Bioactive Compounds from Corymbia citriodora: Steam Distillation Optimization and Antimicrobial Potential in Natural Health Care Applications

Nguyen Thi Diem Hoa[1], Thanh Ngoc Nguyen[2], Huynh Chi Hieu[3], Vy Anh Tran[1*]
[1]Faculty of Applied Science and Technology, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam
[2]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam
[3]Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam
*tavy@ntt.edu.vn

Abstract

This study focuses on investigating the chemical composition and optimizing extraction techniques to produce value-added products from Corymbia citriodora, commonly known as lemon eucalyptus. By analyzing physicochemical parameters, the concentrations of key bioactive compounds-particularly citronellal, citronellol, and isopulegol-were determined to select the most suitable raw materials for further development. Modern extraction methods, including steam distillation and ultrasound-assisted extraction, were evaluated to enhance the recovery of highly active constituents while maintaining compound stability and minimizing degradation. To enhance the recovery of highly active constituents while maintaining compound stability and minimizing degradation, modern extraction methods, including steam distillation and ultrasound-assisted extraction, were systematically evaluated. The research further incorporates structural analysis, chemical profiling, and computational assessment of molecular interactions to explore the potential use of lemon eucalyptus essential oil in natural healthcare products. The findings contribute not only to the valorization of C. citriodora as a rich source of functional phytochemicals but also to the advancement of sustainable extraction strategies, providing a scientific basis for the formulation of safe, effective, and nature-based therapeutic solutions.

Received 17/06/2025
Accepted 04/09/2025
Published 26/11/2025

Keywords
Cinnamomum cassia, bioactive compounds, cinnamaldehyde, eugenol, coumarin reduction, natural products.

1. Introduction

Corymbia citriodora, commonly known as lemon eucalyptus, is a fast-growing tree native to Australia and widely cultivated in tropical and subtropical regions for its aromatic leaves. The predominance of citronellal (70.13%) in the essential oil composition highlights Corymbia citriodora as a promising candidate for antibacterial formulations. Citronellal and related monoterpenes, such as citronellol and isopulegol, exhibit membrane-disrupting properties that impair bacterial integrity, especially in Gram-positive bacteria. This mechanism is consistent with the growing interest in plant-derived antibacterial agents that can overcome antibiotic resistance. Furthermore, the mild aroma and low toxicity of these compounds make them ideal for topical and inhalation therapies. By optimizing the steam distillation process to preserve these volatile components, our study provides a scalable and environmentally friendly method for the production of high-quality essential oil, the essential oil extracted from its leaves is rich in citronellal, a monoterpenoid aldehyde recognized for its powerful antibacterial, anti-inflammatory, insect repellent, and antioxidant properties. This makes *C. citriodora* an attractive candidate for the development of natural health care products, particularly in the fields of dermatology, respiratory support, and personal hygiene (Pu et al., 2025; Rana et al., 2025; Zhang, Lin, & Ye, 2018).

https://doi.org/10.55401/mdc5bg76

In recent years, growing consumer demand for chemical-free, plant-based remedies has prompted renewed scientific interest in the valorization of essential oils from medicinal plants. Corymbia citriodora, with its strong therapeutic efficacy and low toxicity, has emerged as a promising ingredient for various health-supporting formulations. However, the bioactive profile and extraction yield of corymbia citriodora can vary significantly depending on factors such as plant maturity, geographic origin, and the extraction method employed. Therefore, there is a pressing need to develop and optimize modern extraction strategies that can enhance the yield and quality of desired bioactive compounds while ensuring the chemical stability and safety of the final product (Bitwell, Indra, Luke, & Kakoma, 2023; Mohareb et al., 2025; Mungwari, King'ondu, Sigauke, & Obadele, 2025).

The composition and quality of essential oil depend on various environmental and agronomic factors such as climate, soil type, leaf maturity, fertilization regime, and extraction method. Advanced techniques such as steam distillation**,** ultrasound-assisted extraction, and supercritical fluid extraction have shown potential in maximizing recovery efficiency and preserving thermolabile compounds. Moreover, the integration of computational modeling and chemical characterization tools provides a deeper understanding of molecular interactions, enabling the design of more effective and targeted natural formulations (Bhadange, Carpenter, & Saharan, 2024; Dias, de Aguiar, & Rostagno, 2021).

