Valorisation of Lemongrass (*Cymbopogon citratus*) Leaf By-product as a Source of Essential Oil

Nimasnaini Adhawati, Rini Yanti*, Dian Anggraini Suroto

Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology,
Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia
*Corresponding author: Rini Yanti, Email: riniyanti@ugm.ac.id

Submitted: January 24, 2025; Revised: May 12, 2025; Accepted: November 4, 2025

ABSTRACT

This research aimed to investigate the potential of lemongrass (*Cymbopogon citratus*) leaf agricultural by-products as a source of essential oil, and evaluate the antioxidant and antibacterial activities. In this context, the material used was subjected to microwave pre-treatment to enhance the yield. Lemongrass leaf was often discarded as an agricultural by-product. The utilization was examined through different drying methods, including fresh, wilted, and dried treatments. Lemongrass leaf was also treated with microwave (+MV) and non-microwave (-MV) pre-treatments. The essential oils were analysed for physicochemical properties. The chemical composition was determined through gas chromatography-mass spectrometry (GC-MS), and the tissue microstructure was observed by SEM. Antioxidant activity was measured with the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, while MIC and MBC values were assessed using the microdilution method. The results showed that the distillation of dried leaf with microwave pre-treatment provided the highest oil yield of 0.20%. SEM analysis showed that drying and microwave pre-treatment altered the leaf microstructure, enhanced glandular trichomes, and improved extraction efficiency. The physicochemical properties included a bright yellow colour, a fresh citrus aroma, a specific gravity of 0.971 g/mL, a refractive index of 1.486, and a solubility ratio of 1:3 in alcohol. The main components were citral (geranial (44.07–46.81%), neral (35.64 – 36.60%)), geranyl acetate (2.03–5.66%), isogeranial (1.61–2.57%), isoneral (0.96–1.76%), β-pinene (0.16–3.63%). Lemongrass leaf oil exhibited strong antioxidant activity with an IC_{50} of 61.65 to 84.5 μg/mL, antibacterial activity against *E. coli* and *S. aureus* with MIC of 0.3–1.3% and 0.3–1.8%, as well as an identical MBC value of 0.5–2% for both bacteria. This research showed that essential oil extraction increased the value of lemongrass leaf by-products.

Keywords: Agricultural by-product valorisation; antioxidant; *Cymbopogon citratus* leaf; essential oil; microwave

INTRODUCTION

Lemongrass (*Cymbopogon citratus*) is a widely cultivated species for spice and essential oil production. As reported by the BPS (Indonesian Central Statistics Agency), the production in Indonesia reached 36,422 tons in 2021. As a consequence of the considerable potential in a number of industrial sectors, the cultivation of lemongrass has become a rapidly expanding economic activity (Mukarram et al., 2021). However, the concomitant increase in production leads to concerns regarding the management of the organic by-product. The stalk is the only component used as a spice, and the leaf is discarded as an organic by-product. This necessitates the management of the lemongrass leaf and the conversion into high-value products, such as essential oils.

The aroma of cut lemongrass leaf is similar to the stalk, where there is a potential source for essential oil extraction. Yen and Lin (2017) on the extraction of

DOI: https://doi.org/10.22146/agritech.104141
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

lemongrass leaf oil through Clevenger hydrodistillation obtained a result of 1.30%. The yield of essential oil from lemongrass stalks ranges from 1.50% to 2.54%. This shows that the yield from the leaf is not significantly different from the stalk (Naik et al., 2010). Therefore, lemongrass leaf has the potential to be used as a raw material for essential oil production.

The post-harvest handling of plants represents a significant factor influencing the quality of essential oils. Different plant parts necessitate particular treatments, for instance, thinner materials require withering, thicker types are subject to drying and reduction in size, and volatile materials are extracted fresh (Akhihiero et al., 2013). The yield and quality of essential oils are greatly influenced by the condition and size of the material (Lubis et al., 2022). Mulyati et al. (2023) reported that water and steam distillation for a period of 5 hours obtained the highest quantity of essential oil from fresh holy basil (0.62%), in comparison to withered (0.5%) and dried leaf (0.32%).

Hydrodistillation represents a common method in the process of extracting essential oils from plant materials. The process is relatively straightforward but has drawbacks, including lengthy processing times, prolonged distillation durations, and high energy consumption (Nour et al., 2024). An alternative methodology for reducing the time required for the distillation process is to combine hydrodistillation with microwave pre-treatment. Microwave radiation disrupts cell membranes and increases porosity, facilitating the release of essential oils. Radzi and Kasim (2020) reported that pre-treatment of materials with microwave irradiation at 800 kW significantly improved the efficiency of agarwood essential oil extraction. This method reduces the time required for hydrodistillation, while simultaneously decreasing energy consumption and costs. The yield (from 0.0286% without pre-treatment to 0.0877%) and the quality of the essential oil extracted have also been increased.

Based on the description, this research aims to evaluate the effect of drying and microwave pre-treatment on lemongrass leaf before distillation to enhance essential oil extraction efficiency. The essential oil was isolated from lemongrass leaf using water distillation, with the raw materials subjected to drying and microwave pre-treatment. The essential oil was subjected to analysis to determine the compound profile, physicochemical properties, antioxidant activity,

