

Article

Influence of Species, Growing Site, and Postharvest Leaf Handling on the Yield and Chemical Composition of Cajuput Oil (*Melaleuca* spp.) from Seram Island, Indonesia

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Abstract. Cajuput (*Melaleuca* spp.) is an important non-timber forest product from Seram Island, Maluku, widely used in the pharmaceutical, cosmetic, and health industries. Ideally, cajuput oil shows high yield and high 1,8-cineole (eucalyptol) content; however, in practice, yield and composition vary due to species, environment, and post-harvest handling. This variability reduces industrial consistency and highlights the need to identify optimal species, locations, and raw material conditions. This study aimed to evaluate the effects of species, growing location (Hatusua and Eti villages), and leaf condition on cajuput oil yield and composition. Two species, *Melaleuca leucadendra* and *M. cajuputi*, were selected for their contrasting chemotypes, dominated by eucalyptol and methyleugenol, respectively. Leaf samples (fresh and dried) were extracted by steam distillation, and chemical profiles were analyzed using GC-MS. Fresh leaves produced higher yields than dried leaves. The highest yield (1.022%) was obtained from fresh *M. leucadendra* from Eti, whereas drying reduced the yield to 0.008%. *M. leucadendra* oil was dominated by eucalyptol (56.62–58.50%), while *M. cajuputi* contained methyleugenol (81.61%) with a lower yield (0.1213%). Genetic factors determined dominant compounds, whereas environment and post-harvest handling influenced yield and minor constituents. These findings indicate that the use of fresh material, chemotype selection, and environmental optimization are essential for consistent production.

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Universitas Pattimura, Ambon, IndonesiaEmail : berly_mollucas@yahoo.com**1. Introduction**

Cajuput (*Melaleuca* spp.) is a significant non-timber forest product in eastern Indonesia, especially on Seram Island in Maluku Province. This island serves as a major center for cajuput oil production [1]. The oil is extracted from *Melaleuca* leaves and contains 1,8-cineole. It also has other monoterpenes with antibacterial, anti-inflammatory, and antioxidant properties. These characteristics explain its widespread use in pharmaceuticals, cosmetics, aromatherapy, inhalation products, balms, and traditional medicine [2–5]. Demand for natural products is rising, intensifying the need for consistent oil quality and stable production systems [3],[6-7]. However, cajuput oil produced by local communities in West Seram Regency continues to show significant variation in yield and cineole content. This affects its commercial value and compliance with market standards. Such differences can result from species variation, environmental conditions, and pre-distillation handling of raw materials.

On Seram Island, cajuput vegetation mainly consists of two species: *Melaleuca leucadendra* and *M. cajuputi*. These often grow together in similar environments. Although the species are morphologically similar, their essential oil profiles differ, mainly in the concentrations of eucalyptol and methyleugenol. Cajuput oil is usually produced by distilling fresh or dried leaves. The leaf condition strongly affects oil yield and chemical composition. The concentration of 1,8-cineole is the main indicator of cajuput oil quality and determines its commercial grade. According to the Indonesian National Standard (SNI 3954:2014), oils containing 50–<55% cineole are first grade, while those containing 70% or more are medicinal grade. Oils high in methyleugenol are less suitable for medicinal use due to their lower cineole levels and different aroma. Previous studies report that cineole concentrations in *Melaleuca* oils can range from about 17% to 90%, depending on species and chemotype [8–10].

Both genetic and environmental factors shape the chemical composition of *Melaleuca* essential oils. Inter-species differences produce distinct chemotypes. For example, *Melaleuca alternifolia* is known for high terpinen-4-ol content, while other *Melaleuca* species often contain more 1,8-cineole [10–13]. Within *M. leucadendra*, oil yield and composition can vary greatly by location. Environmental variables, such as soil type, rainfall, temperature, altitude, and local stress conditions, further affect biosynthesis of secondary metabolites and essential oils [10–13]. Plants in similar environments may have similar chemical profiles, even if they are different species. The same species in different locations can show substantial variation in metabolite composition.

