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EXTRACTION AND COMPOSITION ANALYSIS OF ESSENTIAL OIL FROM *Eucalyptus* HYBRID (*Eucalyptus pellita* × *Eucalyptus grandis*) LEAVES VIA ULTRASOUND-ASSISTED DISTILLATION

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Abstract. The hybridization of *Eucalyptus* species, known for improved growth rates, wood quality and fibre yield, has attracted interest from the pulp and paper industry. After harvesting for pulp, the leaves are often discarded but can be repurposed for extracting essential oils of pharmaceutical, cosmetic and food value. This study evaluates ultrasound-assisted hydrodistillation (UAHD) for extracting essential oils from *Eucalyptus hybrid* (*E. pellita* × *E. grandis*) leaves, compared with microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD). UAHD achieved the highest yield (8.0 ± 0.19%), followed by MAHD (4.0 ± 0.21%) and HD (3.2 ± 0.21%). Scanning electron microscopy confirmed greater cell disruption after ultrasonic pre-treatment, explaining the enhanced extraction. Gas chromatography–mass spectrometry (GC–MS) identified isopulegol (90.9%) as the dominant component, distinct from typical non-hybrid *Eucalyptus* oils. Isopulegol, a monoterpene alcohol with reported antioxidant, anti-inflammatory and gastroprotective properties, has broad industrial potential. These findings demonstrate UAHD as a more efficient and scalable technique for essential oil production, offering additional economic value to forestry by-products while contributing to sustainable industrial practices.

Keywords: *Ultrasound-assisted hydrodistillation, microwave-assisted hydrodistillation, essential oil, Eucalyptus hybrid, pulp and paper industry.*

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1. Introduction

Eucalyptus leaves, often considered as waste in the pulp and paper industry, are highly potential to be used as a source of essential oils (EOs). They contain a number of valuable compounds with antibacterial, antioxidant and insecticidal properties, making them promising for medicinal, therapeutic and agricultural applications (Dhakad *et al*., 2017; Gallon *et al*., 2020; Kartiko *et al*., 2021). Repurposing *Eucalyptus* leaves for EO extraction presents an eco-friendly and sustainable way to add economic value to forestry by-products.

Hydrodistillation (HD), besides other conventional techniques including steam distillation and hydrodiffusion (Elyemni *et al*., 2019), is a widely used method for EO extraction. It involves the use of water and heat to separate aromatic compounds from plant materials (Katekar *et al*., 2023). Despite its popularity, HD in fact has drawbacks such as the degradation of high-sensitive compounds due to the high heat and more time-consuming process compared to newer methods (Daryasari *et al*., 2012; Elyemni *et al*., 2019). Nevertheless, HD remains widely used in view of its simplicity, historical significance and ability to produce oils with distinct characteristics.

In response to the limitations of HD, researchers have explored several alternative extraction techniques. These include low-pressure solvent extraction, soxhlet extraction, supercritical fluid extraction (SFE) using carbon dioxide, pressurized liquid extraction and advanced methods such as ultrasound-assisted and microwave-assisted extraction (Zhang *et al*., 2018). For instance, SFE offers high EO yields with precise control over the chemical composition of the extract, making it a strong candidate for industrial applications (Elhafez, 2022). Similarly, solvent-free microwave extraction is gaining popularity as it can minimize energy use and environmental impact, while eliminating the need for solvents (Lucchesi *et al*., 2004). This technology also rapidly and evenly enhances efficiency by using microwave energy to heat plant material, reducing extraction time while protecting heat-sensitive constituents Hashemi-Moghaddam et al. (2013). Ultrasound-assisted extraction uses sound waves to disrupt plant cell walls, improving solvent penetration and releasing more oil in less time. It is efficient and well-suited for preserving delicate compounds. These techniques have shown a promise in producing higher yields and better-quality oils compared to traditional ones (Syahir *et al*., 2016).

The *Eucalyptus hybrid* (*Eucalyptus pellita* x *Eucalyptus grandis*) is particularly significant in the pulp and paper industry for its favorable characteristics such as fast growth, high fiber yield and superior wood quality for producing short fiber cellulosic pulp used in high-quality paper and tissue (Hung *et al*., 2015). In Indonesia, Eucalyptus species, especially *E. pellita*, are also known to grow well in peatland areas, making them highly adaptable and sustainable as plantation species under local site conditions. However, to the best of our knowledge, there is limited research on the essential oils extracted from hybrid *Eucalyptus* leaves, particularly using modern extraction technologies.