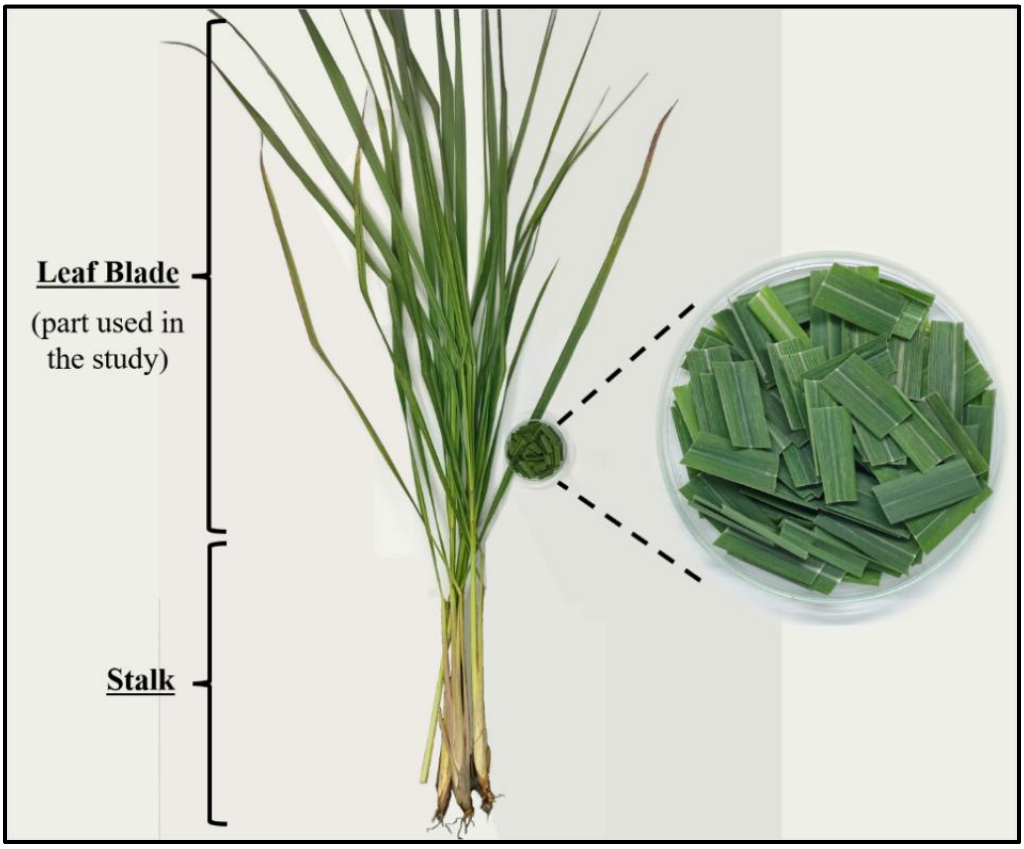


Figure 1. Lemongrass leaf parts that were used in this research

and antibacterial properties. The results offer valuable perspectives for future research focused on the sustainable use of plant by-products.

METHODS

Materials

The materials used comprised lemongrass leaf (*Cymbopogon citratus*) (Fig. 1) procured from a Women's Farmers Group in the Godean, Sleman District, Yogyakarta Special Province, Indonesia. The leaf parts included the base to the tip of the leaf blade and were harvested on the same day as the treatment, with the plants ranging in height from 30 to 60 cm. Distillation used tap water as a solvent, and the chemicals adopted included sodium sulphate (Na_2SO_4), toluene (PA), methanol (PA), alcohol, sodium chloride (NaCl), dimethyl sulfoxide (DMSO), Mueller-Hinton agar (MHA), Mueller-Hinton broth (MHB) from Merck (Germany), and DPPH (Sigma, USA).

Pretreatment and Essential Oil Extraction

A total of 6 kg of fresh lemongrass (*Cymbopogon citratus*) leaves were sorted and weighed. The fresh, wilted (air-dried at 29-31°C for 48 hours), and dried leaf (cabinet-dried at 50°C for 5 hours) were subjected to different treatments. Each sample was chopped into pieces of approximately 3 cm in length. Before distillation, each sample was subjected to a preliminary treatment, namely, heating using a microwave (Electrolux, Sweden) at 450 watts for a period of five minutes. This was carried out in two different modes with (+MV) and without microwave (-MV). The essential oil was extracted using a 2 kg Clevenger water distillation apparatus for a period of 4 hours, commencing from the appearance of the first oil droplets. The resulting hot vapour was condensed and collected, and the remaining water was desiccated using anhydrous sodium sulphate. The extracted oil was stored at 4°C for subsequent analysis, and the yield calculation followed the formula outlined in equation (1).

$$\text{Yield (\% g/g)} = \frac{\text{Oil weight (g)}}{\text{Material weight (g)}} \times 100 \qquad (1)$$

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM JEOL JSM-IT700HR, Japan) was used to analyse the microstructure of lemongrass leaf at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada. The lemongrass samples were placed on aluminium stubs and gold-coated. The SEM settings were configured with an increasing voltage of 5 kV and Secondary Electron (SE) mode. The leaf tissue structures were observed at magnification levels of x100, x200, x300, and x1000.

Physicochemical Properties of Essential Oil

The essential oils' physical properties, such as colour, aroma, specific gravity, refractive index, and alcohol solubility, were evaluated. Colour, aroma, and alcohol solubility were measured manually by direct observation. Specific gravity was determined using a pycnometer (Pyrex, US), while the refractive index was measured with an Abbe refractometer (Atago, Japan). Furthermore, the testing was conducted in accordance with the Indonesian National Standard 8835:2019 (SNI, 2019).

Chemical Composition Identification

The GC-MS (Shimadzu QP 20110S, Japan) was performed at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada. The GC-MS system used an HP-5MS column (30 meters in length, 0.25 mm ID). A 0.2 mL sample of essential oil was dissolved in 1 mL of ethanol in a GC vial and vortexed to achieve a uniform solution. The analysis was conducted with an injector temperature of 300°C, a column temperature of 60°C, helium as the carrier gas, a pressure flow control mode set at 12 kPa, a total flow of 25 mL/min, a column flow of 0.51 mL/min, a linear acceleration of 26 cm/s², a cleaning flow of 3.0 mL/min, and a split ratio of 42. An Agilent DB-1 column (30 m length, 0.25 mm diameter) was used with Electron Impact (EI) ionization at 70 eV.

DPPH Radical Scavenging Assay

The assessment of antioxidant activity followed the method described by Mulyati et al. (2023). Essential oils were prepared in concentrations ranging from 25 to 500 ppm, with 1 mL added to 3 mL of a 40 ppm DPPH methanolic solution. After vortexing, the sample was incubated in the dark at room temperature for 30 minutes. A UV-Vis spectrophotometer was used to measure absorbance (Thermo Scientific, US) at 515 nm. Methanol and ascorbic acid were adopted as a blank and positive control, respectively. The inhibition level was calculated using equation 2.

$$\%Inhibition\ \text{DPPH}\ radical\ activity = \frac{A^{0} - A^{1}}{A^{0}} \times 100\% \qquad (2)$$

Antibacterial Activity

The minimum inhibitory concentration (MIC) was assessed using a 96-well microplate microdilution method. Essential oils were dissolved in 20% DMSO to achieve the specified concentration (0.0625-3%).

Each well contained a total reaction volume of 200 µL, comprising 25 µL of *Escherichia coli* FNCC 0091 or *Staphylococcus aureus* ATCC 25923 inoculum at 1.5 × 10^8 CFU/mL, 105 µL of MHB, and 70 µL at different concentrations. The plates were incubated at 37 °C for 24 hours, and chloramphenicol (100 ppm) was used as the positive control. Turbidity was quantified at 630 nm using a microplate reader (Accuris, US), and the minimum bactericidal concentration (MBC) was determined. A 100 µL aliquot from each MIC well was transferred onto MHA plates and incubated at 37 °C for 24 hours to determine the MBC (M'hamdi et al., 2024; Scotti et al., 2021).