Despite its recognized pharmacological potential, current applications of C. citriodora essential oil remain limited by variability in oil composition and insufficient optimization of extraction techniques. Furthermore, there is a lack of comprehensive studies combining both chemical profiling and application-driven analysis to justify its incorporation into modern natural health care products. Therefore, this study aims to: (1) analyze the chemical constituents of C. citriodora essential oil using GC–MS, (2) optimize the steam distillation process to improve yield and compound stability, and (3) evaluate the potential health-related applications of the oil, particularly its antimicrobial effects that are relevant to topical and inhalation therapies. The significance of this research lies in its contribution to the development of sustainable, plant-based therapeutic agents, offering alternatives to conventional antimicrobials in an era of rising antibiotic resistance. Moreover, the study provides a scientific foundation for integrating C. citriodora oil into eco-friendly personal care products, thus promoting both health and environmental sustainability.

2. Materials and Methods

2.1. Materials

The leaves of lemon-scented eucalyptus (*Corymbia citriodora*) are thoroughly washed to remove dust and impurities, then finely chopped to increase the surface area for extraction. Anhydrous sodium chloride (NaCl) is added to aid in the separation of water, alongside distilled water and vaseline. The steam distillation process employs a typical setup consisting of a two-neck round-bottom flask, a Liebig condenser, and a separatory funnel. The two-neck flask contains both the plant material and water, which is heated to generate steam; one neck is connected to the condenser, while the other holds a thermometer to monitor temperature. The steam carrying volatile essential oil passes through the condenser, where it is cooled and converted into liquid. The resulting distillate, containing both essential oil and water, is collected in the separatory funnel. Here, additional anhydrous NaCl is added to facilitate phase separation, allowing the essential oil to be effectively isolated from the aqueous layer.

In addition, other equipment used includes an Erlenmeyer flask, alcohol thermometer, essential oil separator arm, heating mantle for the round-bottom flask, glass beakers, plastic water hoses, cotton wool, ring clamps for securing the separatory funnel, support stands, and dark-colored glass bottles for storing the essential oil.

2.2. Method

Fresh lemon-scented eucalyptus leaves were used in this study, with younger leaves generally yielding a higher amount of essential oil compared to older ones. Specifically, we utilized the entire batch of young leaves harvested from a garden located in Cai Be District, Tien Giang Province, Vietnam. The fresh leaves featured a vibrant green color and a rich, refreshing aroma reminiscent of early morning mist. After harvesting, the leaves were thoroughly washed, and only those meeting quality standards were selected for the essential oil extraction process (**Figure 1**).

Figure 1. Lemon-scented eucalyptus leaves (*Corymbia citriodora*) were collected and used for the extraction of lemon-scented eucalyptus essential oil.

Steam distillation procedure

The prepared lemon-scented eucalyptus leaves are placed into a two-neck round-bottom flask, and distilled water was added until the plant material is fully submerged. The distillation apparatus is then assembled: the flask is positioned over a heating mantel, with an essential oil separator arm connected to the main neck and an alcohol thermometer inserted into the secondary neck. A Liebig condenser is attached to the outlet of the separator arm, and water hoses are connected following to the "bottom-in, top-out" principle. The top opening of the condenser is lightly plugged with moistened cotton to minimize vapor loss. While the system operates, a separatory funnel is fixed using a ring clamp on a support stand to ensure stability and readiness for the subsequent oil-water separation. Meanwhile, anhydrous sodium chloride is completely dissolved in distilled water, to be used later in the extraction process (**Figure 2**).

Remove the separatory funnel from the ring clamp and open its stopper. Open the valve of the separator arm to allow the distillate mixture of water and essential oil to flow completely into the funnel. Add the NaCl solution into the mixture, then close the funnel and gently swirl it in a circular motion to ensure thorough mixing. Place the funnel back onto the ring clamp and let it rest undisturbed for a few minutes. As the NaCl begins to act, phase separation occurs: the essential oil rises to form the upper layer, while the lower layer consists of clear water. Finally, collect and store the separate essential oil appropriately (**Figure 3**).