Given this gap, the present study aims to evaluate and compare the effectiveness of two emerging extraction methods: ultrasound-assisted hydrodistillation (UAHD) and microwave-assisted hydrodistillation (MAHD) with the conventional HD method for extracting EOs from *E. hybrid* leaves, which are byproducts of the Indonesian pulp and paper industry. The main objectives of the study include (i) to investigate the effects of leaf type, microwave power and extraction time on EO yield using the MAHD method; (ii) to study the influence of leaf type, plant-to-water ratio, amplitude of ultrasound and extraction time on EO yield using the UAHD method; (iii) to compare EO yields obtained from MAHD and UAHD with those from HD under identical distillation conditions and (iv) to analyze the morphology of leaf particles and determine the chemical composition of the UAHD extracted EOs using Gas Chromatography-Mass Spectrometry (GC-MS).

2. Materials and Methods

2.1. Plant materials

The *Eucalyptus hybrid* (*E. pellita* × *E. grandis*) leaves used in this research were supplied by a local pulp and paper industry in Indonesia and air dried for 72 h at room temperature before being used. Depending on the experiment, some leaves were carefully cut into similar pieces (±1 cm size) using scissors and the rest were used directly without further processing. Results with finely powdered leaves were excluded due to the much less yield obtained using this material. The raw leaves were then stored in air-tight plastic bags at the temperature of 4°C. Fresh leaves were also used as a comparison.

2.2. Ultrasound-assisted hydrodistillation process (UAHD)

E. hybrid leaves were placed in an Erlenmeyer flask containing a certain amount of distilled water, followed by submerging a sonotrode from ultrasonic equipment (20 KHz, 250 W, Biobase UCD-250, China). Ultrasonic treatments were initially carried out using dried leaves by varying ultrasonic amplitudes (40-80%) and leaves-water ratio (1:8-1:20) to investigate the effect of these parameters on the EO yields. Subsequently, the impact of four-leaf types on the EO yields was also studied. The ultrasound periods for up to 40 min were performed for all parameters explained.

Hydrodistillation (HD) processing of *E. hybrid* leaves was carried out following the ultrasound treatment using a Clevenger-type apparatus. The resultant solution and leaves from the ultrasound process were combined with distilled water (up to 1.5 L) and heated to 100°C for 2 h (the optimal extraction time). The steam leaving the boiling flask was condensed in a Liebig condenser and water was removed using the same technique in MAHD, i.e. a rotary evaporator. The collected pale-yellow oils were weighed and stored in sealed vials at 4°C for further analysis.

2.3. Microwave-assisted hydrodistillation (MAHD) process

The procedure of the MAHD method in previous studies was followed with an airflow rate of 3 L/min (Kusuma & Mahfud, 2017). Briefly, 25 g of *E. hybrid* leaves were added to 500 mL of deionized water in a 1-liter flask. The flask was placed within the microwave (Krisbow, Indonesia) oven's cavity and the extracted EOs were collected using a condenser placed on top (outside the oven). A separating funnel separated the condensate containing a mixture of EOs and water. Water was removed by means of a vacuum rotary evaporator and EOs were kept in amber vials at the temperature of 4°C before analysis. Three microwave power levels, i.e. 220 W, 450 W and 660 W, were studied to examine the effect of microwave power on EOs using dried-cut leaves. Further, the effect of leaf types i.e. dried-cut, dried-whole, fresh-cut and fresh-whole leaves on the EO yields was investigated at 660 W and samples were periodically taken. A 120-minute experiment was performed to study both parameters (microwave power and time) since EO yield values tended to reach a constant value at the end of this period.

The yields of EOs in both MAHD and UAHD methods were calculated as the amounts of extracted EOs (g) divided by the initial amount of leaf sample (g) (x 100). HD processing alone of dried *E. hybrid* leaves was also carried out using the exact condition of HD explained previously with a plant-to-water ratio of 1:20.

2.4. Scanning electron microscopy (SEM) investigation

SEM micrographs of *E. hybrid* leaves were obtained by means of a JEOL scanning electron microscope (Model JSM-6510, Japan) from an ISO-accredited and integrated laboratory, Diponegoro University, Indonesia. The equipment was operated at an accelerating voltage of 10 kV and a working distance of 10 mm. Leaves' physical characteristics were compared after three stages of the UAHD process, i.e. before and after sonication and after hydrodistillation (at 1000x and 5000x magnifications).