Data Analysis

All the experiments were conducted in triplicate, and the data tests were analyzed using Two-way Analysis of Variance (ANOVA) with IBM SPSS Statistics software version 20 (IBM Corp, USA). Values of $p<0.05$ were considered to show a significant difference.

RESULTS AND DISCUSSION

Microstructure of Lemongrass Leaf Tissue

Lemongrass leaf essential oil is located in glandular trichomes (GT) (Mirzaie et al., 2020). Figure 2 shows lemongrass leaf glandular trichomes distributed across the abaxial (lower) surface of the leaf in proximity to the stomata. Batool et al. (2024) stated that glandular trichomes were predominantly found on the surface. The abaxial side, shielded from direct sunlight and characterised by higher humidity than the adaxial (upper) surface, provides an optimal environment for essential oil storage (Gostin & Blidar, 2024).

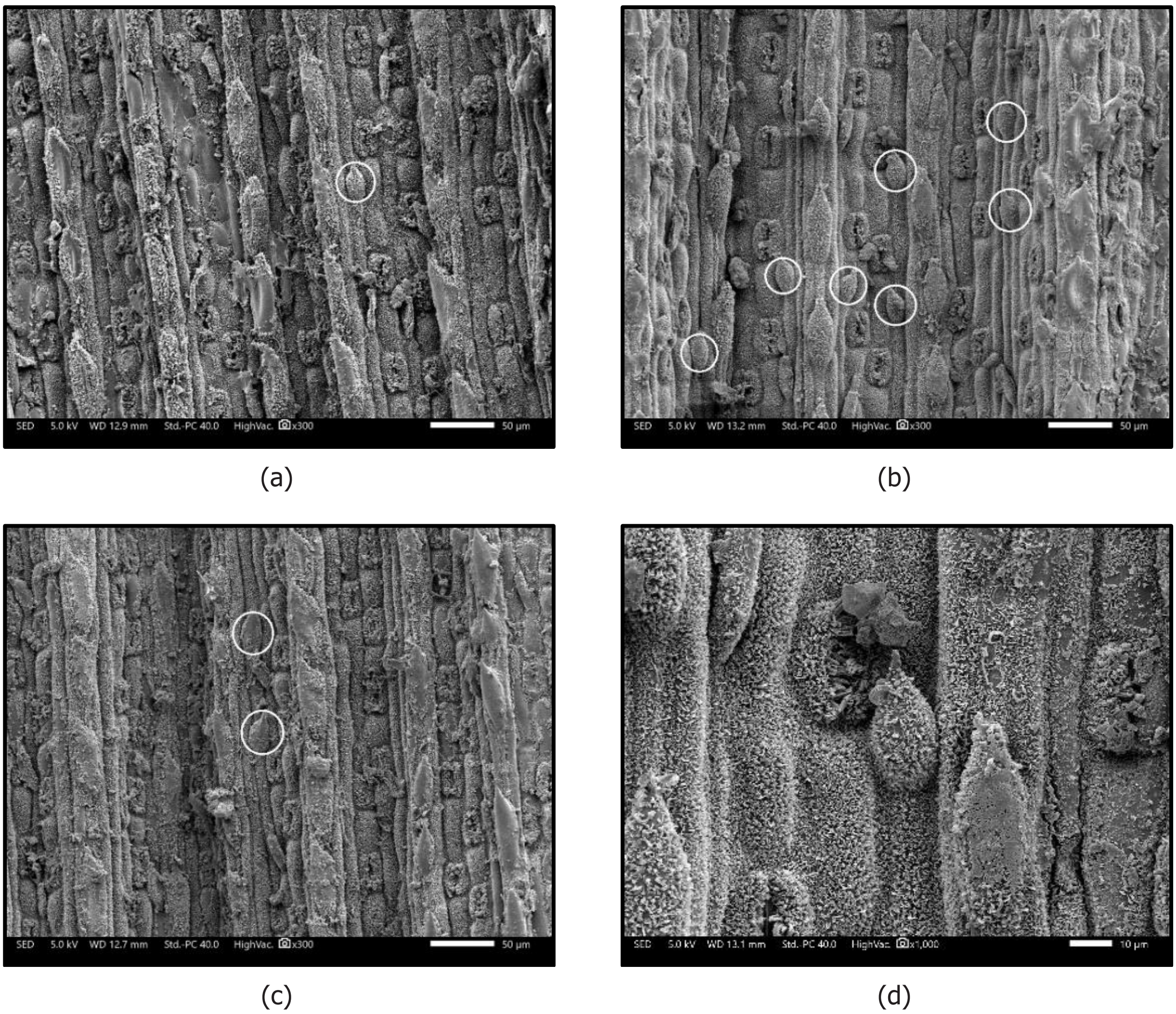
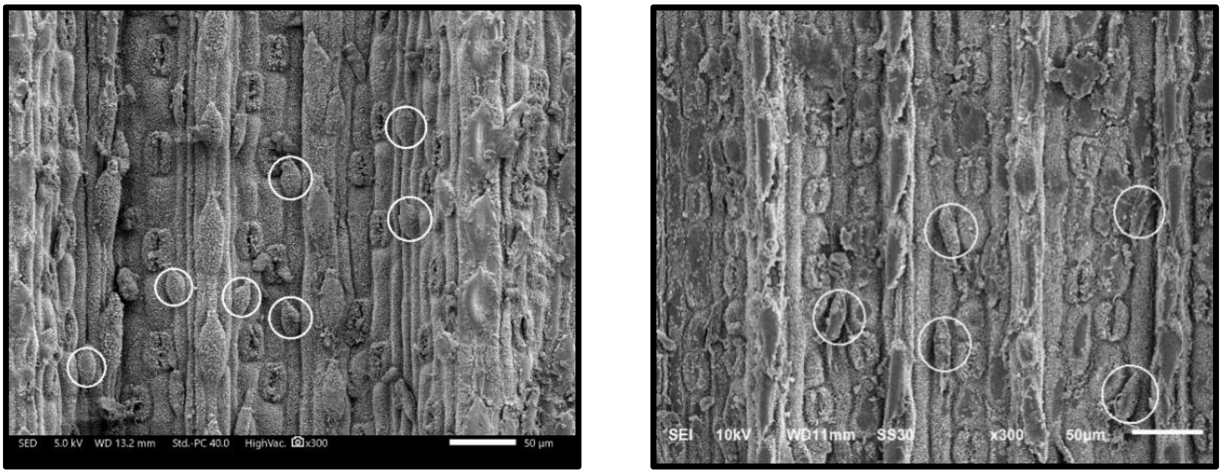


Figure 2. Abaxial microstructure of *Cymbopogon citratus* leaf (a) fresh, (b) withered, (c) dried, and (d) the glandular trichomes of lemongrass leaf. Scale bars (a, b, c) = 50 µm, (d)=10 µm



(a) (b)

Figure 3. The condition of the glandular trichomes on the fresh leaf before microwave treatment (a) and following microwave pre-treatment (b). Scale bars (a,b,c)=50 µm

Lemongrass leaves exhibit the presence of non-glandular trichomes (NGT) distinguished by elongated and slender morphology when compared to the glandular types. NGT on lemongrass leaf are arranged in a regular row pattern, following the furrows or veins, with an orderly distribution across the surface (Fig. 2). These trichomes lack essential oils but fulfil a protective mechanical function and assist in reducing evaporation (Gostin & Blidar, 2024).

