# Asian Journal of Plant Sciences

ISSN 1682-3974

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Asian Journal of Plant Sciences
ISSN 1682-3974
DOI: 10.3923/ajps.2024.467.474

# Research Article
# Comparative Study of Essential Oil Extracted from the Fresh Leaves of Malaysian *Melaleuca leucadendron* Linn. Using Hydrodistillation and Steam Distillation Methods

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## Abstract
**Background and Objective:** Essential oils, also known as etheric or volatile oils, are aromatic compounds extracted from plant parts like leaves, flowers, seeds and roots. These complex mixtures are primarily obtained through various distillation methods, including water, steam, or dry distillation. This study aimed to compare the content of essential oil yield and composition of fresh leaves of *Melaleuca leucadendron* from Malaysia. **Materials and Methods:** Fresh leaf samples were subjected to two different methods of extraction, which were hydrodistillation and steam distillation. The collected essential oils (EOs) were analyzed by gas chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). Collected data were analyzed using the SPSS statistical package software version 17.0. (Chicago, USA). A t-test at $p<0.05$ was used to determine the significance of variation between the two methods of distillation. **Results:** The oil yield was 0.278% for hydrodistillation and 0.378% for steam distillation, with no significant statistical difference. Steam distillation yielded 29 components, while hydrodistillation yielded 28, sharing 16 components (36.4% similarity). Terpinolene (27.79%) was predominant in hydrodistillation, whereas O-Cymene (41.5%) was the major component in steam distillation, highlighting *Melaleuca leucadendron* Linn. as a rich source of O-Cymene. **Conclusion:** Detection of these compounds has revealed that they can be used in the production of pesticides, which is crucial for sustainable agriculture and reducing environmental pollution caused by chemical pesticides.

Key words: *Melaleuca leucadendron*, essential oil, extraction method, Terpinolene, O-Cymene, $\gamma$-Terpinene, sustainability

Citation: Abdelmageed, A.H.A., F. Qamaruz Zaman and K. Jaffel, 2024. Comparative study of essential oil extracted from the fresh leaves of Malaysian *Melaleuca leucadendron* Linn. using hydrodistillation and steam distillation methods. Asian J. Plant Sci., 23: 467-474.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

In 2000, the Malaysian Industry-Government Group on High Technology (MIGHT) came out with a Malaysian Herbal Products Blueprint, a master plan for the development of the herbal products industry[1]. The botanical term defines an herb as a plant that has no woody tissue and that dies down to the ground at the end of a growing season. Usually, herbs are processed for culinary, pharmaceutical, sanitary, cosmetic, medicinal and fragrance purposes. These plant species range from shrubs to large trees[2]. Natural medicines have gained recognition as a safe, effective and cost-efficient alternative to pharmaceuticals, with research supporting these benefits.

Demand for plant essential oil has risen because it is a more natural alternative to disease-fighting medication, biodegradable and less toxic than synthetic preservative agents[3]. Essential oils also known as etheric oil or volatile oil derived from various parts of the plant including leaves, flowers, fruits, seeds and roots. The aromatic components which are complex mixtures of volatile are usually obtained by water distillation, steam distillation, water-and-steam distillation, or dry distillation[3]. Hydrodistillation and steam distillation are the most common techniques that are used for the isolation of EOs. Recent literature proves that hydrodistillation and steam distillation are the primary methods of choice when it comes to the isolation of EOs. Besides, in developing countries, these two methods are often the methods of choice because they are cheap[4]. However, steam distillation is the most common procedure[5]. Other methods such as enfleurage, expression, extraction or fermentation are also used for the isolation of more sensitive compounds. Other methods can be used to extract the essential oil such as rectification extraction[6] and supercritical fluid $CO_2$ extraction[7].

*Melaleuca leucadendron* Linn. (Myrtaceae) which is locally in Malaysia and known as "Gelam" is among the herbs that can be found in Malaysia. It belongs to the family Myrtaceae[8] and is widespread throughout Indo-Malaysian and the Tropical Australian Regions. It is commonly found in open forests or shrubland on swamp edges along the coastal areas, reaching a height of 20-30 m. About 230 species of *Melaleuca* are known in the world[9].