Technical factors such as harvest timing, distillation duration, and leaf condition also impact oil yield and quality. Increased rainfall and flowering can enhance oil production. Extended distillation times may improve oil recovery [14–17]. Drying leaves often reduces yield, but the stability of key compounds depends on both species and drying method. Similar effects of genetic and environmental factors on chemotype have been found in other aromatic plants, such as *Thymus*, *Cinnamomum*, *Juniperus*, and *Lavandula*. These influences are highly specific to each species and compound [1]. In summary, essential oil composition is determined by plant genetics, environmental adaptation, and post-harvest handling of raw materials.

Many studies have investigated the influence of genetics and environment on essential oil composition. Few, however, have compared both cajuput species while also considering growing location and raw material condition, especially on Seram Island. Most earlier research focused on a single species, geographic variation, or technical factors. The combined effects of species, environment, and raw material remain insufficiently understood. There is also little information on

methyleugenol-rich chemotypes in *M. cajuputi* and on their effects on oil quality, as defined by SNI standards. This knowledge gap hinders the development of effective strategies for species selection and raw material management to produce high-quality, commercially valuable cajuput oil. This study investigates the effects of species, location, and raw material condition on the yield and chemical composition of cajuput oil in West Seram Regency.

This study is unique because it compares the effects of genetics, environment, and post-harvest conditions across two co-occurring *Melaleuca* species. Previous research examined these factors separately. This investigation examines species, ecological variation, and raw material condition within a single production area. It also provides new insights into methyleugenol-rich chemotypes and their importance for cajuput oil quality standards. The results are expected to deepen understanding of chemotype formation in *Melaleuca* species. They should also inform more selective, consistent, and sustainable cajuput oil production practices in Maluku.

2. Experimental Section

2.1 Research Design

This study employed a comparative experimental design to evaluate the effects of plant species, growing location, and raw material condition on the yield and chemical composition of cajuput oil. The observed variables included plant species (*Melaleuca leucadendra* and *Melaleuca cajuputi*), growing location, and raw material condition (fresh and dried leaves). The study was designed to compare the chemical profiles and oil yields of the two *Melaleuca* species under different environmental and post-harvest conditions.

2.2 Materials and Equipment

The main materials were leaves of *Melaleuca leucadendra* and *M. cajuputi*. These were collected from several growing locations in West Seram Regency, Maluku Province. Distilled water was used for the distillation process. The equipment included a Global Positioning System (GPS) device to record sampling coordinates, an analytical balance, and a drying oven. A water-steam distillation apparatus, consisting of a distillation kettle, condenser, and oil collection container, was used. Chemical composition analysis was done with a Gas Chromatography–Mass Spectrometry (GC-MS) instrument.

GC-MS analysis was performed using a capillary column (e.g., HP-5MS, 30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min⁻¹. The injector temperature was maintained at 250°C, while the detector temperature was set at 280°C. The oven temperature program was initiated at 60°C, held for 2 min, then increased to 280°C at 5°C min⁻¹ and maintained for 10 min.

2.3 Sampling Procedure

Leaf samples were collected using a purposive sampling method. Selection was based on the presence of two *Melaleuca* species, either growing at the same location or at different locations. The geographical coordinates of each sampling point were recorded using a GPS device. This ensured positional accuracy and helped facilitate environmental analysis. Leaves were collected from plants with similar physiological conditions, including leaf maturity and a healthy appearance. Several individual plants were sampled for each species and location to obtain representative samples.

The collected leaves were grouped by species and growing location, and subsequently divided into two treatments: fresh and dried. The drying treatment was conducted using a laboratory oven at 50°C until the leaves reached relatively constant weight. For each distillation treatment, approximately 5 kg of leaf material was used as the raw sample. This procedure was applied consistently across all treatments to ensure comparability among samples.