The glandular trichomes in fresh lemongrass leaf remain intact, with essential oil content preserved (Fig. 2a). In fresh leaf, high water content preserves tissue integrity, maintaining the physical structure in the natural form (Wagner et al., 2004). The moisture content of the leaf tissue facilitates the retention of structural integrity to maintain the regular arrangement of NGT at intervals exceeding 50 µm (Fig. 2a).

In a wilted leaf, reduced water content results in alterations to the structure of plant tissue. The NGT remain in neat rows at the natural spacing (>50 µm). In other areas, the spacing between the rows is reduced due to shrinkage, with the trichomes becoming closer (<50 µm). In dried leaf, water loss causes the tissue to contract and the leaf structure to tighten, which brings the NGT closer (<50 µm). However, glandular trichomes remain intact in wilted and dried leaves, with the essential oil content preserved (Fig. 2b, 2c). These observations show the impact of water content on leaf microstructure. The processes of withering and drying result in alterations to leaf tissue, but glandular trichomes remain intact, preserving the essential oil content (Iriti et al., 2006; Wagner et al., 2004).

Figure 3a shows that before microwave treatment, glandular trichomes exhibit a mean diameter of approximately 20 µm. However, a reduction in size to less than 20 µm is observed (Fig. 3b) due to the release of essential oils. Microwave radiation produces electromagnetic waves for inducing the rapid vibration of water molecules in the leaf tissue and trichomes. These vibrations lead to intermolecular friction, generating heat that rapidly elevates the temperature of the leaf tissue (Iriti et al., 2006). The accumulation of heat within the trichomes may increase internal pressure, causing the shrinkage of the trichome walls and the subsequent release of essential oils (Iriti et al., 2006; Moradalizadeh et al., 2013). This process shows that microwave pre-treatment enhances the efficiency of the distillation process, reducing the time required and increasing the yield of essential oils.

Drying treatments reduce the water content of lemongrass leaf and maintain the integrity of the essential oils within the glandular trichomes (Wagner et al., 2004). The application of microwave energy facilitates the release of essential oils from trichomes (Iriti et al., 2006) to enhance the efficiency of the distillation process.

Essential Oil Yield

The process of drying is intended to reduce the water content contained within the leaf. The pre-treatment of the leaf by microwave irradiation facilitates the disruption of the cell walls, enabling the more efficient release of essential oils during the distillation process.

The results showed that the microwave pre-treatment of lemongrass leaf increased essential oil yield compared to untreated leaf ($p<0.05$). Fresh, wilted, and dried leaf pre-treated with microwave obtained

Table 1. The yield of lemongrass leaf (*Cymbopogon citratus*) essential oil

Drying Method	Yield (%)	
	Without Microwave	Microwave
Fresh Leaf	0.16±0.03^{Aa}	0.18±0.008^{Ba}
Wilted Leaf	0.14±0.04^{Aa}	0.18±0.02^{Ba}
Dried Leaf	0.17±0.01^{Aa}	0.20±0.01^{Ba}

Description: different uppercase letters represent a significant difference between microwave and without, and identical lowercase letters represent no significant difference between drying methods.

1.13, 1.28, and 1.19 times more essential oil (Table 1). Microwave heating uses electromagnetic radiation to rapidly and uniformly heat the water within the leaf tissue, leading to the generation of internal pressure on the cell walls. The pressure exerted on the cells facilitates the extraction of the essential oils trapped inside (Fouelefack et al., 2024).

The condition of the plant material exerted no significant influence on essential oil yield ($p>0.05$). The results showed that dried leaf produced the highest essential oil yields (0.17% and 0.20%), followed by fresh and withered leaf, respectively. However, statistical analysis suggested that the difference in yield between dry, fresh, and wilted leaves was not significant (Table 4.1). These results were consistent with Syarifuddin et al. (2020), where withering of *Eucalyptus grandis* leaf had no significant effect on essential oil yield. This may be attributed to the distillation process, where the vapour pressure and heat provided are sufficient to extract the essential oil from the leaf tissue, without affecting the results, even though the leaves were weathered or dried. Therefore, withering or drying does not lead to a decline of the extractable essential oil.

Physicochemical Properties of Essential Oil

The physicochemical properties of essential oil were analysed according to the Indonesian National Standard (SNI) 8835:2019 for *Cymbopogon citratus.* This standard was used for comparison purposes due to the absence of a specific reference point for lemongrass essential oil derived exclusively from the leaves.

The lemongrass leaf oil exhibited a bright yellow colour and a fresh citrus aroma, in accordance with SNI standards (Table 2). The essential oil contains natural compounds, including citral (geranial and neral), which contribute to the lemon-like citrus scent. The principal compounds, citral and citronellal, are also responsible for the yellow colour (Oniha et al., 2023). In addition to the composition of the compounds, the distillation process and the conditions of the raw material influence the colour of the essential oil (Cortes-Torres et al., 2019; Oniha et al., 2023).

Specific gravity is the ratio of the oil's weight to water when measured at an equal volume. The specific gravity of lemongrass and *Cymbopogon citratus*' essential oil ranges from 0.893 to 0.975 g/mL and 0.869 to 0.907 g/mL, respectively. The occurrence of specific gravities above the standard may be attributed to the presence of large molecular weight compounds. The essential oil of lemongrass leaf contains sesquiterpenes with a higher specific gravity than monoterpenes (Table 3). The weight fraction is dependent on the length of the molecular chains present in the compounds. Molecules comprising a greater number of atoms are typically of a greater molecular weight.

The refractive index of essential oil ranges from 1.486 to 1.493 in line with the SNI standard of 1.4800 to 1.4930 (Table 2). According to Qorriana et al. (2015), the refractive index is used as a method of detecting the presence of water in essential oils. Water has a refractive index of 1.333, lower than essential oil (1.4800-1.4930). The addition of water to essential oil reduces the refractive index due to light refraction to a greater extent.