2.5. Gas chromatography/mass spectrometry (GC/MS)

Gas chromatography/mass spectrometry (GC/MS) analysis of UAHD extracted EOs was carried out at the same external certified laboratory as explained previously, using the Shimadzu GCMS-QP2010 SE system (Shimadzu Corporation, Tokyo, Japan). The GC specifications included 60°C column oven temperature, 290°C injector temperature, splitless injection mode, 8.4:1 split ratio, linear velocity flow control mode and 2.0 mL/min column flow with helium as the carrier gas at column head pressure of 123.2 kPa. The column oven temperature program started at 50°C for 2 min at 10°C/min and 110°C. The rate was then changed into 5°C/min to reach a final temperature of 290°C with 2 min hold time. Column: Rtx-5ms: length: 30 m, diameter: 0.25 mm and thickness: 0.25μm. The MS specifications included 300°C ion source temperature, 250°C interface temperature, 45-500 m/z scan range, 0.3s event time, 3 min solvent cut time, 3 min start time, 47 min end time and EI mode ionization. The components of the extracted oil were derived by comparing the retention indices (RI), retention time (RT) and m/z ratio using the NIST library and comparing the literature reports.

2.6. Statistical evaluation

Each of process and condition (UAHD, MAHD and HD) was performed in triplicate and the mean values of the extraction yields were reported. A one-way analysis of variance (ANOVA), followed by Tukey’s test at a 95% confidence level ($p<0.05$), was carried out to compare the essential oil (EO) yields: (1) among different leaf types at the same sonication and power level and (2) among different amplitudes and plant-to-water ratios at the same power level after the UAHD process. Furthermore, Tukey’s test was used to compare the EO yields at different microwave power levels, as well as the EO yields from different leaf types and treatment times at the same power level after the MAHD process. In addition, Tukey’s test was also used to compare the EO yields among different processing methods. Data analysis was done using SYSTAT 13 software (Systat Inc., USA) and graphs were drawn using Microsoft Excel 2013 software.

3. Results and Discussion

3.1. UAHD process: Influence of leaf type, plant-to-water ratio, amplitude of ultrasound and sonication time on EOs yield

Figure 1 shows essential oil yields (%) over time for dried-cut, dried-whole, fresh-cut and fresh-whole leaves after UAHD. Dried-cut leaves performed best, peaking at 8.0 ± 0.19% at 20 min, slightly declining thereafter ($p<0.05$). Dried-whole leaves reached ~6.0 ± 0.24% at similar sonication times, while fresh-cut and fresh-whole leaves yielded only ~3.8 ± 0.27% and <3.0 ± 0.32%, respectively. Thus, dried leaves consistently outperformed fresh ones and cutting enhanced solvent accessibility. Across all samples, yields increased sharply within 5-10 min, peaked at ~20 min, then plateaued or declined, indicating optimal extraction occurs within 20 min.

Ultrasonic cavitation, through solvent penetration and cell wall disruption, explains these improvements (Vinatoru, 2001). Increasing amplitude from 40% to 80% significantly enhanced yields confirming the amplitude effect, with the highest (~8.0%) at 80% ($p<0.05$). Past studies reported similar amplitude-dependent effects on EO recovery (Sánchez *et al*., 2019). Extraction time beyond 20 min reduced yields, consistent with thermal degradation or exhaustion of extractable oil (Bhadange *et al*., 2022).

The second inset graph shows that higher plant-to-water ratios (1:16-1:20 g/mL) produced maximum yields (~8.0%), whereas lower ratios (1:8-1:10) provided only 5.8-6.0% ($p<0.05$). Sufficient solvent volume improves heat distribution and ultrasound attenuation, enabling better extraction (Samad *et al*., 2023). However, excessively high ratios can reduce efficiency (Bin Mokaizh *et al*., 2024). Here, the optimal ratio of 1:20 demonstrated higher yields than previous reports (2.5-4.1%) for *Eucalyptus* species using similar methods (Al-Khirsan & Al-Yaqoobi, 2024; Pheko-Ofitlhilea *et al*., 2024).

In conclusion, UAHD with dried-cut leaves, 1:20 ratio, 80% amplitude and 20 min sonication achieved the best results (8.0%), showing the strong potential of ultrasound pretreatment in enhancing EO yield while remaining energy-efficient and environmentally sustainable.

