

Comparative analysis of chemical and biological properties of essential oils from fresh and dried leaves of *Ocimum americanum*

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Abstract. Rusdi M, Rahim A, Rante H, Lallo S. 2025. Comparative analysis of chemical and biological properties of essential oils from fresh and dried leaves of *Ocimum americanum*. *Biodiversitas* 26: 4976-4982. *Ocimum americanum* is a tropical aromatic plant native to Southeast Asia and Africa, widely used in traditional medicine. This study aimed to evaluate the impact of post-harvest drying on the chemical composition and bioactivities of the essential oils (EOs) from the leaves of *O. americanum*. EOs were extracted via steam distillation and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Antioxidant capacity was assessed by DPPH radical scavenging, cupric ion reducing antioxidant capacity (CUPRAC), and ferric reducing antioxidant power (FRAP) assays. Cytotoxicity against MCF-7 breast cancer cells was determined using the MTT assay, while antimicrobial activity was measured using the agar diffusion method. GC-MS analysis revealed that geranial, neral, geraniol, and nerol were the most dominant components in the essential oil from fresh leaves. The most dominant compounds in dried leaves were geranial, neral, linalool, and trans-13-octadecenoic acid methyl ester. The essential oil from fresh leaves showed higher antioxidant capacity in the DPPH radical scavenging assay, with a value of 38.08 ± 2.17 mg TE/g EO. Conversely, the essential oil from dried leaves showed higher antioxidant capacity in the CUPRAC (278.23 ± 1.78 mg TE/g EO) and FRAP (15.59 ± 0.06 mg TE/g EO) assays. Fresh and dried EOs showed antimicrobial activities and cytotoxic activity against MCF-7 cells, and the dried leaf EO demonstrated better anticancer potential, with an IC_{50} value of 22.91 μ g/mL. These findings suggest that drying significantly alters EO composition and modulates its biological activities. The findings indicate that drying extends shelf life and diversifies applications in functional foods, nutraceuticals, and phytopharmaceuticals. Optimizing post-harvest processing could improve the therapeutic value and market potential of *O. americanum*, supporting its sustainable use and conservation.

Keywords: Antioxidant capacity, antimicrobial activity, drying effects, essential oil composition, MCF-7 cytotoxicity

INTRODUCTION

Ocimum americanum L. is an economically aromatic plant of the family Lamiaceae. It's widely cultivated in tropical and subtropical regions of Africa, Asia, and the Americas (Mulugeta et al. 2024). In Indonesia, particularly in Sulawesi, it is locally known as *kamangi* (Makassar) or *camangi* (Bugis) and is consumed as a leafy vegetable and a traditional remedy for fever, headaches, gastrointestinal disturbances, shortness of breath, and diarrhea (Heyne 1987; Ali et al. 2022; Luanda et al. 2023; Chaachouay et al. 2024). Its traditional knowledge highlights the importance of the plant culturally and economically, contributing to complementary healthcare practices and local food security.

Plants of the genus *Ocimum* are well known for their ability to synthesize a wide spectrum of volatile compounds through specialized glandular trichomes distributed on the surface of their leaves and stems. These volatile compounds are not only responsible for the characteristic aroma of *Ocimum* species but also contribute to multiple pharmacological properties, including antifungal, anticancer, antibacterial, anti-inflammatory, and antioxidant activities (Stanojevic et al. 2017; Frezza et al. 2019; Azizah et al. 2023; Scott et al. 2023). Essential oil serves as a valuable

biochemical marker for distinguishing inter- and intraspecific variation within the Lamiaceae from a chemotaxonomic perspective (Vieira 2006).