Lemongrass leaf essential oil is classified as soluble in alcohol but insoluble in water. The oil is soluble in 90% alcohol at a ratio of 1:2 to 1:3, in accordance with the SNI standard, which specifies a ratio less than 1:3 (Table 2). Essential oils with a higher proportion of polar compounds show enhanced solubility in alcohol. Lemongrass leaf essential oil contains compounds primarily composed of oxygenated monoterpenes and sesquiterpenes (Table 3), which exhibit greater polarity than non-oxygenated monoterpenes and sesquiterpenes. The functional groups present in oxygenated monoterpenes and sesquiterpenes, including hydroxyl (-OH), carbonyl (-C=O), and epoxide (-O-), enable the establishment of hydrogen bonds with alcohols, increasing the polarity of the molecules (Cavanagh & Wilkinson, 2002; de Sousa et al., 2023).

The physicochemical properties of the lemongrass leaf essential oil conform to the *Cymbopogon citratus* SNI standard. The characteristics were consistent with the essential oils derived from all parts of the lemongrass plant since only the leaves were used.

Chemical Composition of Lemongrass Leaf Essential Oil

A chemical content analysis was conducted using GC-MS to characterise the compounds present in lemongrass leaf essential oil. Dried leaf without

Table 2. Physical characteristics of lemongrass leaf essential oil

Parameter	Fresh leaf		Wilted leaf		Dried leaf		EOA Standard (*Cymbopogon citratus*)
	+MV	-MV	+MV	-MV	+MV	-MV	
Colour	Bright yellow	Bright yellow	Bright yellow	Bright yellow	Bright yellow	Bright yellow	Pale yellow to yellowish brown
Aroma	Fresh, citrus	Fresh, citrus	Fresh, citrus	Fresh, citrus	Fresh, citrus	Fresh, citrus	
Specific gravity (g/mL)	0.954±0.012^{Aa}	0.933±0.062^{Ba}	0.975±0.064^{Ab}	0.965±0.012^{Bb}	0.971±0.104^{Aa}	0.893±0.161^{Ba}	0.869-0.907
Refractive index	1.488±0.001^{Aa}	1.493±0.002^{Ba}	1.490±0.003^{Aa}	1.488±0.003^{Ba}	1.486±0.002^{Aa}	1.494±0.002^{c}	1.4800-1.4930
Solubility in alcohol	1:3	1:3	1:2	1:2	1:3	1:2	1:3

Description: microwave pre-treatment (+MV) and no pre-treatment (-MV). Different uppercase letters represent a significant difference between microwave and non-microwave, and different lowercase letters represent a significant difference between drying methods.

microwave pre-treatment (-MV) was selected as the control sample. Meanwhile, the sample with microwave pre-treatment (+MV) was analysed for chemical content. The GC-MS analysis detected 33, 32, 31, and 34 compounds in fresh leaf +MV, withered leaf +MV, dried leaf +MV, and dried leaf –MV, respectively. Table 3 shows the compounds identified in lemongrass leaf essential oil.

The principal constituents are citral (comprising geranial (44.07–46.81%) and neral (35.64–36.60%)), geranyl acetate (2.03–5.66%), isogeranial (1.61–2.57%), isoneral (0.96–1.76%), and β-pinene (0.16–3.63%) (Table 3). Geranial (trans-citral, citral A) and neral (cis-citral, citral B) are the two isomeric acyclic monoterpene aldehydes that constitute citral (3,7-dimethyl-2,6-octadienal). From a chemical perspective, citral is comprised of two aldehydes with the same molecular formula, $C_{10}H_{16}O$, but distinct structural characteristics (Hartatie et al., 2020). This is consistent with Yen & Lin (2017), where lemongrass essential oil (*Cymbopogon citratus*) is characterised by a high citral content.

Dried leaf without microwave treatment had lower citral content (79.71%) compared to microwave-treated leaf, including dried (82.09%), withered (81.14%), and fresh (82.54%) (Table 3). According to Kumar et al. (2015), the application of microwave radiation facilitates the oxidation of geraniol to geranial and nerol to neral.

Oxygenated monoterpenes constitute the majority of compounds in lemongrass leaf essential oil, comprising 93.19–96.44%. These include citral (neral and geranial), geranyl acetate, myroxide, linalool, isogeranial, and isoneral (Table 3). According to Moradi et al. (2018), the aroma, flavour, and therapeutic properties of oxygenated compounds serve as key indicators of essential oil quality. Oxygenated compounds contribute more to the olfactory profile of essential oils than monoterpene hydrocarbons, conferring a high degree of value. The higher content in microwave-treated samples suggests an enhanced quality (Moradi et al., 2018).

The concentration of oxygenated compounds in the dried leaf treated with microwave pre-treatment was higher (96.44%) compared to those without microwave treatment (94.19%) (Table 3). Research by Bendahou et al. (2008), Ferhat et al. (2007), Okoh et al. (2010) showed that microwave treatment typically enhanced the oxygenated compound content in essential oils. However, Wang et al. (2006) reported lower oxygenated compound levels in oils obtained through microwave treatment. This suggests that the oxygenated compound content of essential oils is more influenced by the plant species than by the extraction method (Moradi et al., 2018).

Table 3. Chemical composition of lemongrass leaf essential oil under different treatments

Compound components	Dried -MV		Dried +MV		Wilted +MV		Fresh +MV	
	RT (Min)	Area (%)	RT (Min)	Area (%)	RT (Min)	Area (%)	RT (Min)	Area (%)
ß-Pinene	7.95	3.63	7.90	0.32	7.95	2.89	7.90	0.16
Citronellal	12.54	0.21	12.54	0.22	12.55	0.22	12.55	0.12
Isoneral	12.91	1.76	12.91	1.47	12.91	1.40	12.90	0.96
Isogeranial	13.44	2.57	13.44	2.34	13.44	2.22	13.43	1.61
Neral (citral b)	15.52	35.64	15.54	36.60	15.55	36.38	15.53	35.73
Geranial (citral a)	16.13	44.07	16.23	45.49	16.15	44.76	16.23	46.81
Geranyl acetate	18.94	5.66	18.94	2.03	18.94	2.08	18.93	4.04
2,6-Octadiene, 1,1-diethoxy-3,7-dimethyl-	19.80	2.08	19.81	6.25	19.80	4.43	19.80	4.35
Monoterpene hydrocarbons		3.87		1.16		3.60		0.79
Sesquiterpene hydrocarbons		0.06		0.05		0		0
Oxygenated monoterpenes		94.19		96.44		93.19		96.40
Oxygenated sesquiterpenes		0.41		0.74		0.64		0.89
Aldehydes		0.86		1.08		1.29		0.78
Others		0.61		0.53		1.27		1.15
Identification Total		100.00		100.00		100.00		100.00

Description: microwave pre-treatment (+MV) and no pre-treatment (-MV).