Figure 2. Lemon-scented eucalyptus essential oil distillation system

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The essential oil was analyzed using GC–MS with a Shimadzu QP-2010 gas chromatograph equipped with a QP-2010 mass selective detector (MSD), operating in electron ionization (EI) mode at 70 eV electron energy. The scan range was set from 45 to 400 amu with a scan rate of 3.99 scans per second. Data processing was performed using the Shimadzu GC–MS solution software. The GC column used was an HP-5MS fused silica capillary column with a stationary phase of 5% phenyl polymethylsiloxane, measuring 30 m in length, 0.25 mm in internal diameter, and 0.25 µm in film thickness. Helium was used as the carrier gas at a constant flow rate of 1.61 mL/min. The GC oven temperature program began with an initial isothermal hold at 60°C, followed by a ramp from 60°C to 180°C at a rate of 10°C/min, held at 180°C for 2 minutes, then increased to 280°C at 15°C/min and held at 280°C for 4 minutes. The injection port temperature was maintained at 250°C. Sample components were ionized in EI mode (70 eV), with the injector and detector temperatures set at 250°C and 280°C, respectively. A 1.0 µL aliquot of the diluted sample (1:100 v/v in hexane) was injected using an autosampler in split mode with a split ratio of 10:90, as described by Okhale et al. [1](Bhuiyan, Begum, Sardar, & Rahman, 2009).

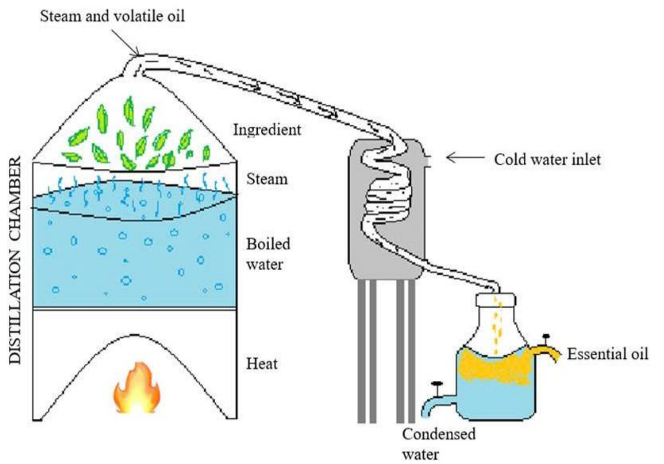

Figure 3. Steam distillation process for essential oil extraction.

3. Results and Discussion

3.1. GC–MS Analysis Results

Quantitative GC–MS Analysis of Bioactive Constituents

A total of more than 20 compounds were identified in the essential oil of Corymbia citriodora using gas chromatography–mass spectrometry (GC–MS). Citronellal (also known as eucalyptol or 1,8-cineole) was the most abundant constituent, accounting for 70.13%, and played a crucial role in contributing to the characteristic aroma and the biological activity of the oil. Other major components included citronellol (6.47%), isopulegol (4.73%), geraniol (3.27%), and limonene (2.36%). A total of more than 20 compounds has been identified in *Corymbia citriodora* essential oil using gas chromatography–mass spectrometry (GC–MS). In this process, plant material is placed above a tank of boiling water inside a distillation chamber. As the water is heated, steam rises and passes through the plant material, carrying with it the volatile compounds (essential oils). The water-oil vapor mixture then enters a condenser, where cold water is circulated to cool the vapor. After condensation, the resulting liquid water and essential oil is collected in a collection flask. Due to the difference in density, the essential oil naturally separates and floats on top of the water, allowing for easy collection. The high concentration of cineole is often considered a defining characteristic that contributes significantly to the distinct properties of the essential oil. The high concentration of cineole is commonly regarded as a defining feature that contributes significantly to the essential oil's distinctive properties.