2.4 Distillation Process and Yield Calculation

Cajuput oil was extracted by water-steam distillation. Fresh and dried leaf samples were separately placed into the distillation kettle and distilled for approximately 6 h or until no additional oil was produced. The condensed distillate was collected, and the oil layer was separated from the water phase and stored in sealed glass containers prior to analysis.

Oil yield was calculated as the percentage (%) of oil extracted, based on the ratio between the weight of oil obtained and the initial weight of the leaf material used for distillation. The following formula was used:
$$\text{Oil Yield (\%)} = \frac{\text{Weight of oil obtained (g)}}{\text{Initial leaf weight (g)}} \times 100$$

2.5 Chemical Composition Analysis

The chemical composition of cajuput oil was analyzed by Gas Chromatography–Mass Spectrometry (GC-MS) to identify and quantify volatile compounds in the oil samples. Oil samples were injected into the GC-MS instrument under previously described operating conditions. Compound identification was conducted based on retention times and mass spectral fragmentation patterns, using standard reference libraries for comparison.

The detected compounds were recorded and expressed as relative percentage peak areas. This described the chemical profiles of cajuput oil from each species, growing location, and raw material condition. This analytical procedure allowed comparison of major and minor constituents among treatments. This supported the evaluation of chemotypic variation in the studied *Melaleuca* species.

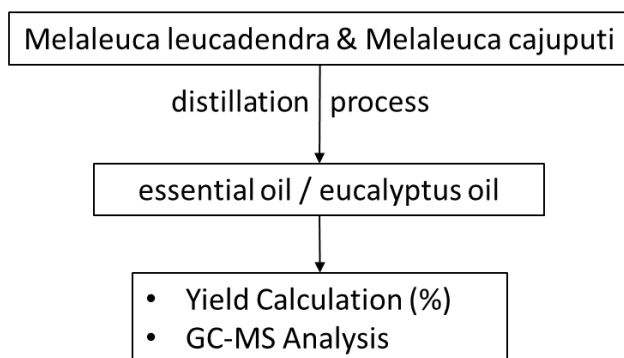


Figure 1. Research method flow chart

3. Results and Discussion

The results demonstrated that cajuput oil yield was strongly influenced by raw material condition, growing location, and plant species. A pronounced decline was observed in *Melaleuca leucadendra* from Hatusua Village, where oil yield decreased sharply from 0.710% in fresh leaves (MKPH1) to 0.008% in dried leaves (MKPH2). This drastic reduction indicates that drying caused severe loss of volatile compounds through evaporation and thermal degradation, particularly oxygenated monoterpenes such as eucalyptol (1,8-cineole), which are highly volatile and thermolabile [18–20].

The extremely low yield observed in dried samples indicates that volatilization is the dominant mechanism, as most monoterpenes are lost before any significant secondary transformation occurs. Similar reductions in essential oil yield under drying conditions have been reported in *Ocimum basilicum* and *Mentha piperita*, where volatilization and oxidative degradation significantly reduced monoterpene abundance [21–22].

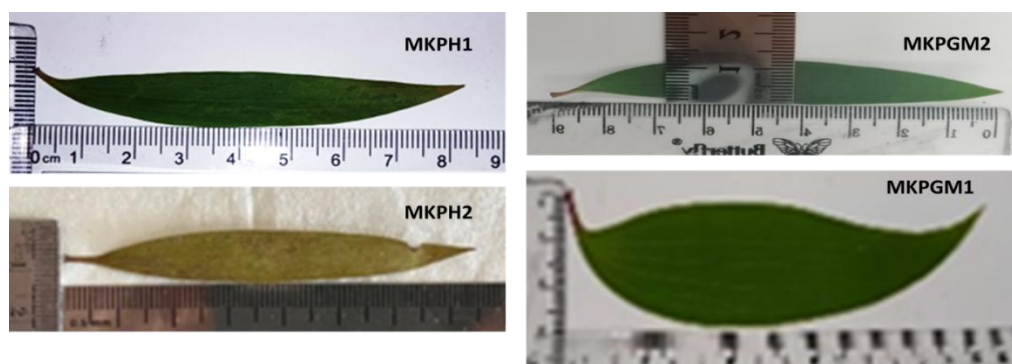


Figure 2. Samples of cajuput leaves, *Melaleuca leucadendra* and *Melaleuca cajuputi*

