The analysis of the essential oil from *E. citriodora* using GC-MS and NMR techniques revealed a predominance of monoterpenes, with citronellal identified as the major constituent, constituting 46.87% of the total composition.

## KEYWORDS

Essential oils, *Eucalyptus citriodora*, citronellal, myrtacea, hydrodistillation, elucidation



## INTRODUCTION

*Eucalyptus citriodora*, commonly known as Lemon-Scented Gum, is a species renowned for its aromatic properties and diverse applications in traditional medicine, aromatherapy and industry[1]. The essential oil derived from *E. citriodora* possesses a distinct citrusy aroma and is recognized for its therapeutic benefits,

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including antimicrobial, antifungal and insecticidal properties[2]. However, the chemical composition of *E. citriodora* essential oil can vary depending on factors such as geographic origin, environmental conditions and extraction methods[2].

Structural elucidation of the essential oil will be conducted using advanced analytical techniques, including Gas Chromatography-Mass Spectrometry (GC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy. These analytical methods provide valuable insights into the molecular composition and structural characteristics of the essential oil components[3].

Through comprehensive chemical analysis, we aim to identify the major constituents and minor compounds present in the essential oil of *E. citriodora* from Kaduna, Nigeria. This research contributes to the existing body of knowledge on the chemical diversity of *E. citriodora* essential oil and provides insights into its potential applications in various industries, including pharmaceuticals, cosmetics and aromatherapy[4-6]. Additionally, by highlighting the unique chemical profile of *E. citriodora* from Kaduna, Nigeria, our study contributes to the sustainable utilization of this valuable natural resource.

Essential oils, renowned for their aromatic properties, are extracted from aromatic plants and utilized in various industries such as pharmaceuticals, food and perfumery due to their diverse biological and pharmacological activities[7,8]. The *Eucalyptus* genus, particularly *Eucalyptus citriodora*, is valued for its rich essential oil content, which includes compounds like citronellol, citronellal, cineole and limonene[9,10]. The essential oil derived from *Eucalyptus citriodora* leaves possesses potent antiseptic and disinfectant properties, making it a common ingredient in nasal decongestants and treatments for colds, flu and skin infections[11,12]. Gas chromatography (GC) and Nuclear Magnetic Resonance (NMR) spectroscopy are analytical techniques used to analyze essential oils, with GC-MS providing insights into volatile compound separation and NMR spectroscopy facilitating molecular structure elucidation without additional purification steps[13,14]. This study aimed to employ GC-MS and NMR spectroscopy to analyze the essential oil extracted from *Eucalyptus citriodora* leaves collected in Kaduna, Nigeria, providing valuable insights into its chemical composition and molecular structure.

## MATERIALS AND METHODS

**Collection of plant materials:** The botanical specimen was gathered from Kaduna, Nigeria, during the month of June in 2017. The plant's identification and validation were carried out by a qualified taxonomist at the Herbarium of the National Institute of Pharmaceutical Research and Development in Abuja, Nigeria. A voucher specimen labeled as (NIPRD/H/7106) was securely stored at the herbarium for future reference.

**Isolation of essential oil:** The dried leaves weighing 500 g were fragmented into small fragments and subjected to hydrodistillation using a Clevenger-type apparatus. The distillation process was conducted over a period of 4 hrs. The resulting colorless oil, with a yield of 3.5% (v/w), was subsequently dried using anhydrous sodium sulfate. Following drying, the oil was filtered through a 0.22-micron filter and stored at 4°C in sealed vials in a dark environment until it was ready for analysis, as outlined in the study by Chinyere *et al.*[15].

**Gas chromatography-mass spectroscopic analysis:** The oil underwent analysis via GC-MS employing a Shimadzu QP-2010 GC equipped with a QP-2010 mass selective detector (MSD), operated in the electron impact (EI) mode with an electron energy of 70eV. The scan range was set from 45 to 400amu with a scan rate of 3.99 scans per second. A Shimadzu GC-MS solution data system was utilized for data processing. The GC column utilized was an HP-5MS fused silica capillary, featuring a (5% phenyl)-polymethylsiloxane stationary phase, with dimensions of 30 m in length, 0.25 mm in internal diameter and 0.25 µm in film thickness. Helium served as the carrier gas with a flow rate of 1.61 mL/min. The GC oven