According to Noor[10], *Melaleuca cajuputi* Powell (MC) which is locally known as "Gelam" or "pokok kayu putih" essential oil is widely used in Malay traditional medicine for the treatment of stomach cramps, cough, acne, insect bites, thrush problems and many more. Embong[8] reported that the oil is fluid and greenish as it contains traces of copper. It tastes bitter, is highly volatile and inflammable and burns without residue. The oil is said to be antispasmodic, diaphoretic, stimulating, antiseptic and anthelmintic. The leaves are very aromatic. It contains essential oils that give a distinct and stimulating odor like camphor or cardamom seeds when crushed. Gelam, a species of *Melaleuca*, is commercially valued for its essential oils, similar to tea tree and cajuput oils. The essential oil of *Melaleuca leucadendron* is recognized for its beneficial properties. Studies have highlighted its fungicidal effects, with potential applications in managing significant fungal pathogens affecting crops like *Ipomoea batatas*[11,12]. These essential oils also offer a sustainable alternative to synthetic fungicides.

In addition, EOs of *Melaleuca* species are known to have antibacterial, antioxidant and anti-inflammatory activities[13,14]. However, the production of EOs is scarce and time-consuming with conventional methods. Despite such wide-ranging studies on the EOs content of *Melaleuca* species, very little work has been done to study this species. Moreover, previous studies on the comparison of *M. leucadendron* oil content between two different methods which are hydrodistillation and steam distillation are very limited. Based on available information, for long, Gelam has been overlooked in Malaysia, with its commercial potential and medicinal benefits often underestimated due to the limited number of studies conducted on these aspects[15].

Therefore, the present study aimed to determine the extraction methods that produced a higher yield of EOs in fresh leaves of *M. leucadendron* using hydro distillation and steam distillation and to determine the profile component of *M. leucadendron* oil yield from both methods.

## MATERIALS AND METHODS

**Study area:** The present study was conducted during 2017-2018 at the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

**Plant specimens:** Leaves part of *M. leucadendron* were investigated for the EOs. The plant samples being undertaken at the Kolej Sultan Alaeddin Suleiman Shah in Universiti Putra Malaysia. The species was identified by the taxonomist Dr. Shmsul Khamis from the Institute of Bioscience, Universiti Putra Malaysia (UPM). A voucher specimen (IR 0127/11) has been deposited in the herbarium of the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia.

**Plant sample preparation:** The plant samples were harvested during the dry season when the yield of oil is generally higher[1]. Only matured leaves were harvested for study. During harvesting, no pests or diseases were observed on any plant. Fresh leaves were cut into small pieces. These plant materials were weighed to obtain the fresh weight for each treatment.

**Extraction of EOs by hydrodistillation method:** Extraction of EOs was carried out on a sample size of 300 g of fresh leaves placed in a flask containing 3 L of distilled water and connected to a Clevenger distillation apparatus. Then electrical heating was used. As the water started to boil, the vapor and the EOs extracted from the plant passed through the cooler, where both the oil and water were condensed and collected. The temperature was set up at 30°C and the operation lasted for 4 hrs using Clevenger apparatus as described previously by Qamaruz Zaman *et al.*[16]. The EOs obtained were dried over anhydrous sodium sulfate (Merck, AR grade) and then stored at 4-6°C before analysis. The experiment was conducted in triplicate.

**Extraction of EOs by steam distillation method:** About 300 g of fresh sample was subjected to steam distillation in a Clevenger-type glass apparatus. The steam was produced in the boiler by electrical heating of 3 L of distilled water. As the water started to boil, the vapor from the boiler went through the fresh plant. Then the vapor and the EOs extracted from the plant passed through the cooler, where both the oil and water were condensed and collected. The extraction process was done for 4 hrs with a temperature of 30°C using Clevenger apparatus as described previously by Qamaruz Zaman *et al.*[16]. The EOs obtained were dried over anhydrous sodium sulfate (Merck, AR grade) and then stored at 4-6°C before analysis. The experiment was conducted in triplicate.

**Gas chromatography (GC) analysis:** Essential oils obtained from the fresh leaves of *Melaleuca leucadendron* were analyzed using a Shimadzu GC-2010 Plus system (Shimadzu Corporation, Kyoto, Japan), featuring a flame ionization detector (FID) and a BPX5 fused silica capillary column (30 m×0.25 mm×0.25 μm film thickness). The sample injection volume was set at 1.0 μL with a split ratio of 13:1 and the injector temperature was maintained at 230°C. Helium served as the carrier gas at a constant flow rate of 1.0 mL/min. The column temperature was programmed to start at 30°C (held for 3 min), then increased at a rate of 8°C/min to 230°C (held for 5 min) and finally held at 245°C for 10 min.