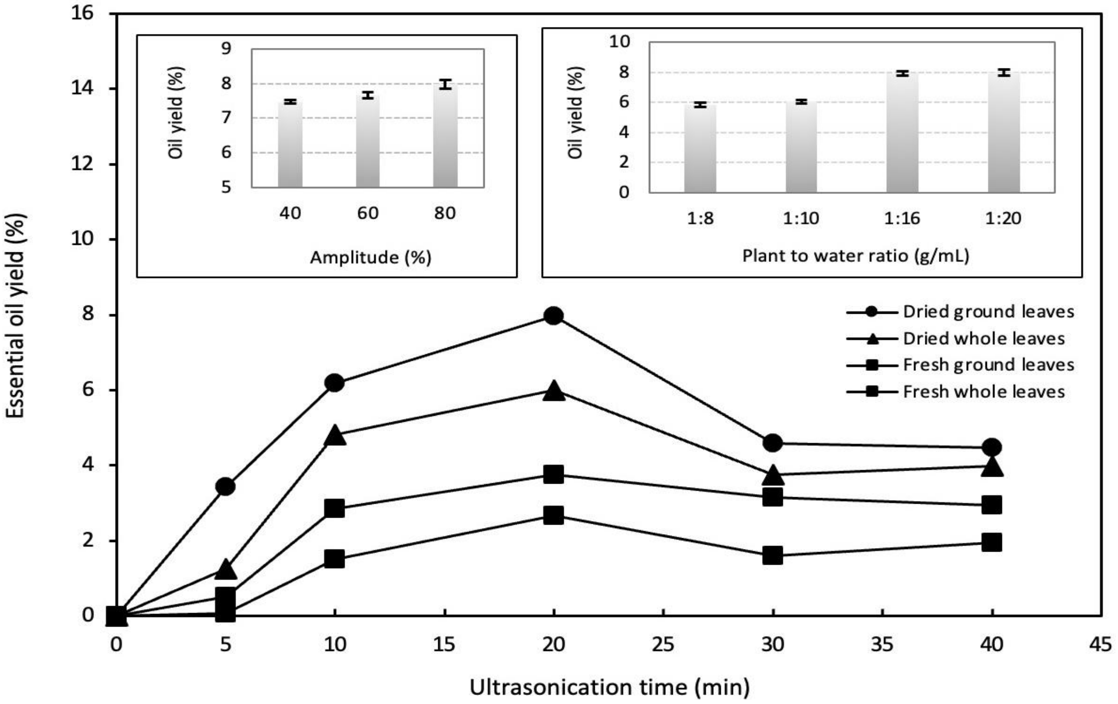

Figure 1. Effect of leaf type, amplitude, ultrasonication time and plant-to-water ratio on essential oil yield after UAHD process (inset graphs present results with dried leaves and a 120-min hydrodistillation time)

3.2. MAHD process: effect of leaf type, microwave power and processing time on EO yield

The inset in Figure 2 shows that microwave power affected oil yield, with 450-660 W giving ~4.0 ± 0.14% compared to 3.7 ± 0.07% at 220 W. No significant difference was observed between 450 and 660 W ($p>0.05$), suggesting that increasing power beyond 450 W offers little benefit. Extraction kinetics revealed rapid oil recovery within the first 30 min, followed by slower release and a plateau after ~60 min, which indicates a diffusion-controlled mechanism where equilibrium yield is reached (Rezvanpanah *et al*., 2008). Among the materials, dried-cut leaves produced the highest yield (4.0 ± 0.14%), whereas fresh-whole leaves gave the lowest (2.5 ± 0.27%). This supports the role of drying and cutting in reducing moisture, disrupting cell walls and increasing surface area, all of which facilitate more efficient oil release (Zhang *et al*., 2021).

Previous studies report similar trends. Rezvanpanah et al. (2008) obtained <3.2% yield from dried *Satureja* spp., while Kusuma and Mahfud (2017) achieved ~3% from patchouli at 600 W. Under optimized ratios, higher yields have been noted, such as 5% from patchouli (1:12, 400 W, 60 min) (Abdurahman & Sundarajan, 2019), while citronella yielded only ~1% under 450 W for 90 min (Putri *et al*., 2023). Variations in yield across plants such as *Litsea cubeba* and *Ferulago angulata* (Huang *et al*., 2020; Mollaei *et al*., 2019) highlight the sensitivity of MAHD to material type, ratio and power. Despite its advantages, MAHD may suffer from overheating, loss of volatiles or degradation of thermolabile compounds (Akhbari *et al*., 2018; Azar *et al*., 2013; Pavlić *et al*., 2022).