Table 1: Result of the GC-MS analysis of the essential oil of *E. citriodora* leaves

| Peak no. | Name | Retention time | Composition (%) |
|---|---|---|---|
| 1 | 2-Methylpropyl-2-methylpropionate | 3.195 | 0.45 |
| 2 | α-Pinene | 3.497 | 0.40 |
| 3 | β-Pinene | 4.033 | 1.25 |
| 4 | Myrcenol | 4.689 | 0.73 |
| 5 | Eucalyptol | 4.739 | 0.96 |
| 6 | 2,6-Dimethyl-5-heptenal | 4.997 | 2.85 |
| 7 | Linalool | 5.646 | 0.50 |
| 8 | Citronellal | 6.563 | 46.87 |
| 9 | Isopulegol | 6.648 | 7.68 |
| 10 | Menthone | 6.724 | 0.59 |
| 11 | 5-Caranol | 6.777 | 1.70 |
| 12 | Dihydrocarveol | 6.892 | 0.60 |
| 13 | Cyclohexylacetone | 7.330 | 1.71 |
| 14 | Citronellol | 7.530 | 7.47 |
| 15 | 7-Methyl-1,6-octadiene | 7.822 | 1.02 |
| 16 | Geraniol | 7.898 | 0.35 |
| 17 | 3-Tetradecanol | 7.991 | 4.98 |
| 18 | Citronellyl formate | 8.190 | 0.47 |
| 19 | 9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol | 8.725 | 0.91 |
| 20 | Bicyclo[3.3.1]nonan-9-ol,9-methyl- | 8.767 | 1.91 |
| 21 | Citronellic acid | 8.883 | 4.31 |
| 22 | Citronellol epoxide | 9.017 | 0.54 |
| 23 | p-Methane-3,8-diol | 9.113 | 3.83 |
| 24 | Citronellyl acetate | 9.283 | 2.67 |
| 25 | p-Methane-1,8-diol | 9.422 | 1.78 |
| 26 | Geranyl acetate | 9.699 | 0.35 |
| 27 | Methyleugenol | 10.006 | 0.38 |
| 28 | Caryophyllene | 10.379 | 1.41 |
| 29 | Caryophyllene oxide | 12.573 | 1.34 |

temperature protocol initiated with an initial isothermal phase set at 60°C, succeeded by a gradual increase from 60 to 180°C at a pace of 10°C/min, maintained at 180°C for 2 min, subsequently elevated from 180 to 280°C at a rate of 15°C/min and then sustained at 280°C for 4 min. Meanwhile, the injection port temperature remained constant at 250°C. Sample components underwent ionization in the EI mode (70eV), with injector and detector temperatures set at 250 and 280°C, respectively. Helium was employed as the carrier gas at a flow rate of 1.61 mL/min. A volume of 1.0 µL of the diluted sample (1/100 in hexane, v/v) was injected using an autosampler in the split mode, with a split ratio of 10:90, as described by Okhale *et al*.[16].

**Identification of constituent compounds:** To identify the individual components present in the essential oil, their mass spectra were compared with known compounds from the National Institute of Standards and Technology (NIST) mass spectral library and the Flavour and Fragrance Natural and Synthetic Compounds mass spectral library database[3]. Confirmation of the structure of citronellal, the primary component of the oil, was achieved through 2D NMR analysis Chinyere *et al*.[15]. Quantitatively, the area percentage of each component from the GC-MS analysis was reported as raw percentage based on the total ion current without standardization. Results were presented in Table 1.

**NMR spectroscopic analysis:** The $^{1}H$ and $^{13}C$ NMR and 2D NMR spectra were obtained by a JEOL-LA 400 MHz NMR spectrometer system using deuterated $CDCl_3$ ($CDCl_3$-d) as solvent Chinyere *et al*.[15].

**Statistical analysis:** Statistical analysis was carried out with the statistical package BMDP, using the BMDP 2R program (stepwise multiple regression). Results were expressed as mean of triplicate analysis.

## RESULTS AND DISCUSSION

**Gas chromatography-mass spectral analysis:** The gas chromatography-mass spectral analysis of the essential oil extracted from *Eucalyptus citriodora* leaves, as presented in Table 1, unveiled twenty-nine distinct constituents, collectively constituting 100% of the total volatile oils. Notably, citronellal emerged as the primary monoterpene constituent, accounting for 46.87% of the composition, followed by isopulegol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%) and citronellic acid (4.31%). The total ion chromatogram was depicted in Fig. 1, where peak 8, corresponding to citronellal, prominently represents *E. citriodora* leaf essential oil Okhale *et al.*[17]. Identification of constituents was accomplished by matching their mass spectra with known compounds in the National Institute of Standards and Technology (NIST) mass spectral library.

**NMR spectral analysis:** The Nuclear Magnetic Resonance (NMR) analysis assignment shown in (Table 2) indicated the characteristic citronellal proton NMR spectra comprising of one aldehydic proton 9.64 (1H, s, H-1), three methyl protons: 0.87 (3H, d, H-10), 1.50 (3H, s, H-8) and 1.58 (3H, s, H-9); three methylene protons: 1.22 (2H, m, H-4), 1.92 (2H, m, H-6) and 2.30 (2H, dd, H-2) and two methine protons: 1.96 (1H, d, H-3) and 4.99 (1H, t, H-6). The $^{13}$CNMR absorptions for citronellal (δ, $CDCl_3$) consisted of 203.3 (C-1), 51.2 (C-2), 27.7 (C-3), 37.0 (C-4), 25.5 (C-5), 124.1 (C-6), 131.7 (C-7), 17.6 (C-8), 25.6 (C-9) and 19.95 (C-10).