In addition to raw material condition, the growing location significantly affected oil yield. *M. leucadendra* from Eti Village produced a higher yield (1.022%) compared to Hatusua Village, despite identical species identity and extraction procedures. This indicates that environmental factors regulate biomass-level accumulation of essential oils rather than altering extraction efficiency. Soil fertility, water availability, light intensity, and microclimatic conditions influence photosynthetic performance and carbon allocation toward secondary metabolism. These factors collectively regulate terpenoid precursor availability, thereby affecting oil yield magnitude [14],[16],[23].

However, the observed variation should be interpreted cautiously, as the dataset reflects site-specific environmental conditions rather than controlled factorial experimentation. Therefore, the role of the environment in yield variation is supported but not yet fully quantifiable in isolation. Mild environmental stress may enhance secondary metabolite accumulation, whereas excessive stress suppresses metabolic activity and reduces yield [23–27], indicating a nonlinear environmental response.

Table 1. Sample codes, locations, gps coordinates, species, and oil yield

Sample Code	Sample Condition	Location	GPS Coordinates	Species	Yield (%)
MKPH1	Fresh sample	Hatusua Village	Lat -3.327549; Long 128.336167	<i>Melaleuca leucadendra</i>	0.7100
MKPH2	Dried sample	Hatusua Village	Lat -3.321176; Long 128.344090	<i>Melaleuca leucadendra</i>	0.0080
MKPGM2	Fresh sample	Eti Village	Lat -3.064216; Long 128.143594	<i>Melaleuca leucadendra</i>	1.0220
MKPGM1	Fresh sample	Eti Village	Lat -3.064216; Long 128.143594	<i>Melaleuca cajuputi</i>	0.1213

Building on this, genetic factors strongly determined interspecific variation in essential oil yield under similar environmental conditions. In Eti Village, *M. leucadendra* (MKPGM2) yielded 1.0220%, whereas *M. cajuputi* (MKPGM1) produced only 0.1213%. This large disparity confirms that biosynthetic capacity is genetically encoded, particularly through differential expression of enzymes regulating terpenoid biosynthesis pathways. These include terpene synthases and precursor-modifying enzymes that determine flux toward monoterpene or phenylpropanoid products. Such genetic control governs both total yield and dominant chemotype expression. High heritability of essential oil traits has also been reported in *Atractylodes lancea*, where sesquiterpene composition shows strong genetic stability across environments [28].

Similar genotype-driven chemotypic dominance has been documented in basil and thyme [29-30]. This clear separation supports the concept of chemotype stability under genetic control, with environmental variation acting primarily as a quantitative modulator rather than a determinant of chemical identity.