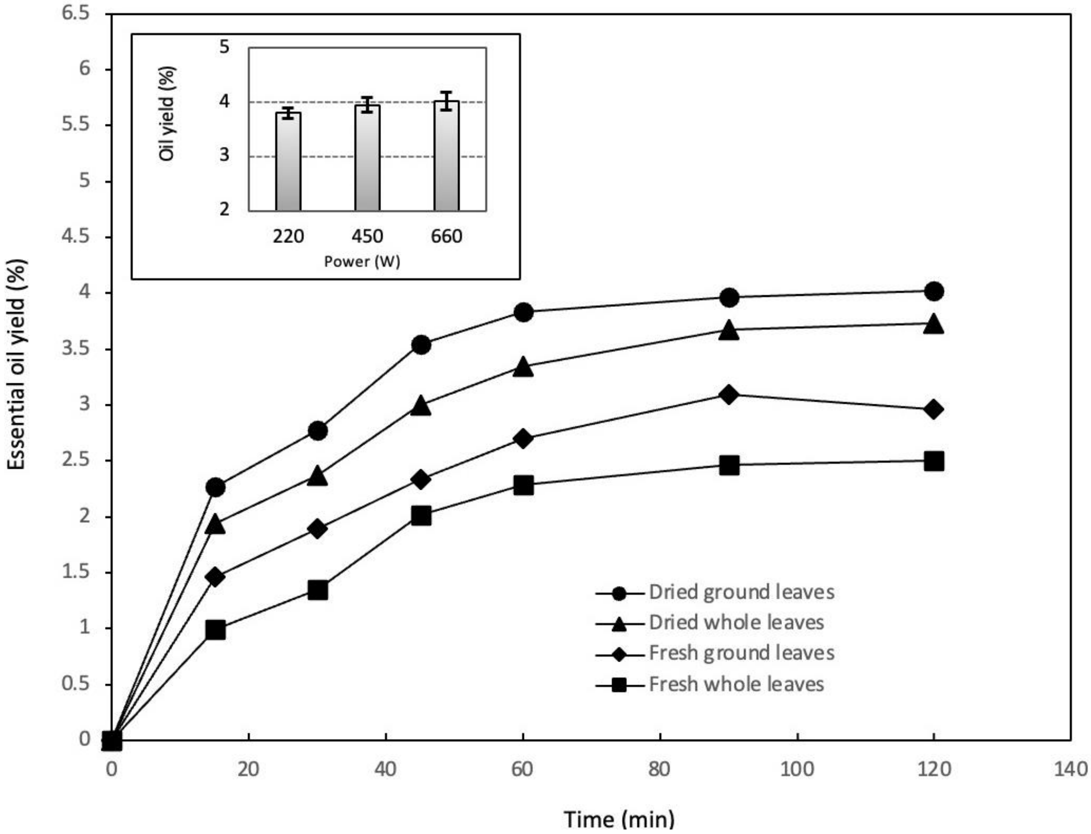

Figure 2. Effect of power level, MAHD duration and leaf type on essential oil yields (with the main graph presenting results at 660 W)

3.3. Comparison of UAHD, MAHD and HD processes

Figure 3 compares the yields obtained from HD, MAHD and UAHD. The UAHD process produced the highest EO yield (8.0 ± 0.19%, $p<0.05$), followed by MAHD (4.0%) and HD (3.2%). In this study, UAHD enhanced yield by ~60% compared with HD, confirming the effectiveness of ultrasound pretreatment in facilitating oil recovery. Previous reports demonstrated similar outcomes, in which ultrasound-assisted extraction improved EO yields by up to 40% in citrus peels and *Ocimum* spp. (Miljanović *et al*., 2020; Rasouli *et al*., 2021; Zorga *et al*., 2020) and by ~10% in *Thymus vulgaris* and *Mentha piperita* (Mahfud *et al*., 2022). The mechanism is generally attributed to cavitation effects that disrupt cell walls, improve solvent penetration and accelerate the release of volatile compounds.

Microwave-assisted methods also showed superior performance compared to HD. Mahfud et al. (2022) reported that both MAHD and solvent-free microwave extraction increased yields by ~13% and improved EO recovery quality. Similarly, Benmoussa et al. (2016) observed better efficiency of microwave-based processes compared with HD and steam distillation, while Tyśkiewicz et al. (2018) and Wintola et al. (2019) highlighted differences in both yield and oil composition between MAHD and HD.

Overall, these findings indicate that assisted-hydrodistillation methods, particularly UAHD, are more efficient than conventional HD, producing higher yields within shorter times while potentially influencing oil quality and composition.