The composition and biological efficacy of essential oils (EOs) are shaped by a complex interplay of intrinsic and extrinsic factors. Intrinsic factors include genetic makeup, plant chemotypes, and the stage of plant development, all of which determine the biosynthetic pathways leading to volatile production (Mann et al. 2012; Zahran et al. 2020). Extrinsic factors, on the other hand, involve ecological and environmental conditions such as geographic origin, altitude, soil fertility, rainfall, and seasonal variations, which significantly influence the quantity and quality of EOs (Bernhardt et al. 2015; Dhifi et al. 2016). In addition, post-harvest handling—including drying, storage, and extraction methods—plays a critical role in maintaining the stability of volatile constituents. Among these, drying is particularly decisive, as exposure to heat, light, and oxygen can trigger degradation, oxidation, or isomerization of sensitive compounds, thereby reshaping the chemical profile and altering bioactivities such as antioxidant or antimicrobial potential (Mieso et al. 2022; Ray et al. 2022; Arpiwi et al. 2023). Thus, the same plant species may yield

markedly different EO profiles depending on both its growth conditions and the way the plant material is processed.

Previous investigations have documented phytochemistry and biological activities of *O. americanum* essential oils, identifying citral, linalool, geraniol, and other terpenoids as dominant constituents with promising pharmacological activities (Parida et al. 2014; Pandey et al. 2014; Mahendran and Vimolmangkang 2023). However, comparative studies between fresh and dried leaves remain scarce. Most published works have emphasized oils obtained from fresh leaves, which are widely considered to better preserve volatile terpenes but are less practical due to rapid spoilage and limited storage stability. By contrast, dried materials, while easier to store and transport, are often assumed to have diminished quality. Yet emerging evidence suggests that drying may not only preserve but in some cases enhance certain bioactivities, possibly due to the transformation of unstable molecules into more stable derivatives with new pharmacological properties (Altay et al. 2024). This knowledge gap highlights the need for systematic comparisons to determine how drying influences the balance between chemical integrity, biological efficacy, and practical usability of the oils. Addressing this gap is vital for both scientific understanding and the optimization of industrial applications. This study addresses this gap by conducting a comprehensive comparison of the chemical composition and bioactivity of essential oils (EOs) from fresh and dried *O. americanum* leaves.

By comparing the essential oils of fresh and dried leaves, this study suggests that drying may alter the volatile composition and biological activity of these oils. The results offer insight into optimal post-harvest strategies, support the development of functional foods, nutraceuticals, and phytopharmaceutical applications, and contribute to the sustainable utilisation and conservation.

MATERIALS AND METHODS

Study area

The research was conducted from June 2023 to January 2024 at the Hasanuddin University Medical Research Center (HUMRC) laboratory, the Chemistry Laboratory at the Faculty of Science and Technology, and the Pharmaceutical Biology and Microbiology Laboratories at the Faculty of Medicine and Health Sciences, Universitas Islam Negeri Alauddin Makassar, Indonesia.

Plant material

Ocimum americanum (basil) leaves were collected from cultivated plants in Gowa Regency, South Sulawesi, Indonesia (longitude of 119°23'58.5"E and latitude of 5°15'53.2"S). The plant material was documented as photos and sent to Herbarium Bandungense at the School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia, for plant identification. It was officially identified and verified as *Ocimum americanum*, a member of the Lamiaceae family. The authentication process is documented in doc no. 7587/IT1.C11.2/TA.00/ 2024.

Sample preparation and essential oil extraction

Fresh leaves were washed, chopped, and directly subjected to steam distillation. For dried leaf samples, the leaves were oven-dried at 40°C until they reached a constant weight, and then they were chopped before distillation. Approximately 75 g of each sample was distilled in 600 mL of distilled water for 3 hours using water-steam distillation apparatus. Oils were collected in airtight vials.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Essential oils were diluted in n-hexane and analyzed using a Thermo Scientific Trace 1310-ISQ 7000 GC-MS system equipped with a TG-5MS column. Compound identification was performed by comparing mass spectra with the NIST library and retention indices from the literature.

