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# Quality Analysis and Anti-termite Activity of Essential Oils from the Leaves, Stems, and Roots of *Litsea firma* (Blume) Hook F

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**Abstract**

*Litsea firma* (Blume) Hook. f. has long been utilized as a traditional medicine plant. This research aims to analyze the quality of *L. firma* (Blume) Hook F essential oils extracted from its leaves, stems, and roots and to evaluate their anti-termite activity. The essential oils were obtained using the steam-water distillation method for 8 hours in triplicate. The quality of the essential oils was determined by their yield, specific gravity, refractive index, and optical rotation angle, which was compared with the previously reported quality data of *L. cubeba* essential oil. The results showed that all *L. firma* essential oils possessed a transparent colour, a distinctive aroma, and a bitter taste. The quality assessment revealed that essential oil yields ranged from 0.16% - 2.42%. The specific gravity of leaves essential oil was 0.83. Furthermore, the refractive index ratio ranged from 1.43 to 1.44, and the optical rotation angle varied between +15.3° to +16.8°. Phytochemical analysis revealed the presence of terpenes in *L. firma* essential oil. Essential oil of *L. firma* leaves exhibited a pronounced anti-termite activity with a percentage mortality of 100% and paper weight loss of 11.6 %. Thus, *L. firma* essential oils, particularly from leaves, had promising potential for natural termite control.

**Keywords:** *Litsea firma* (Blume) Hook F; Medang pirawas; No-choice test; Physicochemical analysis; Phytochemistry screening.

**Abbreviations:** TLC = thin layer chromatography; d = diameter.

## INTRODUCTION

Essential oils are extensively utilized in medicine and food preservation. In Indonesia, essential oils are a significant export commodity, contributing significantly to profits reaching up to 172.9 million US dollars (International Trade Center, 2022). Maximizing the market value of essential oils necessitates attaining high levels of purity, thereby ensuring excellent quality that commands a superior valuation (Rahman et al., 2019). Consequently, subjecting the produced essential oils to rigorous quality testing is imperative before engaging in enhanced domestic and international trading opportunities while effectively mitigating the risks associated with counterfeit products.

The quality of essential oils is ascertained through adherence to national and international quality standards. Several factors influence the quality of essential oils, including the variety of raw materials, plant cultivation methods, initial treatment of plant materials, oil extraction techniques, and post-production processes. The Indonesian National Standard (SNI) regulates quality standards for essential oils, which encompasses both general and specific requirements. General requirements for essential oils contain parameters such as odour, colour, specific gravity, refractive index, optical rotation, and solubility of essential oils in alcohol. On the other hand, specific requirements vary depending on the essential oils being tested, including factors such as active ingredient content, acid number, ester number, and fatty oil content (Satuhu & Yulianti, 2012).

Genus Litsea has been known as an aromatic plant (Ammar et al., 2020; Kusprandini et al., 2021). Numerous studies have been conducted on the quality of essential oils derived from the Litsea genus. Kuspradini (2021) investigated Litsea spp. Originating from Kalimantan, that is *L. angulata*, *L. elliptica*, and *L. rubiginosa*, focusing on the quality and antimicrobial of essential oils extracted from various parts of the plants through steam-water distillation. Meanwhile, the essential oil of *L. firma* (Blume) Hook. f. leaves and stems were reported previously for their chemical composition (Jamal, 2009). There was a difference in chemical composition between leaves and stems essential oils from the latter study. The leaf's essential oil was dominated by non-terpenes, including 2-undecanoate (39.45%) and 9-decen-2-one (34.33%), while the stems contained a higher concentration of undecanoate

(28.73%) and 7-hydroxy-3,7-dimethyloctanal (28.73%). Another variant of *L. firma*, *L. firma* var. austroannmensis from Vietnam, was reported to contain quite different chemical composition. Those compounds are oxygenated monoterpenes (33.2%), sesquiterpene hydrocarbons (24.8%), monoterpene hydrocarbons (22.4%), and oxygenated sesquiterpenes (14.8%) (Dai et al., 2020). Nonetheless, there is no report hitherto on the physicochemical properties of essential oil from *L. firma* (Blume) Hook. F., particularly from different parts of the plant. Thus, it could suggest the best source of essential oil from *L.firma* (Blume) Hook F.