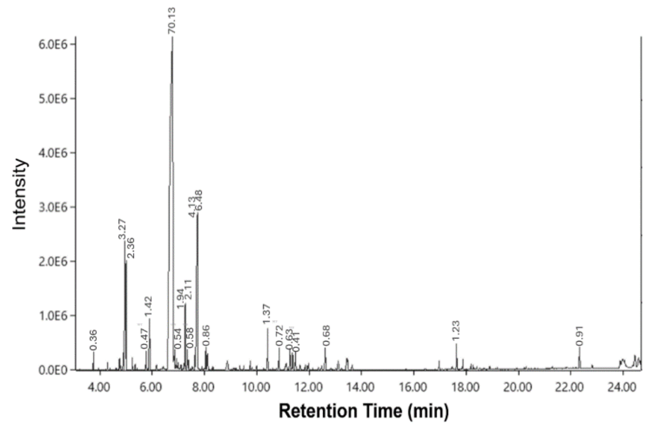

Figure 4. The GC–MS chromatogram of Corymbia citriodora essential oil was obtained using gas chromatography–mass spectrometry analysis.

These compounds are primarily classified as monoterpenes and sesquiterpenes, ~~which are~~ known for their prominent biological activities such as antibacterial, anti-inflammatory, antioxidant, and insect-repellent properties. According to Daizy R. Batish et al. (Batish, Singh, Kohli, & Kaur, 2008), essential oils are complex mixtures composed of various monoterpenes and sesquiterpenes. Monoterpenes consist of 10 carbon atoms and are typically characterized by their strong aromatic scent. They are major constituents in many essential oils, such as lemon and peppermint oils, and are known for their antibacterial, anti-inflammatory properties, making them widely used in healthcare and cosmetic products. Sesquiterpenes, with a more complex structure containing 15 carbon atoms, often possess a stronger aroma and are applied in both medicinal and industrial fields (**Figure 4**). They are recognized for their antibacterial, antifungal, and anti-inflammatory activities.

Table 1. Gas Chromatography–Mass Spectrometry Analysis Results of Lemon-Scented Eucalyptus Essential Oil

Peak	Active substance	Retention time	Composition (%)
1	Citronellal	6.54	70.13
2	Citronellol	7.23	6.48
3	Isopulegol	7.80	4.73
4	Geraniol	8.10	3.27

5	Limonene	5.92	2.36
6	β-caryophyllene	1.53	2.11
7	Eucalyptol (1,8-Cineole)	6.13	1.94
8	α-terpineol	10.21	1.42
9	β-pinene	5.12	1.37
10	α-pinene	4.83	1.23
11	α-humulene	15.03	0.91
12	Nerol	8.32	0.86
13	Myrcene	5.35	0.72
14	Terpinen-4-ol	10.03	0.68
15	Sabinene	4.64	0.63
16	Spathulenol	16.47	0.58
17	γ-terpinene	6.28	0.54
18	Aromadendrene	13.84	0.47
19	Viridiflorol	17.06	0.41
20	Caryophyllene oxide	14.59	0.36
21	Borneol	9.10	0.33
22	Camphene	4.51	0.31
23	α-thujene	4.32	0.28
24	δ-cadinene	14.93	0.26
25	α-copaene	12.35	0.24
26	Linalool	6.74	0.22
27	trans-β-ocimene	5.47	0.19
28	γ-elemene	12.81	0.17
29	p-Cymene	5.78	0.14

In addition to terpenes, essential oils also contain a variety of other compounds such as phenols, oxides, esters, alcohols, ethers, aldehydes, and ketones. Due to the presence of constituents such as 1,8-cineole, p-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α-terpineol, alloocimene, and aromaendrene, lemon-scented eucalyptus essential oil exhibits potent pesticidal, antifungal, and antimicrobial activities (Barbosa, Filomeno, & Teixeira, 2016). The antibacterial activity of lemon-scented eucalyptus essential oil is attributed to the presence of certain low molecular weight phenols, terpenes, and aldoketones. Each ~~type of~~ essential oil contains distinct antimicrobial components, and their antibacterial efficacy varies depending on the specific microbial strains (Muhizi, Manizabayo, Uwamariya, Nkuranga, & Umereweneza, 2025).