Antioxidant capacity assay

Antioxidant capacity was evaluated using the DPPH radical scavenging capacity, cupric ion reducing antioxidant capacity (CUPRAC), and ferric reducing antioxidant power (FRAP) following standard protocols. Each test was performed in triplicate (n=3). Trolox was used as the positive control, while methanol served as the negative control. Absorbance was read at 517 nm (DPPH), 450 nm (CUPRAC), and 595 nm (FRAP) using a UV-Vis spectrophotometer. Results were expressed in mg Trolox equivalent (TE) per gram of essential oil (Apak et al. 2008; Kicel et al. 2015; Irawan et al. 2022; Wu et al. 2023; Paula et al. 2023).

Cytotoxic activity assay

Cell culture

Cell culture of MCF-7 cells was obtained from the Hasanuddin University Medical Research Center (HUMRC). The cells were cultured in DMEM with 0.5% amphotericin B, 10% FBS, and 1% Pen-Strep at 37°C in a CO₂ incubator (5% CO₂ - 95% humidified air). A sterile polyethersulfone filter membrane with a pore size of 0.22 µm was used for sterilizing the enriched medium, which was then stored at 4°C.

Cell viability assay

The cytotoxicity of the essential oils was assessed against MCF-7 breast cancer cells using the MTT assay as described by Tanumihardja et al. (2020), with minor modifications. Cells (1 × 10⁵ cells/mL) were seeded in 96-well plates and incubated for 24 h, then treated with EO at varying concentrations (1-1000 µg/mL) or DMEM (control). After 24 h, MTT (0.5 mg/mL) was added and incubated for 4 h, followed by the addition of 10% SDS to stop the reaction. Absorbance was read at 595 nm, and cell viability was expressed as % inhibition:

$$\text{Inhibition (\%)} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\%$$

The inhibitory concentrations (IC₅₀) were graphed on the x-axis and y-axis to align the data with a linear regression.

Antimicrobial activity assay

The disc diffusion method was applied against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. The EO solution (200 µg/mL) was applied to sterile paper discs (6 mm) and placed on inoculated agar plates. Clindamycin (bacteria) and nystatin (fungi) serve as positive controls; DMSO serves as a negative control. The zone of inhibition, including the diameter of the disc, was measured after incubation. All tests were conducted in triplicate.

Data analysis

Data are expressed as mean ± standard deviation (SD). A one-way ANOVA was used to compare means, with a significance level of $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics version 27.

RESULTS AND DISCUSSION

Identified chemical compound of *Ocimum americanum* essential oil

The GC-MS analysis identified major differences between the volatile profiles of essential oils (EOs) obtained from fresh and dried leaves of *Ocimum americanum*. In the fresh leaf EO, citral isomers were dominant, with geranial (18.77%) and neral (17.25%) as the most abundant compounds, followed by geraniol (6.19%) and nerol (6.38%). In the dried leaf EO, the concentration of citral isomers slightly decreased, while linalool (5.27%) and fatty acid methyl esters such as hexadecanoic acid methyl ester (4.54%) and trans-13-octadecenoic acid methyl ester (6.41%) became more prominent (Table 1, Figure 1). These results indicate that post-harvest drying caused a shift in the chemical profile, particularly by reducing citral-related compounds and increasing oxygenated monoterpenes and fatty acid derivatives.

Table 1. Identified compounds of the essential oils of dried and fresh leaves of *Ocimum americanum* by GC-MS analysis

Compounds	Essential oil of dry leaves			Essential oil of fresh leaves		
	RT	SI	Relative area %	RT	SI	Relative area %
Linalool	6.316	954	5.27	6.296	961	0.65
Nerol (cis-geraniol)	8.612	900	0.63	8.615	948	6.38
Neral	8.846	882	13.65	8.884	875	17.25
Geraniol	9.057	809	0.16	9.125	955	6.19
Geranial	9.363	930	18.30	9.377	937	18.77
α-Bisabolene	13.989	880	0.91	13.999	940	2.90
Hexadecanoic acid, methyl ester	19.427	920	4.54	19.424	936	1.16
Trans-13-Octadecenoic acid, methyl ester	21.641	928	6.41	21.641	946	1.34