Antioxidant Activity of Lemongrass Leaf Essential Oil

The antioxidant capacity of a given substance is categorised according to the IC_{50} value, with the categories of very strong (<50 μg/mL), strong (50-100 μg/mL), moderate (101-150 μg/mL), and weak (>150 μg/mL) (Saroyo & Arifah, 2021). Lemongrass leaf essential oil exhibits strong antioxidant activity, with IC_{50} values ranging from 61.65 to 84.5 μg/mL (Table 4). This result is consistent with Lawrence et al. (2015), who reported strong antioxidant activity for lemongrass essential oil with an IC_{50} value of 75.83 μg/mL.

Microwave pretreatment of lemongrass leaf significantly increased the antioxidant activity of essential oil ($p < 0.05$). This was evidenced by a decline in IC_{50} values in microwave-treated lemongrass leaf (61.65 - 70.93 μg/mL) in

Table 4. IC_{50} value of lemongrass leaf essential oil

Drying Method	IC_{50} Value (µg/mL)		Antioxidant Activity
	Without Microwave	Microwave	
Fresh Leaf	70.39±1.505^{Ab}	70.93±1.015^{Bb}	Strong
Wilted Leaf	84.5±5.390^{Ac}	70.86±0.970^{Bc}	Strong
Dried Leaf	68.37±1.980^{Aa}	61.65±0.765^{Ba}	Strong
Ascorbic Acid	10.45±0.025		Very Strong

Description: Different uppercase letters represent a significant difference between microwave and non-microwave, and different lowercase letters represent a significant difference between drying methods.

comparison to non-microwave treatment (68.37 - 84.5 µg/mL) (Table 4). A positive correlation was observed between the increase in antioxidant activity and the content of oxygenated compounds. In this context, the content of oxygenated compounds is higher in the microwave-treated leaf (96.44%) than in the non-treated leaf (94.19%) (Table 3).

The antioxidant activity of the essential oil was significantly influenced by the initial condition (fresh, wilted, dried) of the lemongrass leaf ($p < 0.05$). This is showed by lower IC_{50} values in fresh (70.39–70.93 µg/mL) and dried (61.65–68.37 µg/mL) leaf compared to wilted leaf (70.86–84.5 µg/mL). The discrepancy was associated with a higher abundance of oxygenated compounds in the essential oils of dry (97.18%) and fresh (97.29%) leaf, in comparison to wilted leaf (93.83%).

Lemongrass leaf essential oil exhibits strong antioxidant potential, largely attributable to the elevated concentration of compounds such as neral and geranial. Citral functions as an antioxidant by scavenging free radicals and preventing oxidative damage. The donation of electrons or hydrogen atoms (H^+) by the essential oil enables the neutralisation of DPPH free radicals, leading to the stabilisation of radicals as DPPH-H (Lawrence et al., 2015). This reaction results in a colour change from purple to yellow, showing a reduction in the concentration of free radicals (Santos-Sánchez et al., 2019).

Antibacterial Activity of Lemongrass Leaf Essential Oil

The MIC value shows the minimum concentration needed to inhibit bacterial growth. Meanwhile, the MBC value represents the minimum concentration necessary to eradicate the bacterial population. The minimum concentration of essential oils considered effective in inhibiting MIC and MBC typically ranges from 0.03% to 2% (v/v or w/v) and 0.5% to 5% (v/v or w/v), respectively. The exact range varies depending on the essential oil and the type of bacteria (Burt, 2004; Oyedeji et al., 2009).

The MIC values against *E. coli* and *S. aureus* ranged from 0.3% to 1.3% and 0.3% to 1.8%, respectively (Table 5). This showed that lemongrass leaf essential oil effectively inhibited bacterial growth. However, the values are higher than the result reported by Gao et al. (2020) for lemongrass stalk essential oil, with a MIC against *S. aureus* of 0.039–0.125%. The MBC values against *E. coli* and *S. aureus* were identical at 0.5%-3% (Table 5) since the essential oil was effective in bactericidal activity. These values were also higher than the result reported by Naik et al. (2010) for lemongrass stalk essential oil, where the MBC for *E. coli* and *S. aureus* were 0.12% and 0.06%, respectively. Therefore, essential oil produced from the lemongrass stalk has greater antimicrobial activity than the leaf.

Citral disrupts peptidoglycan synthesis in the cell wall, specifically in Gram-positive bacteria such as *S. aureus*, by downregulating the enzymes MurE and Pbp1 indispensable for peptidoglycan biosynthesis (Gao et al., 2020). This constituent has been shown to inhibit the expression of the *acpP, accA,* and *fapR* genes implicated in fatty acid biosynthesis, altering the bacterial membrane structure and leading to cell death through ion leakage (Purwanto & Irianto, 2022). Citral has been reported to impede quorum sensing, the bacterial communication system regulating the expression of virulence-related genes, including *hla*, which encodes α-toxin. Citral impairs the bacteria's capacity to colonise and form biofilms by inhibiting the master regulator, AgrA (Gao et al., 2020).

Essential oils from dried and fresh leaf exhibit higher antibacterial activity (MIC 0.3–1%; MBC 0.5–1.8%) compared to withered leaf (MIC 0.6–1.8%; MBC 2.3–3%) (Table 5). This is associated with the higher oxygenated compound content in essential oils from dried (97.18%) and fresh leaf (97.29%) compared to withered leaf (93.83%) (Table 3). Furthermore,

Table 5. MIC and MBC value of lemongrass leaf essential oil

Bacterial Strains	MIC (v/v %)						MBC (v/v %)					
	Fresh		Wilted		Dried		Fresh		Wilted		Dried	
	+MV	-MV	+MV	-MV	+MV	-MV	+MV	-MV	+MV	-MV	+MV	-MV
E. coli	0.3±0.00[a]	1.0±0.00[cd]	0.6±0.14[b]	1.3±0.43[d]	0.5±0.06[ab]	0.3±0.00[a]	0.6±0.14[a]	1.8±0.29[b]	2.3±0.29[c]	3.0±0.00[d]	0.6±0.14[a]	0.5±0.06[a]
S. aureus	0.3±0.00[a]	1.0±0.00[cd]	0.92±0.14[c]	1.8±0.29[e]	0.4±0.06[ab]	0.3±0.00[a]	0.6±0.14[a]	1.8±0.29[b]	2.3±0.00[c]	3.0±0.00[d]	0.6±0.14[a]	0.5±0.00[a]

Description: microwave pre-treatment (+MV) and no pre-treatment (-MV).

oxygenated compounds, such as alcohols, ketones, esters, and aldehydes, contain active oxygen groups (-OH, =O) interacting more strongly with bacterial cell membranes (Angane et al., 2022). The interactions are more effective due to the ability of oxygenated compounds to disrupt lipid integrity in the membrane, increase permeability, and cause leakage of intracellular components, inducing oxidative stress (Angane et al., 2022; Guimarães et al., 2019).