Table 2: $^{13}$C and $^{1}$H NMR spectral assignment of citronellal ($C_{10}H_{18}O$)

| Position | $\delta_C$, type | $\delta_H$ (J in Hz) |
|---|---|---|
| 1 | 203.3, CHO | 9.64, s |
| 2 | 51.2, $CH_2$ | 2.30, dd |
| 3 | 27.7, CH | 1.96 |
| 4 | 37.0, $CH_2$ | 1.22, m |
| 5 | 25.5, $CH_2$ | 1.92, m |
| 6 | 124.1, CH | 4.99, t |
| 7 | 131.7, C | - |
| 8 | 17.6, $CH_3$ | 1.50, s |
| 9 | 25.6, $CH_3$ | 1.58, s |
| 10 | 19.95, $CH_3$ | 0.87, d |

s: Singlet, d: Doublet, m: Multiplet, t: Triplet and dd: Doublet of doublet

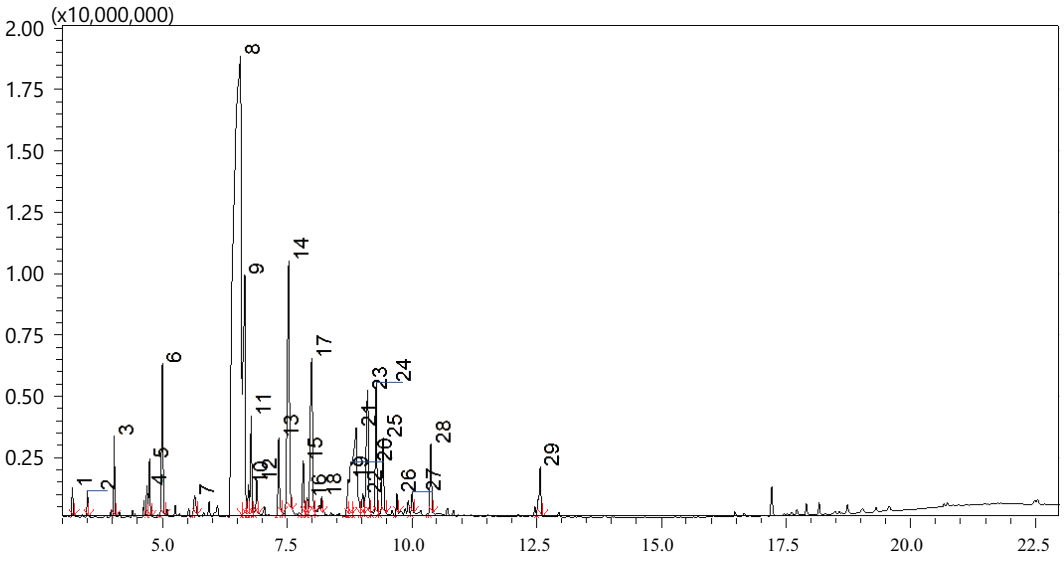


Fig. 1: GC-MS chromatogram of *E. citriodora* essential oil analyzed on GC-MS (Shimadzu, Japan) using a capillary column (HP 5MS) coupled to mass selective detector

Chromatogram showed peaks representing chemical components found in the *E. citriodora* and peak 8 is citronellal, the most dominant peak of the *E. citriodora* essential oil[18]

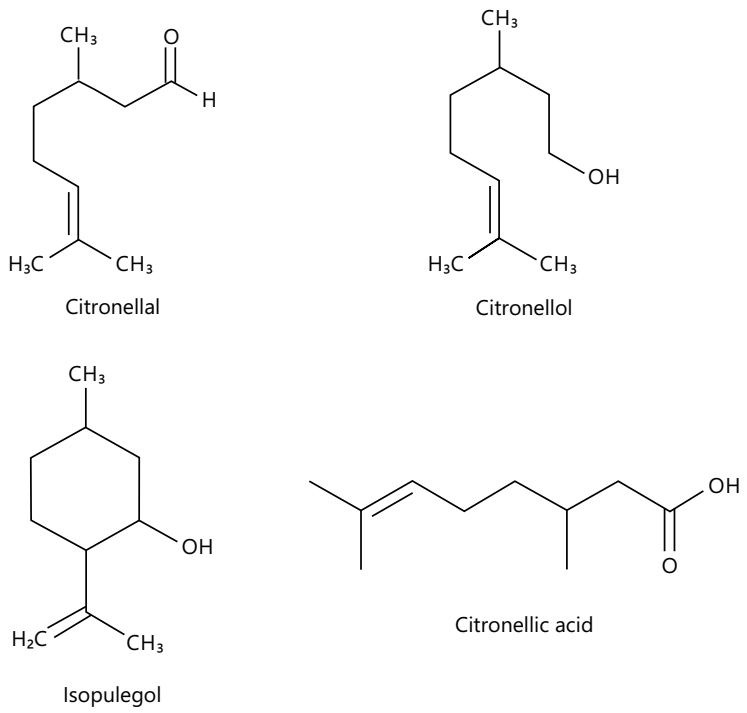


Fig. 2: Structure of some major chemical constituents of *E. citriodora* leaf essential oil