Table 2. Comparison of Major Essential Oil Compounds in *Corymbia citriodora*

Compound	**This Study (%)**	**Mohareb et al. (2025) (Mohareb et al., 2025)**	**Barbosa et al. (2016) (Barbosa et al., 2016)**	**Miguel et al. (2018) (Miguel et al., 2018)**	**Notes on Variation**
Citronellal	70.13	65.32	61.45	68.75	Major compound; varies by leaf age and extraction temperature
Citronellol	6.48	5.29	7.12	5.93	By-product of citronellal reduction

Isopulegol	4.73	3.76	2.90	4.15	Formed during oxidative rearrangement
Geraniol	3.27	2.58	3.19	2.94	Present in most *C. citriodora* chemotypes
Limonene	2.36	1.95	2.42	2.81	Common monoterpene: concentration varies by geography
1,8-Cineole	1.42	1.80	1.23	1.39	Typical in eucalyptus species; slightly lower in this study
β-Caryophyllene	2.11	2.33	1.98	2.26	Stable across samples; sesquiterpene marker

Table 3. Detailed statistical parameters from the lemon-scented eucalyptus essential oil distillation process.

No.	**Lemon-scented eucalyptus leaves (g)**	**Time (minutes)**	**Temperature (ºC)**	**Solvent**	**Water separation**	**Essential oil volume (mL)**
1	218,29	113	92	Water	NaCl	3.1
2	191,14	98	86	Water	NaCl	2.9
3	256,85	125	91	Water	NaCl	5.3

Given its rich composition of bioactive monoterpenes and sesquiterpenes, Corymbia citriodora essential oil demonstrates significant potential for incorporation into a wide range of natural health care products. Its high citronellal and cineole content endows it with strong antimicrobial, anti-inflammatory, and insect-repellent properties, making it suitable for use in topical formulations such as antiseptic creams, wound-healing balms, and anti-acne treatments.

Steam distillation plays a critical role in extracting volatile bioactive compounds from Corymbia citriodora leaves without causing significant thermal degradation. In this process, steam penetrates the plant matrix and ruptures glandular structures, releasing essential oils. These volatile compounds then co-distill with steam, allowing their separation at temperatures significantly lower than their boiling points. One of the key advantages of steam distillation is its ability to preserve thermolabile compounds such as citronellal and isopulegol. Unlike direct heat or solvent-based extraction, steam distillation avoids prolonged exposure to high temperatures, reducing oxidation and chemical decomposition. In our optimized setup, we maintained the distillation temperature around 90–92°C and controlled the duration (~90–125 minutes), which was ~~found to be~~ ideal for releasing essential oil from young leaves while maintaining compound integrity. Furthermore, the addition of anhydrous NaCl into the aqueous phase enhances the oil–water phase separation, improving the clarity and purity of the final essential oil. The steam distillation method also allows continuous separation of oil as it forms, minimizing reabsorption by plant tissues and loss of volatile components. This optimized approach resulted in higher essential oil yield and a better retention of key constituents, especially citronellal. This demonstrates the efficiency of steam distillation in capturing both the quantity and bioactivity of C. citriodora oil, supporting its application in high-quality natural healthcare formulations.

Additionally, its pleasant and refreshing aroma, coupled with its low toxicity, supports its application in aromatherapy, stress-relief sprays, and respiratory support products like chest rubs or inhalants. The oil's antifungal and antibacterial actions also offer potential for use in oral care products, such as natural mouthwashes or toothpaste. These diverse applications underscore the value of lemon eucalyptus essential oil as a multifunctional and sustainable ingredient in the development of nature-based therapeutic and personal care solutions (Hassan et al., 2020; Miguel et al., 2018; Shiekh et al., 2025). Based on the high content of citronellal and other monoterpenes, the essential oil of Corymbia citriodora can be formulated into several types of natural health care products. For instance, citronellal's strong antibacterial effect supports its use in antiseptic gels or acne creams. The cooling and decongestant effects of 1,8-cineole make the oil suitable for chest rubs and inhalants in respiratory therapy. Furthermore, the pleasant lemony aroma, combined with insect-repellent properties, supports its use in stress-relief sprays, roll-ons, and natural mosquito repellents. These potential uses align with growing market trends for chemical-free, plant-based products in personal and home care (de Araújo-Filho et al., 2018; Miguel et al., 2018).