CONCLUSIONS

In conclusion, the highest yield (0.20%) of essential oil is produced by microwave-treated dried leaf, which exhibited physicochemical properties. Citral is the main chemical constituent of lemongrass leaf essential oil, with neral and geranial contents ranging from 35.64% to 36.60% and 44.07% to 46.81%, respectively. The antioxidant and antibacterial activities of lemongrass leaf essential oil are comparable to the stalk, which has been classified as a strong antioxidant for inhibiting bacterial growth.

CONFLICT OF INTEREST

The authors reported no potential conflict of interest concerning the submission of the manuscript entitled ' Potential of Lemongrass (*Cymbopogon citratus*) Leaf Byproduct as Source of Essential Oil ' for consideration in the Journal of Agritech.

REFERENCES

Akhihiero, E., Ayodele, B., & Akpojotor, G. (2013). Effect of Particle Size and Temperature Variation on the Yield of Essential Oil from Lemon Grass Using Steam Distillation †. *African Journal of Physics*, *66*, 105–112.

Angane, M., Swift, S., Huang, K., Butts, C. A., & Quek, S. Y. (2022). Essential Oils and Their Major Components: An Updated Review on Antimicrobial Activities, Mechanism of Action, and Their Potential Application in the Food Industry. *Foods*, *11*(3), Article 3. https://doi.org/10.3390/foods11030464

Batool, T., Zafar, M., Elshikh, M. S., Mustafa, A. E.-Z. M. A., Ahmad, M., Makhkamov, T., Islamov, S., Sotiboldiyeva, D., Yuldashev, A., De Oliveira, M. S., Majeed, S., Bakhramov, I., & Musthafa, M. M. (2024). Foliar epidermal micromorphology: A contribution to the taxonomy of family Oleaceae. *Genetic Resources and Crop Evolution*. https://doi.org/10.1007/s10722-024-02060-w

Bendahou, M., Muselli, A., grignon-dubois, M., Benyoucef, M., Desjobert, J.-M., Bernardini, A.-F., & Costa, J. (2008). Antimicrobial activity and chemical composition of Origanum glandulosum Desf. essential oil and extract obtained by microwave extraction: Comparison with hydrodistillation. *Food Chemistry*, *106*, 132–139. https://doi.org/10.1016/j.foodchem.2007.05.050

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, *94*(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Cortes-Torres, A. G., López-Castillo, G. N., Marín-Torres, J. L., Portillo-Reyes, R., Luna, F., Baca, B. E., Sandoval-Ramírez, J., & Carrasco-Carballo, A. (2023). Cymbopogon citratus Essential Oil: Extraction, GC–MS, Phytochemical Analysis, Antioxidant Activity, and In Silico Molecular Docking for Protein Targets Related to CNS. *Current Issues in Molecular Biology*, *45*(6), Article 6. https://doi.org/10.3390/cimb45060328

Ferhat, M., Meklati, B., & Chemat, F. (2007). Comparison of Different Isolation Methods of Essential Oil from Citrus Fruits: Cold Pressing, Hydrodistillation and Microwave 'Dry' Distillation. *Flavour and Fragrance Journal*, *22*, 494–504. https://doi.org/10.1002/ffj.1829

Fouelefack, F. R., Tapan, K. P., Dongmo, L. J. B., Ndonkeu, M. G., Mekam, P. N., & Nguefack, J. (2024). Correlation between the chemical composition of fresh and dried Cymbopogon citratus essential oil fractions and their antifungal effects against the causal agents of brown spot and bakanae diseases of rice. *European Journal of Plant Pathology*. https://doi.org/10.1007/s10658-024-02938-6

Gao, S., Liu, G., Li, J., Chen, J., Li, L., Li, Z., Zhang, X., Zhang, S., Thorne, R. F., & Zhang, S. (2020). Antimicrobial Activity of Lemongrass Essential Oil (Cymbopogon flexuosus) and Its Active Component Citral Against Dual-Species Biofilms of Staphylococcus aureus and Candida Species. *Frontiers in Cellular and Infection Microbiology*, *10*, 603858. https://doi.org/10.3389/fcimb.2020.603858

Gostin, I. N., & Blidar, C. F. (2024). Glandular Trichomes and Essential Oils Variability in Species of the Genus Phlomis L.: A Review. *Plants*, *13*(10), Article 10. https://doi.org/10.3390/plants13101338

Hartatie, E. S., Prihartini, I., Widodo, W., & Wahyudi, A. (2020). Short Communication: Detection of bioactive compounds in essential oil from lemongrass cultivated in Ngantang, Malang, East Java, Indonesia. *Biodiversitas Journal of Biological Diversity*, *21*(6), Article 6. https://doi.org/10.13057/biodiv/d210659

Idrees, M., Hakkim, L., Naikoo, G. A., & Hassan, I. (2019). *Recent Advances in Extraction, Characterization, and Potential Use of Citral* (pp. 225–236). https://doi.org/10.1007/978-981-13-7438-8_9

Iriti, M., Colnaghi, G., Chemat, F., Smadja, J., Faoro, F., & Visinoni, F. A. (2006). Histo-cytochemistry and scanning electron microscopy of lavender glandular trichomes following conventional and microwave-assisted hydrodistillation of essential oils: A comparative study. *Flavour and Fragrance Journal*, *21*(4), 704–712. https://doi.org/10.1002/ffj.1692

Kumar, V., Tewari, R., & Singh, K. (2015). Comparative Studies of Drying Methods on Yield and Composition of the Essential Oil of Cymbopogon citratus. *Journal of Essential Oil Bearing Plants*, *18*(3), 744–750. https://doi.org/10.1080/0972060X.2014.935069

Lawrence, R., Lawrence, K., Srivastava, R., & Gupta, D. (2015). *Antioxidant activity of lemon grass ESSENTIAL OIL (Cympopogon citratus) grown in North Indian plains*. *4*.