The essential oil extracted from *Eucalyptus citriodora* leaves exhibited a complex composition, comprising twenty-nine distinct volatile components, as outlined in Table 1, along with their respective retention times and percentage compositions. Among these constituents, citronellal dominated the composition at 46.87%, followed by isopulegol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%) and citronellic acid (4.31%). Minor constituents such as citronellyl acetate, p-methane-1,8-diol, cyclohexylacetone, 5-caranol, caryophyllene, caryophyllene oxide, β-pinene, eucalyptol, myrcenol, menthone, citronellol epoxide, linalool, citronellyl formate, α-pinene, methyleugenol, geraniol and geranyl acetate were also identified.

Table 1 presented the results of the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the essential oil extracted from *Eucalyptus citriodora* leaves[19-21]. The lists of the identified components are shown in Table 1, their retention times and their percentage compositions in the essential oil. Notably, citronellal emerged as the predominant constituent, constituting 46.87% of the total composition, followed by other significant components such as isopulegol, citronellol, 3-tetradecanol and citronellic acid. These findings provide valuable insights into the chemical composition of *E. citriodora* essential oil. Figure 1 depicted that GC-MS chromatogram of the *E. citriodora* essential oil, showing the peaks corresponding to the various chemical components identified in the analysis. Notably, peak 8 corresponds to citronellal, which emerges as the most dominant peak in the chromatogram.

Table 2 presented the $^{13}C$ and $^{1}H$ Nuclear Magnetic Resonance (NMR) spectral assignments of citronellal, the major constituent of *E. citriodora* essential oil. The table provides information on the chemical shifts and types of carbon and hydrogen atoms present in citronellal, aiding in its structural elucidation[22-27]. Figure 2 illustrated the structures of some major chemical constituents found in *E. citriodora* leaf essential oil, providing visual representations of these important compounds.

The mass spectrum analysis of citronellal indicated its molecular composition as $C_{10}H_{18}O$, with significant ions observed at various mass-to-charge ratios (m/z), including 139 ($C_{10}H_{18}O$, $M^+$-$CH_3$), 121, 111, 95, 83, 69, 55, 41 and 27 (Fig. 2). To further confirm the structure of citronellal, 2D-NMR analysis was

conducted, revealing characteristic signals for aldehydic proton and various methyl, methylene and methine protons[28-32].

The dominance of citronellal and the overall chemical composition of the *E. citriodora* leaf essential oil observed in this study aligns with previous research findings from various regions globally[33-35]. For instance, samples from Algeria, Thailand and Mali exhibited similar dominant constituents, albeit in varying proportions, underscoring the species' chemical diversity across different geographical locations[36]. These findings contribute to the understanding of the chemical profile of *E. citriodora* essential oil and its potential applications in various industries[37].

## CONCLUSION

The analysis of the essential oil from *E. citriodora* using GC-MS and NMR techniques revealed a predominance of monoterpenes, with citronellal identified as the major constituent, constituting 46.87% of the total composition. Additionally, other significant constituents included isopulegol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%) and citronellic acid (4.31%). These findings are in line with previous research conducted on the chemical composition of *E. citriodora* leaf essential oils from various geographic regions. Notably, the identified components contribute to the characteristic odor profile associated with *E. citriodora* leaves.

## SIGNIFICANCE STATEMENT

The utilization of Gas Chromatography-Mass Spectrometry (GC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy in tandem offers a powerful approach for comprehensive chemical analysis, providing insights into both the molecular weight and chemical structure of compounds. In this study, GC-MS and NMR spectroscopy was applied to investigate the essential oil extracted from dried leaves of *Eucalyptus citriodora* sourced from Kaduna, Nigeria. Through hydrodistillation extraction (HDE) coupled with GC-MS (HDE-GC-MS), we identified twenty-nine different components in the essential oil, with citronellal emerging as the predominant monoterpene constituent. The results of 2D NMR analysis further confirmed citronellal as the major component. This study enhances our understanding of the chemical composition of *E. citriodora* essential oil and underscores the potential of GC-MS and NMR spectroscopy as complementary tools for comprehensive chemical characterization.

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