**Gas Chromatography-Mass Spectrometry (GC-MS):** The essential oils were also examined using a Shimadzu GC-MS QP2010 Plus system (Shimadzu Corporation, Kyoto, Japan), fitted with a BPX5 fused silica capillary column (30 m×0.25 mm×0.25 μm film thickness). Helium, at a flow rate of 1.0 mL/min, was used as the carrier gas. The ion source and interface temperatures were maintained at 200 and 310°C, respectively. The mass detector operated under the following conditions: Acquisition start time of 6.00 min, end time of 49.00 min, scan mode with a 0.50 sec event time, scan speed of 1000 and a scan range of m/z 35.00-500.00. The temperature programming for the GC-MS oven was identical to that of the GC analysis.

**Identification of chemical components:** The individual constituents of the EOs were identified by comparing their retention times and mass fragmentation pattern with those standards[17] and NIST (National Institute of Standards and Technology) library[18]. The percentage of constituents was obtained from the GC peak without any correction factors[19,20].

**Statistical analysis:** The experiments were conducted in triplicate. The data were analyzed by performing an One-way Analysis of Variance (ANOVA) using SPSS statistical package software version 17.0. (Chicago, USA). A t-test was used to determine the significance of variation between the two methods of distillation. The statistical significance was determined at $p<0.05$.

## RESULTS AND DISCUSSION

**Yield of EOs:** Fresh leaves of *M. leucadendron* were subjected to hydrodistillation and steam distillation for 4 hrs to extract their EOs. The oil obtained is pale yellow-green and gives a distinct and stimulating odor like camphor. In this study, the EOs obtained from all samples were less dense than water. The yields of EOs obtained from the extraction were different in both methods.

As shown in Fig. 1, the total oil yield for hydrodistillation was 0.278% while for steam distillation was 0.378%. Statistically, the hydrodistillation method and steam distillation method have the same rate of producing EOs of *M. leucadendron*. Even though there are no significant differences between these two methods, the content of *M. leucadendron* oil showed there are differences in major chemical constituents after undergoing these two different methods. According to Sakasegawa *et al.*[11], the EOs yield of Gelam in steam distillation ranged from 1.6-3.6%. Silva *et al.*[21] mentioned that the average yields of *M. leucadendron* in Brazil using hydrodistillation methods were 1.5%.

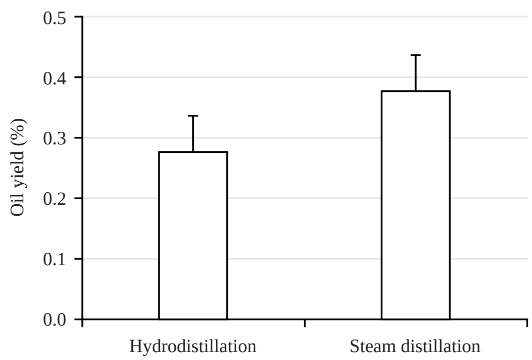


Fig. 1: Essential oil yield (%) obtained from fresh leaves of *M. leucadendron* in hydrodistillation and steam distillation

Many factors can affect the oil yield and the chemical components of the EOs such as genetic structure[22], seasonal[23,24] and the location in which the plant grows. The extraction product can vary in quality, quantity and composition according to climate, soil composition, plant organ, age and vegetative cycle stage[25-27].

Different types of extraction techniques also have a great influence on oil production, quality of oil, chemical constituents and oil characteristics.

Previous study shows that hydrodistillation produces higher oil yield than steam distillation[28-30]. However, according to Boutekedjiret *et al.*[31], the yield of rosemary EOs obtained by the steam distillation is higher than the yield of the hydrodistillation. Whatever extraction techniques were chosen, the raw material is the starting point that affects the production of EOs[32]. "This was probably because the situation of plant material, including type, mode of comminution, mode of charging and grade of insulation, is much more critical than the distillation methods".

**Comparison of chemical constituents of *M. leucadendron* in hydrodistillation and steam distillation:** In hydrodistillation analysis, EOs showed the presence of 28 chemical constituents (Table 1). The major chemical component found was Terpinolene (27.79%) followed by γ-Terpinene (23.20%), M-cymene (13.76%), 2-Carene (4.57%), α-Phellandrene (4.11%) and Limonene (4.11%).