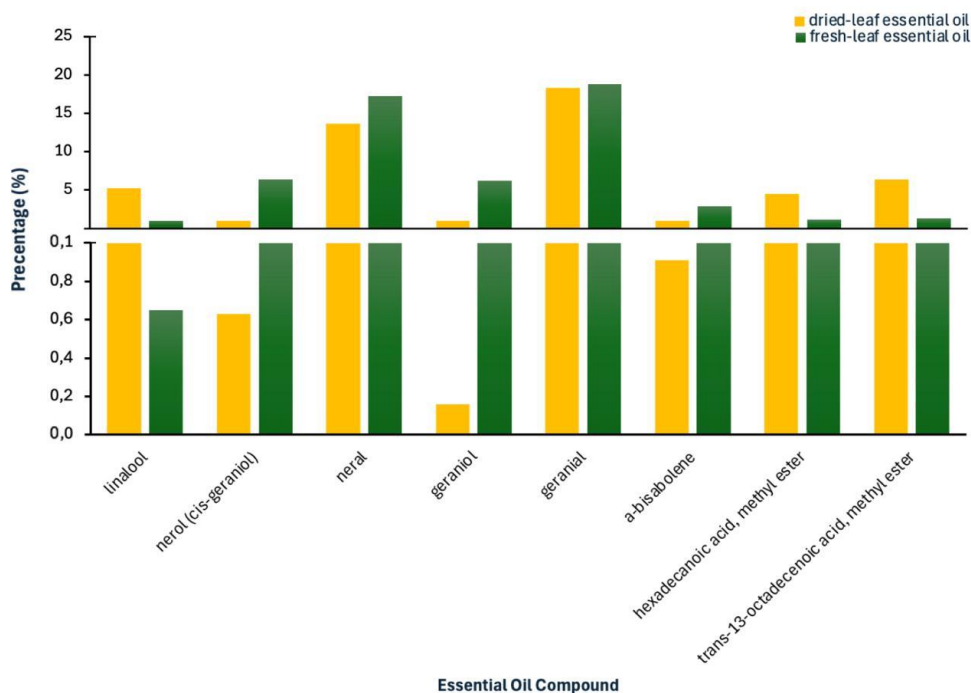


Figure 1. Percentage composition of the essential oils of *Ocimum americanum*

Antioxidant capacity of the leaf essential oil of *Ocimum americanum*

The antioxidant activity of the oils was evaluated using three complementary assays: DPPH radical scavenging, CUPRAC, and FRAP. The EO from fresh leaves exhibited the strongest radical scavenging capacity in the DPPH assay, with a value of 38.08±2.17 mg TE/g, compared to only 5.22±0.20 mg TE/g in the dried leaf oil (Table 2). By contrast, dried leaf EO showed significantly higher reducing power, with CUPRAC and FRAP values of 278.23±1.78 and 15.59±0.06 mg TE/g, respectively, compared to 253.7±4.72 and 6.18±0.02 mg TE/g for the fresh oil.

Statistical analysis confirmed that these differences were significant (p<0.01). The fresh leaf essential oil exhibited a markedly higher DPPH radical scavenging capacity compared to the dried leaf oil. In contrast, the CUPRAC and FRAP assays showed that the dried leaf essential oil possessed significantly greater reducing capacity than the fresh oil (p<0.01).