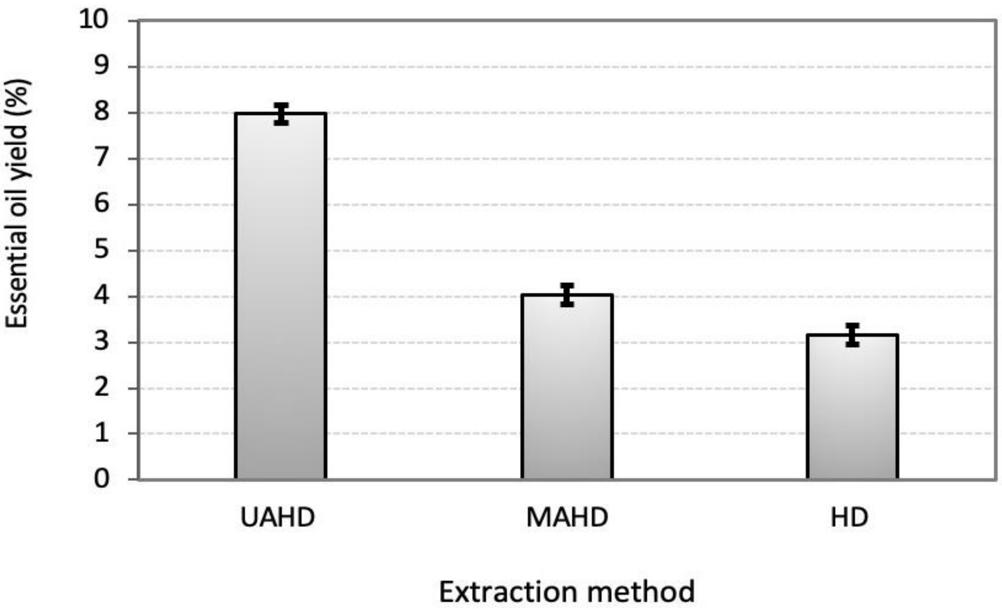

Figure 3. Comparison of essential oil yields from UAHD, MAHD and conventional HD using dried leaves with a plant-to-water ratio 1:20

3.4. Morphological characterization of Eucalyptus leaf particles (UAHD process)

Figure 4 (a-f) illustrates the SEM images of *E. hybrid* leaf particles before and after ultrasound treatment and hydrodistillation. The untreated samples (Figure 4a, 4d) exhibited intact trichomes, stomata, cuticular waxes and organized mesophyll structures, reflecting a robust protective architecture (Healey, 2021; Sasaki *et al*., 2019). Similar intact surfaces with strong lignocellulosic layers were also reported in *Cymbopogon nardus* leaves prior to UAHD (Limam, 2020).

Following ultrasound pretreatment (Figure 4b, 4e), significant structural modifications were observed, including disrupted cell walls, reduced cuticle thickness and altered trichome and stomatal morphology. These changes enlarge the surface area and enhance solvent penetration, thereby improving access to oil reservoirs (Sánchez *et al*., 2019; Insuan & Chahomchuen, 2020).

After HD (Figure 4c, 4f), further disintegration of tissues and depletion of secretory cavities indicated substantial EO removal. Overall, the SEM analysis confirmed that ultrasound pretreatment induces microstructural changes that facilitate hydrodistillation efficiency, underscoring its role in maximizing essential oil yields.