Twenty-nine components were identified in the analysis of *M. leucadendron* in steam distillation (Table 2). The most abundant component was O-Cymene (41.5%). The second most abundant component was Terpinolene (13.93%). Other major compounds identified also included γ-Terpinene (10.06%), α-Phellandrene (7.59%), α-Pinene (5.72%) and Caryophyllene (2.94%).

Based on Table 1, the EOs of *M. leucadendron* showed a predominance of monoterpene hydrocarbons (88.05%) followed by sesquiterpene hydrocarbons (6.91%). Oxygenated monoterpenes (3.34%) and oxygenated sesquiterpenes further compose this oil in hydrodistillation. In steam distillation, the EOs contains 88.08% monoterpene hydrocarbons followed by oxygenated monoterpenes (5.71%), sesquiterpene hydrocarbons (5.04%) and other aromatic compounds (oxygenated sesquiterpenes by 0.56%) (Table 2). These results showed that monoterpene hydrocarbons (Terpinolene, O-Cymene) are marker constituents in the EOs of the *Melaleuca leucadendron* species grown in Malaysia.

The steam distillation method offered higher percentage amounts of oxygenated monoterpenes than the hydrodistillation method, while hydrodistillation offered higher amounts of sesquiterpene hydrocarbons, compared to the steam distillation method (Table 1-2).

There were 16 same components identified in oil extracted using both methods. The percentage of similarity of chemical components found between these two methods was 36.4%. It can be seen that these oils were characterized by the presence of γ-Terpinene, Terpinolene, α-Phellandrene, α-Pinene and α-Thujene but the quantitative differences were observed in the contents of these components. The major chemical component found in hydrodistillation was Terpinolene (27.79%) and the most abundant component in steam distillation was O-Cymene (41.5%).

The EOs of *M. leucadendron* were categorized as the methyl eugenol-rich type, the isomethyl eugenol-rich type[14] and the terpenoids-rich type. However, in this experiment, methyl eugenol and isomethyl eugenol were not detected in gelam EOs. This might be due to the different chemotypes expressed in different environments. According to Sakasegawa *et al.*[11], the major constituents of gelam oil in Japan were α-Terpinene, γ-Terpinene, terpinolene, α-Phellandrene and α-Caryophyllene. All of these constituents also can be found in this study. Silva *et al.*[21] mentioned that the main components of *M. leucadendron* in Brazil were 1, 8-cineole, methyl eugenol and viridiflorol. *Melaleuca* species grown in Brazil express chemotypes different from those found in this study and in other environments such as Australia. Furthermore, Fall *et al.*[33] stated that the major constituents of the EOs of *Melaleuca leucadendron* leaves from Senegal were 1.8-cineole, epiglobulol, α-pinene, limonene and α-terpineol, respectively. Something very interesting was noticed about the chemical composition of the EOs in this study showed the presence of O-Cymene (41.5%). While, this component hasn't been reported in the oils of chemotypes from Indonesia, Brazil and Senegal mentioned here.