Cytotoxic activity of the leaf essential oil of *Ocimum americanum*

The cytotoxic effects of the essential oils (EOs) were evaluated against human breast cancer (MCF-7) cells using the MTT assay (Table 3). The EO from dried leaves exhibited stronger cytotoxicity, with an IC₅₀ value of 22.91 µg/mL, compared to 30.23 µg/mL for the fresh leaf oil. As expected, the standard anticancer drug doxorubicin was considerably more potent, with an IC₅₀ of 3.51 µg/mL. Although less active than the positive control, both oils demonstrated cytotoxic potential that falls within the range of 21-200 µg/mL, which is categorized as reasonably active for in vitro anticancer assays. These findings suggest that post-harvest drying enhanced the anticancer activity of *O. americanum* EO, while confirming its relevance as a natural source of bioactive compounds with promising therapeutic potential.

Antimicrobial activity of the leaf essential oil of *Ocimum americanum*

The antimicrobial assay demonstrated that both fresh and dried leaf essential oils inhibited the growth of Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungi (*Candida albicans*, *Aspergillus niger*) (Table 4). The inhibition zones produced by the oils

ranged from 7.63±0.76 mm to 9.67±0.58 mm, values that were slightly lower than those of the positive controls—clindamycin for bacteria and nystatin for fungi.

Despite these variations, statistical analysis revealed no significant differences (p>0.05) between the antimicrobial activities of fresh and dried oils. This suggests that the drying process did not markedly alter the antimicrobial potency of *O. americanum* essential oil, and that both forms retain comparable broad-spectrum inhibitory effects against bacteria and fungi.

Discussion

Post-harvest drying significantly alters the chemical composition of *O. americanum* essential oil (EO), resulting in significant differences between the profiles of fresh and dried leaf essential oils (Table 1, Figure 1). Both EOs are dominated by citral isomers—geranial and neral—but the fresh leaf EO contains a higher proportion of citral, geraniol, and nerol. The dried leaf EO shows higher linalool and fatty acid methyl esters such as trans-13-octadecenoic acid, methyl ester, and hexadecanoic acid, methyl ester (Table 1). This change is consistent with reports that drying can alter the chemical composition of essential oils (Bernhardt et al. 2015; Ray et al. 2022; Altay et al. 2024). Changes in composition influence the stability, aroma, and bioactivity of EO; therefore, processing can be used as a strategy to obtain specific EO properties for particular applications.

Table 2. Antioxidant capacities of the essential oil of *Ocimum americanum* leaves

Antioxidants capacity	RSC-DPPH ^a	CUPRAC ^a	FRAP ^a
EO of dry leaves	5.22±0.2	278.23±1.78	15.59±0.06
EO of fresh leaves	38.08±2.17	253.7±4.72	6.18±0.02

Note: ^a: Trolox equivalent (mg TE/g EO). IC₅₀ DPPH of trolox is 43.56 mg/L

Table 3. The IC₅₀ value of *Ocimum americanum* leaf essential oil against MCF-7 cancer cells

Sampel	IC ₅₀ (µg/mL)
EO of dry leaves	22.91
EO of fresh leaves	30.23
Doxorubicin	3.51

Table 4. Antimicrobial activity of the leaf essential oil of *O. americanum*

Microorganisms	Zone of inhibition (mm)			
	EO of fresh leaves	EO of dry leaves	Positive control	Negative control
Gram positive bacteria			Clindamycin	DMSO
<i>Staphylococcus aureus</i>	9.33±0.29	9.67±0.58	10	0
<i>Bacillus subtilis</i>	8.67±1.15	7.63±0.76	10	0
Gram negative bacteria				
<i>Escherichia coli</i>	9±1	9.67±0.58	10	0
<i>Pseudomonas aeruginosa</i>	9.67±0.29	9±1	10	0
Yeasts			Nistatin	DMSO
<i>Candida albicans</i>	8.75±0.29	7.75±0.87	9	0
<i>Aspergillus niger</i>	7.83±0.29	7.83±0.76	9	0