*Litsea firma* (Blume) Hook. F., commonly known as medang pirawas or medang piawas by locals, is abundantly found in West Kalimantan. This plant has been traditionally used by local communities for various medicinal purposes, both internally and externally. The leaves of medang pirawas are utilized to eliminate fishy odour for postpartum mothers or to treat skin allergies in infants. All parts of the *L. firma* plant possess a distinctive aroma and are reputed to repel insects. Nevertheless, there is no report regarding the anti-termite activity from this plant's essential oil. On the other hand, essential oils obtained from *L. cubeba* (Kamle et al., 2019) and *L. elliptica* (Roszaini et al., 2022) have been reported to exhibit anti-termite properties, making them promising natural solutions for termite control. Thus, *L.firma* essential oil could likely have the potential to be an anti-termite.

Hence, this study explored the potential of essential oils derived from *L. firma* (Blume) Hook. f. plants for their quality and utility. The aims of this study were to determine the quality of essential oils from the leaves, stems, and roots of *L. firma* (Blume) Hook. f. compared to the quality of *L. cubeba* essential oil based on their physicochemical properties and to evaluate the anti-termite activity of *L. firma* (Blume) Hook F essential oils toward *Coptotermes curvignathus* Holmgren, a rubber termite.

## MATERIALS AND METHODS

### Materials

Plant materials, such as leaves, stems, and roots of *L. firma* (Blume) Hook F., were collected in Melawi district, West Kalimantan, in January 2023. The plant voucher was determined and deposited at the Research Center for Biology, Cibinong, Indonesia with the number letter B-1559/IPH.3/KS/XII/2020. Chemicals used included distilled water ($H_2O$), anhydrous sodium sulfate ($Na_2SO_4$) (Merck), methanol ($CH_3OH$ (99%) (Merck), *n*-hexane($C_6H_{14}$) (Merck), ethyl acetate ($C_4H_8O_2$) (Merck), silica gel 60 coated with fluorescence indicator $F_{254}$ aluminium TLC plate, and sulfuric acid ($H_2SO_4$ 10%). *Coptotermes curvignathus* Holmgren was employed for anti-termite assay. The equipment used consisted of a set of steam-water distillation apparatus, hot plate (IKA C-MAG HS 7, Germany), Abbe Atago DR-A1 refractometer, pycnometer, digital analytical balance, and Kernand wxg-4 polarimeter (Advanx AI003, Singapura).

### Procedures

***Plant sample preparation***

The leaves, stems, and roots of *L. firma* (Blume) Hook F. were cleaned from dirt with clean water and drained. Each part of the plant was aerated and roughly chopped.

***Extraction***

Leaves (200 grams), stems (200 grams), and roots (150 grams) were extracted using the steam-water distillation method for 8 hours, as described previously (Putri et al., 2023) with slight modification. The distillate was collected in a separatory funnel and separated from the water layer to yield essential oil. Anhydrous $Na_2SO_4$ was added to dry the essential oil from the remaining water. The pure essential oil was collected in a vial bottle (Figure 1).


**Figure 1.** Essential Oil of *L. firma* (Blume) Hook. f. leaves (a), roots (b), and stems (c).

***Physicochemical testing***

Quality analysis of L. firma essential oils was carried out through a series of physicochemical tests. these included determining the yield amount, specific gravity, optical angle of rotation, and refractive index of each essential oil based on procedures described by Kuspradini (2021). The Physicochemical data of L. firma essential oil was compared to the reported physicochemical data of essential oil were of L. cubeba, a commercial essential oil.