Table 1: Chemical constituents of *M. leucadendron* oil in hydrodistillation

| Number | Compounds | RT[a] | RI[b] | RI[c] | Area[d] (%) |
|---|---|---|---|---|---|
| 1 | α-Thujene | 7.984 | 924 | 928 | 3.74 |
| 2 | α-Pinene | 8.155 | 932 | 934 | 3.13 |
| 3 | β-Pinene | 8.946 | 943 | 974 | 1.12 |
| 4 | 2-Carene | 9.068 | 948 | 1001 | 4.57 |
| 5 | α-Phellandrene | 9.206 | 964 | 1002 | 4.11 |
| 6 | Sabinene | 9.592 | 968 | 975 | 0.72 |
| 7 | Myrcene | 9.775 | 989 | 990 | 0.73 |
| 8 | Limonene | 9.948 | 1018 | 1024 | 4.11 |
| 9 | α-Ocimene | 10.006 | 1023 | 1032 | 0.34 |
| 10 | 1,3,5-tris(methylene)cycloheptane | 10.065 | 1035 | 1039 | 0.12 |
| 11 | cis-β-Ocimene | 10.258 | 1037 | 1039 | 0.3 |
| 12 | m-Cymene | 10.516 | 1042 | 1082 | 13.76 |
| 13 | Terpinolene | 10.987 | 1052 | 1086 | 27.79 |
| 14 | γ-Terpinene | 11.149 | 1054 | 1054 | 23.2 |
| 15 | α-ρ-dimethylstyrene | 11.199 | 1073 | 1099 | 0.43 |
| 16 | Linalool | 11.517 | 1082 | 1098 | 0.47 |
| 17 | 1-Terpinen-4-ol | 12.645 | 1137 | 1156 | 2.07 |
| 18 | α-Terpineol | 12.879 | 1143 | 1186 | 0.68 |
| 19 | Perillaldehyde | 15.445 | 1207 | 1269 | 0.25 |
| 20 | α-Copaene | 15.596 | 1221 | 1374 | 0.17 |
| 21 | Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1methylethenyl) | 16.102 | 1398 | 1580 | 1.04 |
| 22 | 1-methyl-2,4-bis(1-methylethylidene)-1-vinylcyclohexane | 16.591 | 1465 | 1558 | 0.34 |
| 23 | δ-Cadinene | 16.910 | 1469 | 1522 | 0.24 |
| 24 | Isocaryophyllene | 17.042 | 1494 | 1464 | 3.22 |
| 25 | Caryophyllene oxide | 17.095 | 1507 | 1582 | 0.39 |
| 26 | Germacrene D | 17.306 | 1515 | 1485 | 0.33 |
| 27 | Spathulenol | 18.169 | 1536 | 1578 | 0.16 |
| 28 | α-Humulene | 18.261 | 1579 | 1608 | 1.32 |
| | **Class composition** | | | | |
| | I-Monoterpene hydrocarbons (1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15) | | | | 88.05 |
| | II-Oxygenated monoterpenes (10, 16, 17, 18) | | | | 3.34 |
| | III-Sesquiterpene hydrocarbons (19, 20, 21, 22, 23, 24, 26, 28) | | | | 6.91 |
| | IV-Oxygenated sesquiterpenes (25, 27) | | | | 0.55 |
| | Total identified compounds (%) | | | | 98.85 |

[a]Retention time, [b]Experimental retention index, [c]Retention index retrieved from literature review and [d]Concentration of the chemical compounds (%)

The constituents of EOs are influenced by internal and external factors. Internal factors are such as genetic structures and external factors such as ecological conditions and geographical area. Altitude, type of soil, temperatures and weather conditions are also some influences in the extraction process. These variables are according to the physiological process of the plant itself. Another factor that may affect the EOs yield and composition is whether the plant materials were extracted freshly or went through the drying process[34]. The EOs are the oil that easily evaporates[32]. In this situation, the oil yield percentage may be influenced by the crushing and the cutting size of plant materials. The small pieces of materials caused the oil to evaporate easily because the exposed area of the plant materials to the air is increased and it also increases the probability of the oils being lost into the environment.

Regarding extraction techniques, Binzet *et al.*[35] reported that the quantitative differences in the oil composition of rosemary extracted using different extraction methods were due to the usage of different extraction processes. According to Babu and Kaul[29], composition of EOs was influenced by the type of distillation apparatus used. The total average percentage yield of *M. leucadendron* oil using hydrodistillation was 0.278% while the EOs obtained using steam distillation was 0.378%. However, there was no significant difference between hydrodistillation and steam distillation methods. The GC-MS identified 28 and 29 chemical constituents from fresh leaves of hydrodistillation and steam distillation, respectively. The percentage of similarity of chemical components found between these two methods was 36.4%. The most abundant components in fresh hydrodistillation were Terpinolene (27.79%) while in fresh steam distillation was O-Cymene (41.50%).

The major compounds detected in the EOs of *Melaleuca leucadendron* Linn. in this study like O-Cymene (41.50%) and Terpinolene (27.79%) were reported to function as insecticidal and repellent[36], fungicidal[12,37] disinfectants against bacteria[15]. Based on the properties of the identified major compounds detected in the EOs of *Melaleuca leucadendron* Linn. in this