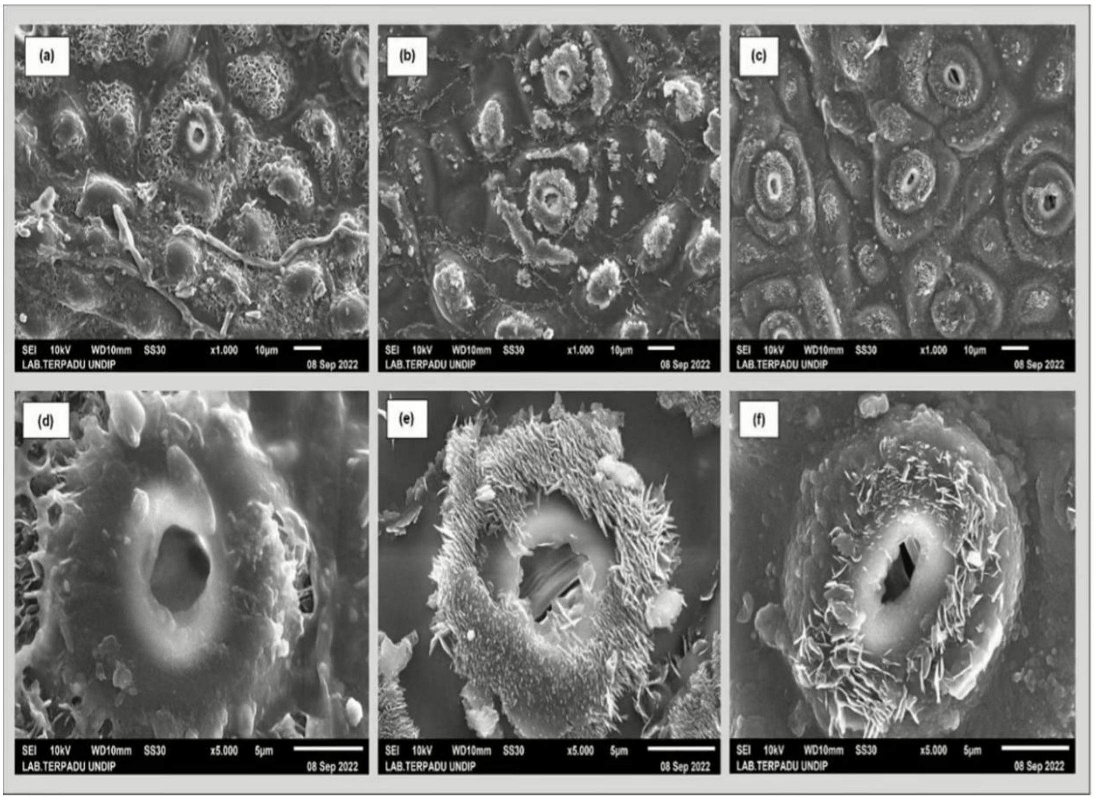

Figure 4. The SEM images of *E. hybrid* leaf particles: (left) before sonication, (middle) after sonication and (right) after hydrodistillation. Images (a-c) are captured at 1000x magnification, while images (d-f) are at 5000x magnification

3.5. Chemical characterization of essential oil

A pale-yellow essential oil was obtained from *E. hybrid* leaves via UAHD (Figure 5), consistent with the coloration commonly reported for *Eucalyptus* oils extracted by HD and other methods (Insuan & Chahomchuen, 2020; Limam, 2020; Rachel *et al*., 2023). GC/MS analysis identified twelve compounds accounting for 100% of the oil, with isopulegol (90.9%) and β-citronellol (6.8%) as the dominant constituents (Figure 6, Table 1). While eucalyptol (1,8-cineole, 40-94.9%) is typically the major compound in most *Eucalyptus* species, exceptions exist such as *E. citriodora*, which is rich in citronellal (Surbhi *et al*., 2023). The predominance of isopulegol in this hybrid likely reflects genetic and biochemical variations arising from hybridization, as previously reported for *Eucalyptus grandis* × *E. tereticornis* showing distinct profiles and absence of expected terpenes (Naspi *et al*., 2017). Isopulegol is valued for applications in flavor, fragrance and medicine (Le *et al*., 2020; Lê & Szakonyi, 2021). The oil also exhibited favorable physicochemical properties, including density (0.88 g/mL), viscosity (0.56 cP) and good ethanol solubility. These compositional differences highlight the uniqueness of hybrid-derived essential oils compared to conventional *Eucalyptus* species.