***Phytochemistry***

The TLC test on *L. firma* essential oil used an eluent system of *n*-hexane and ethyl acetate (97:3). The resulting spot from elution was sprayed with $H_2SO_4$ (10%) and heated until the stain was formed, and observed under UV light at 254 nm.

***Anti-termite assay***

The Anti-termite evaluation was conducted against subterranean termites, Coptotermes curvignathus Holmgren, using the no-choice test described previously (Adfa et al., 2017) with modification. Briefly, the essential oil's concentration was prepared for 10% (v/v) in ethanol. thirty workers and 3 soldiers of C. curvignathus Holmgren were fed with essential oil-impregnated filter paper Whatman no. 1 (d = 3 cm).As a negative control, the same amounts of termites were fed with Whatman filter paper without essential oil in it. For 21 days, termite condition was observed each day and the death termites were calculated. at the end of day 21, percentage mortality and percentage paper weight loss were determined. Percentage mortality was calculated using the formula as follows:

$$\%\, termite\ mortality = \frac{Ndta}{Ntb} \times 100\% \quad (1)$$

where Ndta is the number of dead termites after treatment and Ntb is the number of living termites before treatment. Percentage paper weight loss was calculated as follows:

$$\%\, paper\ weight\ loss = \frac{Pwb - Pwa}{Pwb} \times 100\% \quad (2)$$

where Pwb is the paper weight before treatment and Pwa is the paper weight after treatment. Then, the absolute coefficient was determined using the formula:

$$A = \frac{KK - EE}{KK + EE} \times 100\% \quad (3)$$

where *A* is the percentage of absolute coefficient, *KK* is paper weight loss of control paper, and *EE* is paper weight loss of tested paper.

**Data analysis**

Extraction, physicochemical tests, and anti-termite activity measurements were carried out in triplicate. Essential oil quality data obtained was expressed as a mean (±) and standard deviation (*SD*), with a confidence level of $p < 0.05$ considered statistically significant. Anti-termite data was represented as a mean and a standard deviation. The mean difference between obtained anti-termite data was determined using a t-test with a p-value < 0.05.

## RESULTS AND DISCUSSION

**Physicochemical properties**

The essential oils obtained from all parts of *L. firma* possessed a characteristic aromatic aroma, bitter taste, and clear colour (Figure 1). Essential oil quality parameters observed in this study were based on physicochemical properties, including yield, specific gravity, refractive index, and optical rotation angle, which were compared to other Litsea genera, particularly *L. cubeba*. *Litsea cubeba* essential oil, commonly known as "May Chang," has gained recognition as an export commodity from China. Therefore, it is a suitable benchmark for assessing the quality of *L. firma* essential oil. Physicochemical data of *L. firma* essential oils showed that they differed significantly in yield (Table 1.)

**Table 1.** Quality analysis data of essential oils from *L. firma* (Blume) Hook F.

| Sample | Yield (%, b/b) [a] | Specific gravity [a] | Refractive Index[a] | Optical Rotation Angle [a] |
|---|---|---|---|---|
| Leaves | 2.42 ± 0.32[b] | 0.83±0.00 | 1.43 ± 0.00[c] | +15.8°±0.3 |
| Stems | 0.16 ± 0.38[b,c] | n.d[d] | 1.44 ± 0.00[c] | +16.8°±2.5 |
| Roots | 0.37 ± 0.16[b,c] | n.d[d] | 1.44 ± 0.00[c] | +15.3°±1.1 |

[a]as a mean with *n*=3 and SD,
[b]significantly difference (p-value < 0.05, t-test)
[c]not significantly difference (p-value > 0.05, t-test)
[d]not determined

*Yield* is a crucial parameter that indicates the quantity of essential oil obtained through the distillation process. Knowledge of the amount of yield facilitates calculations in preparing fresh ingredients according to the expected yield of essential oils (Adisa et al., 2022). Plant parts and the extraction duration will significantly influence the yield of essential oil produced. In this study, the essential oil from *L. firma* leaves exhibited the highest yield at a percentage of 2.42%, followed by roots essential oil (0.37%) and stems essential oil (0.16%) (Table 1). In this study, *L. firma* roots proved to contain a pronounced quantity of essential oil that has not been reported previously. The variation in yield among different plant parts can be attributed to the distinct chemical compositions of the leaves, stems, and roots (Putri et al., 2023). It seemed that *L. firma* essential oil was dominantly deposited in plant leaf tissue.