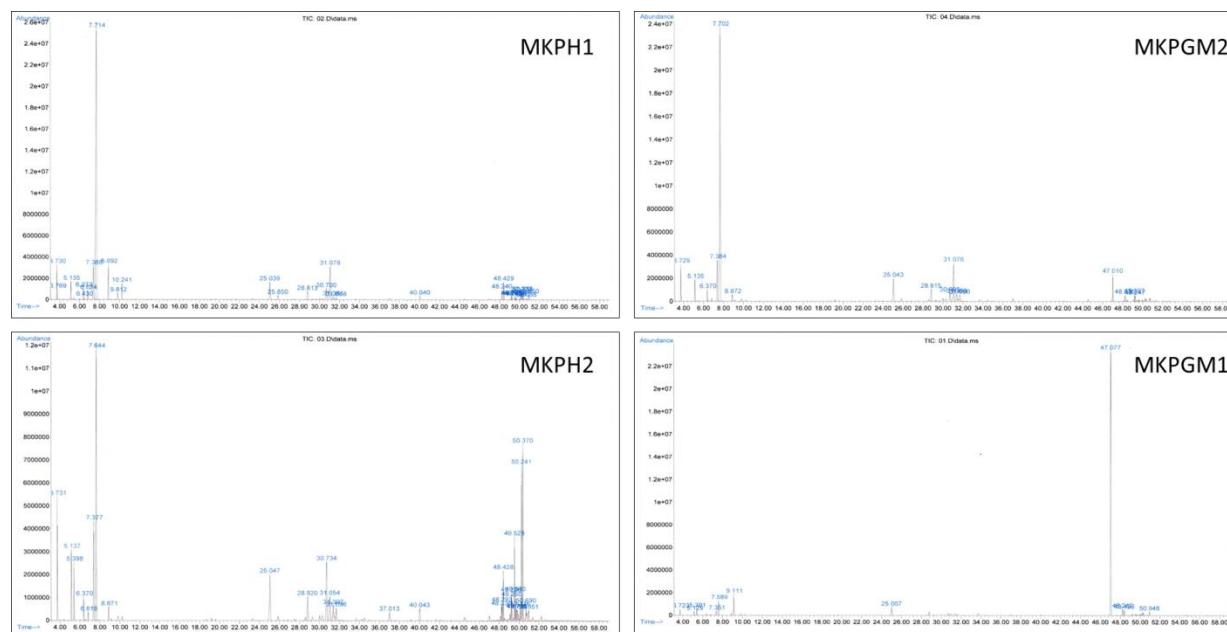


Figure 3. GC-MS chromatograms of cajuput oil from *Melaleuca leucadendra* and *Melaleuca cajuputi*

Beyond yield, significant variation in chemical composition was observed. Fresh leaves of *M. leucadendra* from Hatusua Village (MKPH1) were dominated by eucalyptol (56.62%), along with α -terpineol, d-limonene, and γ -terpineol, indicating a typical oxygenated monoterpene profile associated with antimicrobial activity. In contrast, dried leaves (MKPH2) exhibited reduced eucalyptol content (22.35%) and the emergence of compounds such as safrole, naphthalene, and 2-naphthalenemethanol. The appearance of safrole and naphthalene derivatives is unlikely to reflect true biosynthesis in fresh tissue, but rather to reflect chemical transformation during drying. These compounds may arise from thermal rearrangement, oxidation of phenylpropanoid precursors, or pyrolysis-related degradation of lignin-derived structures. In particular, naphthalene-type compounds are commonly associated with thermal decomposition of plant biomass, indicating that drying induces artifact formation rather than metabolic synthesis.

Drying effects involve not only physical volatilization but also coupled biochemical and chemical transformations. In plant matrices such as grains, ginseng, and mushrooms, drying has been shown to induce enzymatic activation, oxidative reactions, and restructuring of secondary metabolites involving amino acids, lipids, and phenolic compounds [31-33]. In this context, compositional changes in essential oils arise from interactions among volatilization loss, oxidative degradation, and thermally induced molecular rearrangements rather than from simple moisture removal.

Table 2. Chemical composition (%) of cajuput oil identified by GC–MS

Compound Name	MKPH1	MKPH2	MKPGM2	MKPGM1
Alpha pinene	2.54	5.31	3.00	–
Beta pinene	1.63	3.74	2.13	–
Bicycle[3.1.0]hexane, 4-methylene-1-(1-methylene-1-(1-methylethyl	–	3.03	–	1.19
Beta myrcene	1.22	1.48	1.34	–
d-Limonene	5.58	7.22	6.97	–
Eucalyptol (1,8-cineole)	56.62	22.35	58.50	3.47
Gamma terpineol	4.44	–	–	–
Cyclohexene, 1-methyl-4-(1-methylethylidene)	2.19	–	–	–
Beta ocimene	–	–	–	5.02
Caryophyllene	3.76	5.88	5.47	3.47
Humulene	1.67	2.90	2.75	–
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl	2.25	7.14	1.80	–
Alpha terpineol	6.96	2.96	8.63	–
Naphthalene	–	1.92	1.68	–
Safrole	–	1.56	–	–
Guaiol	1.44	2.33	–	–
2-Naphthalenemethanol	–	7.71	–	–
Methyleugenol	–	–	3.71	81.61