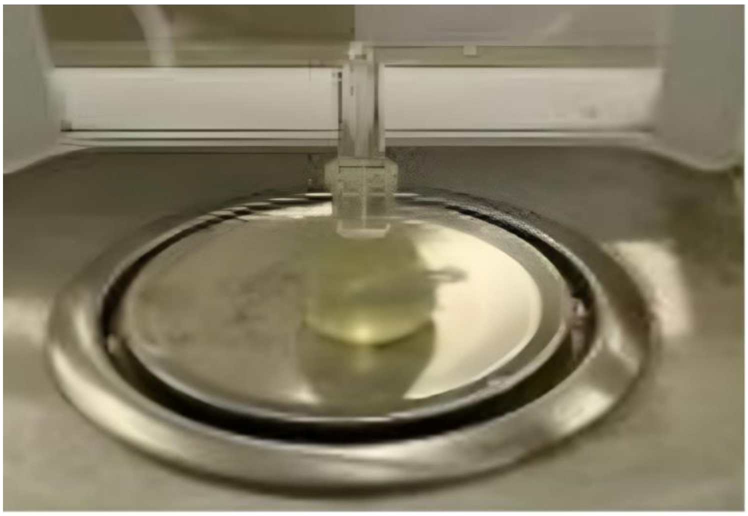

Figure 5. Essential oil obtained via UAHD method

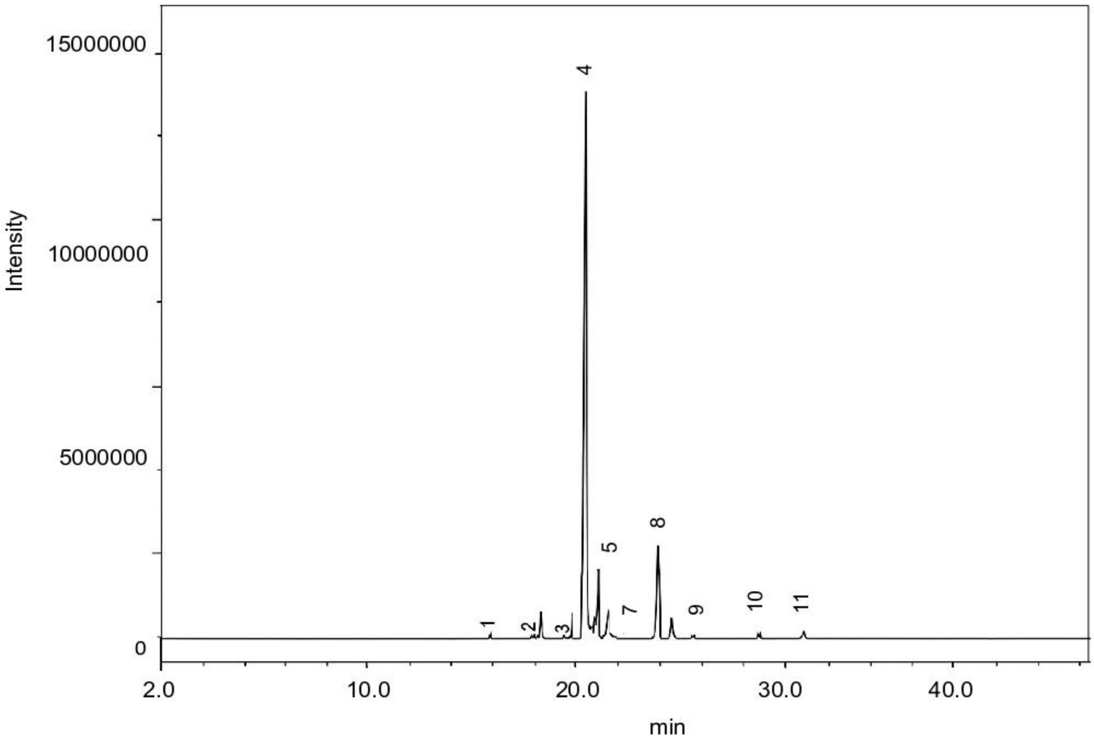

Figure 6. GC-MS chromatogram results of *E. hybrid* leaves' essential oil extract

Table 1. Chemical components of the EOs from *E. hybrid* (*E. pellita* x *E. grandis*)

Residence time (min)	Area	Relative content (%)	Height	Compound
14.428	3580502	0.17	949902	2,6-Dimethyl hept-5-en-1-al
16.372	5378400	0.26	964305	Linalool L
17.484	1578580	0.08	367805	.beta.-Citronellol
18.019	1816949837	87.34	142319218	Isopulegol 1
18.241	73134372	3.52	16301826	(-)-Isopulegol
18.553	8078789	0.39	1455333	Cyclohexanol, 5-methyl-2-(1-methylethenyl)- (CAS)

18.769	1162013	0.06	318449	Isopulegol 1
20.373	140990662	6.78	23916981	.beta.-Citronellol
21.086	9595852	0.46	1539923	6,7-Epoxy-3,7-dimethyloctanal
23.996	8360060	0.40	1113922	Cyclohexanemethanol, 2-Hydroxy-.alpha., .alpha., 4-Trimethyl-
25.217	8568626	0.41	1489707	Benzene, 1,2-dimethoxy-4-(2-propenyl)- (CAS)
25.503	2834386	0.14	614339	trans-Caryophyllene

4. Conclusion

A pale-yellow oil was obtained from *E. hybrid* (*Eucalyptus pellita* × *Eucalyptus grandis*) leaves via the ultrasound-assisted hydrodistillation (UAHD) method. The UAHD process yielded a higher amount of essential oil compared to conventional hydrodistillation under identical conditions (plant-to-water ratio of 1:20 and 120 minutes of distillation) and also outperformed microwave-assisted hydrodistillation under its optimal settings (2 hours, 660 W). These findings highlight UAHD as a promising and potentially scalable method for essential oil production, as it combines improved yield with reduced extraction time and energy requirements. The enhanced extraction efficiency is further supported by SEM observations showing pronounced cell disruption after ultrasonic pre-treatment and subsequent hydrodistillation.

GC-MS analysis revealed isopulegol (90.9%) as the major component, with potential applications in the food, pharmaceutical and cosmetics industries. Future work should focus on pilot- and industrial-scale trials to evaluate the feasibility of UAHD in large-scale operations, as well as comprehensive bioactivity assessments of *E. hybrid* oil to explore its antimicrobial, antioxidant and therapeutic potentials. Together, these directions would strengthen the case for commercial utilization of *E. hybrid* essential oil as a novel, value-added product.

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