The difference in antioxidant profiles between fresh and dried leaf essential oils reflects this compositional variation (Table 2). The essential oil from fresh leaves showed a higher DPPH radical scavenging capacity, likely due to its higher citral and geraniol content (Ben Ammar 2023). Conversely, the essential oil from dried leaves showed higher CUPRAC and FRAP values, which may be related to the higher linalool and fatty acid methyl ester content—compounds that are more active through a single-electron transfer mechanism (Maczka et al. 2022). These findings are consistent with previous research showing that DPPH, CUPRAC, and FRAP assays yield different responses depending on the phytochemical composition (Yıldırım et al. 2001; Diniz do Nascimento et al. 2020; Roslan et al. 2020; Khodaei et al. 2021), confirming the complementary nature of these assays and the importance of correlation between phytochemical composition and antioxidant mechanisms.

EOs from fresh and dried leaves exhibited cytotoxic activity against MCF-7 breast cancer cells (Table 3), with the dry leaf EO showing better activity. This higher activity may be due to the synergism of citral, linalool, and methyl hexadecanoate, which have been reported to induce apoptosis through mitochondrial membrane disruption, oxidative stress formation, and caspase pathway activation (Chandrasekaran et al. 2011; Astiti and Ramona 2021; Elbe et al. 2022; Mostofa et al. 2023). The IC₅₀ values of *O. basilicum* and *O. canum* extracts were reported to be between 21-200 µg/mL, which is categorized as reasonably active in in vitro anticancer tests (Al-Kalaldeh et al. 2010; Anusmitha et al. 2022; Kancherla et al. 2023), thus supporting its potential as a natural anticancer agent.

The growth inhibition of both EOs against Gram-positive and Gram-negative bacteria and fungi (Table 4) showed no significant differences. However, there are specific variations in organisms: fresh EO tends to have wider inhibition against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans*, while dried EO is slightly more effective against *Staphylococcus aureus* and *Escherichia coli*. This variation may be related to the higher citral content in fresh EO, which can damage microbial membranes and increase permeability (Gutiérrez-Pacheco et al. 2023), as well as the higher linalool content in dried EO, which can provide additional antimicrobial mechanisms (Jabir et al. 2018; Maczka et al. 2022). The multicomponent nature of EO *O. americanum* is likely the basis for its broad-spectrum antimicrobial potential and may help reduce the risk of microbial resistance.

The findings of this study are consistent with those in other *Ocimum* species and chemotypes, indicating that drying typically reduces citral content and increases oxygenated monoterpenes such as linalool (Bernhardt et al. 2015; Arpiwi et al. 2023). Changes in antioxidant activity have also been reported in other aromatic plants such as *Rosmarinus officinalis*, suggesting that drying could be a common strategy for modifying antioxidant activity and optimizing specific functional properties. Overall, these findings indicate that fresh and dried EO of *O. americanum* have different but complementary bioactive profiles. Dry EO, with a higher linalool and trans-13-Octadecenoic acid,

methyl ester content, offers greater reduction capacity, higher cytotoxic potential, and better storage stability. The different activities may support the diversification of *O. americanum* utilization, enabling the development of various products from the same raw material. Optimizing post-harvest handling can increase the economic and therapeutic value of *O. americanum*, and also support sustainable production and biodiversity conservation.

In conclusion, postharvest drying has a significant impact on the chemical and biological properties of the essential oil of *Ocimum americanum*. The EO of fresh leaves was high in citral and geraniol and more effective in scavenging free radicals as well as exhibiting selective antimicrobial activity, making it suitable for antioxidant-focused applications and products with a fresh aroma. Conversely, the dried leaf EO had high linalool and fatty acid methyl ester content, a stronger reducing capacity, higher cytotoxic potential, and broader antimicrobial coverage, supporting its potential in functional foods, nutraceuticals, and phytopharmaceuticals. Drying extends shelf life, facilitates storage and distribution, and can enhance certain bioactivities. The simultaneous utilization of fresh and dried EO allows for targeted product development and market diversification. This strategy can increase the economic value of *O. americanum*, especially for local communities, and encourage the sustainable utilization.

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