Lubis, A., Mandang, T., Hermawan, W., & Sutrisno. (2022). Characterization of The Yield and Quality of Patchouli Oil Based on The Size of Chopping and Drying Type. *IOP Conference Series: Earth and Environmental Science*, *1038*(1), 012075. https://doi.org/10.1088/1755-1315/1038/1/012075

M'hamdi, Z., Bouymajane, A., Riffi, O., Rhazi Filali, F., Ettarchouch, M., ELhourri, M., & Amechrouq, A. (2024). Chemical composition and antibacterial activity of essential oil of *Pelargonium graveolens* and its fractions. *Arabian Journal of Chemistry*, *17*(1), 105375. https://doi.org/10.1016/j.arabjc.2023.105375

Mirzaie, M., Ladanmoghadam, A. R., Hakimi, L., & Danaee, E. (2020). Water Stress Modifies Essential Oil Yield and Composition, Glandular Trichomes and Stomatal Features of Lemongrass (Cymbopogon citratus) Inoculated with Arbuscular Mycorrhizal Fungi. *Journal of Agricultural Science and Technology*, *22*(6), 1575–1585.

Moradalizadeh, M., Samadi, N., & Rajaei, P. (2013). Comparison of Hydrodistillation, Microwave Hydrodistillation and Solvent Free Microwave Methods in Analysis of the Essential Oils from Aerial Parts of Haplophyllum Robustum Bge. By GC/MS Method. *International Journal of Advanced Biological and Biomedical Research*, *1*(9), 1058–1067.

Moradi, S., Fazlali, A., & Hamedi, H. (2018). Microwave-Assisted Hydro-Distillation of Essential Oil from Rosemary: Comparison with Traditional Distillation. *Avicenna Journal of Medical Biotechnology*, *10*(1), 22–28.

Mukarram, M., Choudhary, S., Khan, M. A., Poltronieri, P., Khan, M. M. A., Ali, J., Kurjak, D., & Shahid, M. (2021). Lemongrass Essential Oil Components with Antimicrobial and Anticancer Activities. *Antioxidants (Basel, Switzerland)*, *11*(1), 20. https://doi.org/10.3390/antiox11010020

Mulyati, H., Yanti, R., & Supriyadi, S. (2023). Physicochemical Properties and Antioxidant Activity of Essential Oil from Fresh, Wilted, and Dried Leaf of Holy Basil (Ocimum tenuiflorum L.) Planted in Yogyakarta. *agriTECH*, *43*, 218. https://doi.org/10.22146/agritech.72545

Naik, M., Fomda, B., Jeyakumar, E., & Bhat, J. (2010). Antibacterial activity of lemongrass (Cymbopogon citratus) oil against some selected pathogenic bacterias. *Asian Pacific Journal of Tropical Medicine*, *3*, 535–538. https://doi.org/10.1016/S1995-7645(10)60129-0

Nour, A. H., Modather, R. H., Yunus, R. M., Elnour, A. A. M., & Ismail, N. A. (2024). Characterization of bioactive compounds in patchouli oil using microwave-assisted and traditional hydrodistillation methods. *Industrial Crops and Products*, *208*, 117901. https://doi.org/10.1016/j.indcrop.2023.117901

Okoh, O., Sadimenko, A., & Afolayan, A. (2010). Comparative evaluation of the antibacterial activities of the essential oils of Rosmarinus officinalis L. obtained by hydrodistillation and solvent free microwave extraction methods. *Food Chemistry*, *120*, 308–312. https://doi.org/10.1016/j.foodchem.2009.09.084

Oniha, M. I., Ahuekwe, E. F., Akinpelu, S. O., Oniha, M. I., Ahuekwe, E. F., & Akinpelu, S. O. (2023). Phytochemical Contents of Essential Oils from Cymbopogon Species: A Tropical Medicinal Plant. In *Tropical Plant Species and Technological Interventions for Improvement*. IntechOpen. https://doi.org/10.5772/intechopen.105396

Oyedeji, O. O., Lawal, O. A., Shode, F. O., & Oyedeji, A. O. (2009). Chemical Composition and Antibacterial Activity of the Essential Oils of Callistemon citrinus and Callistemon viminalis from South Africa. *Molecules*, *14*(6), Article 6. https://doi.org/10.3390/molecules14061990

Radzi, N. C., & Kasim, F. A. (2020). Effect of Microwave Pretreatment on Gaharu Essential Oil Using Hydrodistillation Method. *Indonesian Journal of Chemistry*, *20*(4), Article 4. https://doi.org/10.22146/ijc.43191

Santos-Sánchez, N. F., Salas-Coronado, R., Villanueva-Cañongo, C., Hernández-Carlos, B., Santos-Sánchez, N. F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). Antioxidant Compounds and Their Antioxidant Mechanism. In *Antioxidants*. IntechOpen. https://doi.org/10.5772/intechopen.85270

Saroyo, H., & Arifah, I. A. N. (2021). Antioxidant Activity Using DPPH & Frap Method and Their Correlation with The Levels of Phenolic and Flavonoid Compounds from Nemba Plants (Azadirachta Indica A. Juss). *Journal of Nutraceuticals and Herbal Medicine*, *3*(2), Article 2. https://doi.org/10.23917/jnhm.v3i2.15658

Scotti, R., Stringaro, A., Nicolini, L., Zanellato, M., Boccia, P., Maggi, F., & Gabbianelli, R. (2021). Effects of Essential Oils from Cymbopogon spp. And Cinnamomum verum on Biofilm and Virulence Properties of Escherichia coli O157:H7. *Antibiotics (Basel, Switzerland)*, *10*(2), 113. https://doi.org/10.3390/antibiotics10020113

Syarifuddin, S., Ellysa, E., Busthan, M., & Thalib, A. (2020). Pengaruh Waktu Penjemuran terhadap Rendemen pada Proses Ekstrasi Minyak Atsiri Eucalyptus dengan Metode Kohobasi. *Jurnal Riset Industri*, *16*(2), 64–71. https://doi.org/10.29360/mb.v16i2.6217

Wagner, G. J., Wang, E., & Shepherd, R. W. (2004). New approaches for studying and exploiting an old protuberance, the plant trichome. *Annals of Botany*, *93*(1), 3–11. https://doi.org/10.1093/aob/mch011

Wang, Z., Ding, L., Li, T., Zhou, X., Wang, L., Zhang, H., Liu, L., Li, Y., Liu, Z., Wang, H., Zeng, H., & He, H. (2006). Improved solvent-free microwave extraction of essential oil from dried *Cuminum cyminum* L. and *Zanthoxylum bungeanum* Maxim. *Journal of Chromatography A*, *1102*(1), 11–17. https://doi.org/10.1016/j.chroma.2005.10.032

Yen, H. Y., & Lin, Y. C. (2017). Green extraction of Cymbopogon citrus essential oil by solar energy. *Industrial Crops and Products*, *108*, 716–721. https://doi.org/10.1016/j.indcrop.2017.07.039