Environmental factors also influenced chemical composition in *M. leucadendra* from Eti Village (MKPGM2), where eucalyptol reached 58.50% alongside the highest yield (1.022%). This suggests strong activation of the MEP pathway (2-C-methyl-D-erythritol 4-phosphate pathway, which creates monoterpenes) responsible for monoterpene biosynthesis. Minor variations, including the presence of methyleugenol, indicate metabolic flexibility without altering the dominant chemotype (the main chemical profile present). Essential oils are synthesized through coordinated MEP (monoterpene) and MVA (mevalonate, producing sesquiterpenes) pathways, as well as the shikimate pathway (producing phenylpropanoids) [34].

Environmental modulation (changes driven by environmental factors) may alter the relative flux among these pathways without altering the primary genetic chemotype. Abiotic factors such as temperature and salinity may regulate gene expression levels (the degree to which certain genes are active) of key biosynthetic enzymes, thereby modifying metabolite proportions (relative amounts of chemical products) without altering core biosynthetic identity [26],[35].

Clear interspecific differences were observed at the same location. *M. leucadendra* was dominated by eucalyptol (58.50%), reflecting MEP pathway dominance, whereas *M. cajuputi* was dominated by methyleugenol (81.61%), indicating strong activation of the shikimate-derived phenylpropanoid pathway. The exceptionally high methyleugenol content suggests elevated O-methyltransferase (EOMT) activity, which catalyzes the methylation of eugenol to methyleugenol [36–38].

This divergence demonstrates a clear chemotype distinction driven primarily by genetic factors. Importantly, methyleugenol at high concentrations has been reported as a compound of toxicological concern due to its hepatocarcinogenic and genotoxic potential in experimental systems. This aligns with regulatory concerns in several international safety frameworks, where methyleugenol-rich essential oils are subject to restricted use or mandatory reduction in cosmetic and food applications. Therefore, although *M. cajuputi* exhibits high phenylpropanoid yield, its industrial utilization requires

careful compositional control. This clear chemotypic divergence confirms that genetic factors primarily determine biosynthetic direction, while environmental factors fine-tune expression intensity [15],[39].

Overall, the results confirm that cajuput oil yield and composition are determined by the interaction of raw material condition, environmental factors, and genetic background. Fresh plant material is essential to preserve volatile compounds and maximize yield, while environmental conditions influence productivity through physiological regulation of secondary metabolism. Genetic factors determine chemotype identity and dominant biosynthetic pathways, whereas post-harvest drying introduces physicochemical transformations that may significantly distort volatile profiles through volatilization and thermal degradation. Therefore, optimized cajuput oil production requires an integrated management approach that includes selecting appropriate species, controlling harvesting conditions, and standardizing post-harvest handling to minimize compositional artifacts and maximize industrial value.

4. Conclusion

The yield and chemical composition of cajuput oil (*Melaleuca* spp.) depend on raw material condition, environment, and genetics. Fresh *Melaleuca leucadendra* leaves yield more oil (0.710–1.022%), with eucalyptol (1,8-cineole) as the main compound. Drying sharply reduces the yield to 0.008% due to evaporation and thermal breakdown of oil components. *Melaleuca cajuputi* yields less oil (0.1213%) but has more methyleugenol (81.61%), reflecting strong genetic control of metabolic pathways and chemotype. The environment mainly affects the oil amount, making only minor changes to the main compounds. Thus, oil yield and quality depend on stable chemotypes, environmental effects on metabolite accumulation, and post-harvest handling. Using fresh material, selecting proper chemotypes, and managing the environment are key to consistent industrial production.

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