Table 2: Chemical constituents of *M. leucadendron* in steam distillation

| Number | Compounds | RT[a] | RI[b] | RI[c] | Area[d] (%) |
|---|---|---|---|---|---|
| 1 | α-Thujene | 7.965 | 924 | 928 | 2.19 |
| 2 | α-Pinene | 8.136 | 932 | 934 | 5.72 |
| 3 | β-Pinene | 8.931 | 943 | 974 | 0.61 |
| 4 | α-Phellandrene | 9.052 | 964 | 1002 | 7.59 |
| 5 | Sabinene | 9.191 | 968 | 975 | 0.56 |
| 6 | β-Phellandrene | 9.580 | 969 | 1025 | 0.53 |
| 7 | Myrcene | 9.761 | 989 | 990 | 0.68 |
| 8 | α-Terpinene | 9.933 | 998 | 1014 | 1.7 |
| 9 | γ-Terpinene | 9.991 | 1014 | 1054 | 10.06 |
| 10 | D-Limonene | 10.050 | 1018 | 1024 | 2.78 |
| 11 | O-Cymene | 10.503 | 1042 | 1026 | 41.5 |
| 12 | Terpinolene | 10.975 | 1052 | 1086 | 13.93 |
| 13 | 3-Cyclohexene-1-carboxaldehyde | 11.140 | 1069 | 1273 | 0.71 |
| 14 | α-p-dimethylstyrene | 11.186 | 1073 | 1099 | 0.23 |
| 15 | Linalool | 11.978 | 1082 | 1095 | 0.25 |
| 16 | 1-Terpinen-4-ol | 12.633 | 1137 | 1174 | 1.99 |
| 17 | α-Terpineol | 12.750 | 1143 | 1186 | 0.54 |
| 18 | ρ-Cymen-8-ol | 12.868 | 1197 | 1182 | 0.67 |
| 19 | Copaene | 15.435 | 1221 | 1374 | 0.44 |
| 20 | Cyclohexane, 2,4-diisopropenyl-1-methyl-1-vinyl | 15.586 | 1398 | 1401 | 1.11 |
| 21 | α-amorphene | 16.092 | 1440 | 1483 | 0.12 |
| 22 | δ-Cadinene | 16.581 | 1469 | 1513 | 0.2 |
| 23 | α-Selinene | 16.755 | 1474 | 1498 | 0.25 |
| 24 | α-Guaiene | 16.898 | 1490 | 1439 | 0.38 |
| 25 | Caryophyllene | 17.033 | 1494 | 1466 | 2.94 |
| 26 | 2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-2,6-diene | 17.105 | 1499 | 1499 | 0.14 |
| 27 | Caryophyllene oxide | 17.298 | 1507 | 1582 | 0.36 |
| 28 | Spathulenol | 18.155 | 1536 | 1578 | 0.2 |
| 29 | α-Humulene | 18.250 | 1579 | 1608 | 1.01 |
| | **Class composition** | | | | |
| | I-Monoterpene hydrocarbons (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14) | | | | 88.08 |
| | II-Oxygenated monoterpenes (13, 15, 16, 17, 18, 19, 20) | | | | 5.71 |
| | III-Sesquiterpene hydrocarbons (21, 22, 23, 24, 25, 26, 29) | | | | 5.04 |
| | IV-Oxygenated sesquiterpenes (27, 28) | | | | 0.56 |
| | Total identified compounds (%) | | | | 99.39 |

[a]Retention time, [b]Experimental retention index, [c]Retention index retrieved from literature review and [d]Concentration of the chemical compounds (%)

study. It appears that the oil of this Malaysian *Melaleuca* species could have enormous potential in various fields like pesticides industry. Further studies are required to test its medicinal and aromatic properties.

## CONCLUSION

The study demonstrated that oil yields were 0.278% for hydrodistillation and 0.378% for steam distillation, with no significant statistical difference. Steam distillation yielded 29 components, while hydrodistillation produced 28, with 16 shared components (36.4% similarity). Terpinolene (27.79%) was dominant in hydrodistillation, whereas O-Cymene (41.5%) was the major compound in steam distillation, highlighting *Melaleuca leucadendron* Linn. as a valuable source of O-Cymene. These findings suggest that the identified compounds have significant potential for pesticide production, contributing to sustainable agriculture and reducing the environmental impact of chemical pesticides. Research should focus on evaluating the pesticidal efficacy of these compounds and optimizing distillation methods for industrial-scale applications.

## SIGNIFICANCE STATEMENT

This study highlights the significance of comparing essential oil yields and compositions from *Melaleuca leucadendron* leaves using steam distillation and hydrodistillation methods. The findings reveal that while steam distillation yielded 29 components and hydrodistillation yielded 28 components, both methods shared 16 components with 36.4% similarity. Notably, Terpinolene was predominant in hydrodistillation and O-Cymene was the major component in steam distillation. The detection of these bioactive compounds underscores their potential application as natural pesticides, promoting sustainable agriculture and reducing environmental pollution associated with chemical pesticides.

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