4. Conclusions

This study comprehensively analyzed the chemical composition of Corymbia citriodora essential oil and successfully identified citronellal as the predominant bioactive compound, followed by isopulegol and citronellol. Through the application of an optimized steam distillation process, the research achieved enhanced extraction efficiency while preserving the structural integrity of thermolabile constituents. GC–MS profiling confirmed a diverse array of monoterpenes and sesquiterpenes, known for their broad-spectrum biological activities, including antimicrobial, antioxidant, anti-inflammatory, and insect-repellent effects. The high concentration of citronellal (70.13%) and the consistent presence of functionally active terpenoids underscore the potential of C. citriodora essential oil as a valuable natural resource for the formulation of therapeutic agents. The integration of advanced extraction techniques with analytical characterization not only enhances product quality but also aligns with the growing demand for safe, sustainable, and plant-based solutions in healthcare and cosmetic industries. Moreover, the results provide a scientific foundation for the further development of lemon eucalyptus essential oil in various applications, such as antiseptic creams, respiratory relief products, natural insect repellents, and aromatherapy formulations. Future research may focus on formulation stability, in vivo efficacy, and safety assessments to facilitate the transition from laboratory findings to commercial natural healthcare products.

Acknowledgments

We acknowledge Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam for supporting this study.

Conflicts of Interest

The authors declare no conflict of interest.

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Các hợp chất hoạt tính sinh học từ Corymbia citriodora: Tối ưu hóa chưng cất lôi cuốn hơi nước và tiềm năng kháng khuẩn trong ứng dụng chăm sóc sức khỏe

Nguyễn Thị Diễm Hoa[1], Thanh Ngọc Nguyễn[2], Huỳnh Chí Hiếu[3], Vy Anh Trần[1]*
[1]Khoa Khoa học Ứng dụng và Công nghệ, Trường Đại học Nguyễn Tất Thành, TP. Hồ Chí Minh 700000, Việt Nam
[2]Viện Kỹ thuật Công nghệ cao NTT, Trường Đại học Nguyễn Tất Thành, TP. Hồ Chí Minh 700000, Việt Nam
[3]Viện Ứng dụng Công nghệ và Phát triển Bền vững, Trường Đại học Nguyễn Tất Thành, TP. Hồ Chí Minh 700000, Việt Nam
*tavy@ntt.edu.vn

Tóm tắt Nghiên cứu này tập trung khảo sát thành phần hóa học và tối ưu hóa các kỹ thuật chiết xuất nhằm tạo ra những sản phẩm giá trị gia tăng từ Corymbia citriodora, thường được biết đến với tên gọi bạch đàn chanh. Thông qua việc phân tích các thông số lý-hóa, nồng độ của những hợp chất hoạt tính sinh học chính đặc biệt là citronellal, citronellol và isopulegol đã được xác định để lựa chọn nguồn nguyên liệu phù hợp nhất cho các bước phát triển tiếp theo. Các phương pháp chiết xuất hiện đại, bao gồm chưng cất lôi cuốn hơi nước và chiết xuất hỗ trợ bằng sóng siêu âm, được đánh giá nhằm nâng cao hiệu suất thu hồi các hoạt chất có độ hoạt tính cao trong khi vẫn đảm bảo tính ổn định cấu tử và hạn chế sự phân hủy. Những kỹ thuật này được triển khai và so sánh một cách hệ thống. Bên cạnh đó, nghiên cứu còn tích hợp phân tích cấu trúc, định danh hóa học và đánh giá tính toán về tương tác phân tử nhằm khai thác tiềm năng ứng dụng tinh dầu bạch đàn chanh trong các sản phẩm chăm sóc sức khỏe có nguồn gốc tự nhiên. Các kết quả thu được không chỉ đóng góp vào việc nâng cao giá trị của C. citriodora như một nguồn phong phú các hợp chất thực vật chức năng mà còn thúc đẩy sự phát triển của các chiến lược chiết xuất bền vững, cung cấp cơ sở khoa học cho việc xây dựng các giải pháp trị liệu an toàn, hiệu quả và thân thiện với tự nhiên.

Từ khóa Cinnamomum cassia, hợp chất hoạt tính sinh học, cinnamaldehyde, eugenol, coumarin reduction, sản phẩm có nguồn gốc tự nhiên.