The yield of essential oils from *L. firma* (Blume) Hook F. leaves and stems in this study demonstrated higher values compared to the findings reported by

Jamal (2009). In her investigation, the yield of essential oil leaves and stems obtained using the steam-water distillation method for 5 hours was 1.68% and 0.23% respectively. These phenomena suggested that extraction duration time affects the yield quantity. However, it should be noted that excessively long distillation time can affect the quality of the essential oil produced (Utomo & Mujiburohman, 2018). Plant materials' drying and storage processes significantly influence the resulting essential oil yield. In this study, the plant samples were stored for 27 days and dried for three days, likely resulting in a decrease in the essential oil yield of *L. firma*. Drying at excessively high temperatures and for a prolonged duration can cause a reduction in the essential oil content (Satuhu & Yulianti, 2012). Therefore, it is necessary to conduct further studies on the drying variations for *L. firma* plant tissue to obtain its essential oil.

Despite the difference in stem-water distillation duration used, comparison to essential oil reported previously from other Litsea genera revealed that the yield of essential oil of *L. firma* leaves was higher than that of *L. angulata* leaves (0.90%), *L. elliptica* leaves (0.70%), *L. rubiginosa* leaves (0.17%) (Kusprandini et al., 2021). Nevertheless, the essential oil yield of *L. cubeba* leaves (9.33%) (Suwandhi et al., 2014) was higher than that of *L. firma* leaves (Figure 2.). Thus, the position *L. firma* leaves essential oils in the Litsea genus was promising.

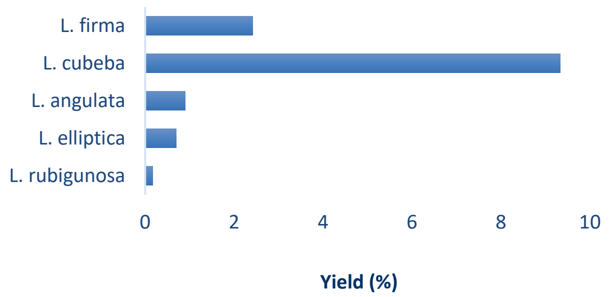



**Figure 2**. Essential oil yield from several Litsea species.

The steam-water distillation method is highly effective in obtaining substantial essential oil. This process involves heating water vapour, which then permeates the plant tissue, facilitating the release of essential oil onto the surface. The essential oil subsequently evaporates and travels toward the condenser. This essential oil extraction method offers several advantages, including using steam and heat under constant pressure. Consequently, the distillation time is expedited while the risk of essential oil degradation is minimized (Nugraheni et al., 2016).

*Specific gravity*, defined as the ratio of the weight and volume of essential oil compared to that of water, is an essential parameter for assessing the water content in essential oils. *Specific gravity* can also be defined as the ratio of the density of the essential oil to the density of water (Kua et al., 2021). Consequently, specific gravity offers valuable insights into the water content present within essential oils. Higher water content in essential oil corresponds to lower specific gravity values, whereas lower water content leads to higher specific gravity values. Furthermore, specific gravity is influenced by various factors, including the composition of compounds within the essential oil, such as molecular weight and carbon chain (Guenther, 2006). In the study, *specific gravity* measurement was conducted solely on essential oil obtained from leaves, due to the lack of essential oil from stems and roots.

The analysis revealed that the specific gravity of *L. firma* essential oil from leaves was 0.83 (Table 1), which was still below the range of specific gravity standard for *L. cubeba* essential oil (0.86-0.91 at 20°C) and *L. angulata* (0.86 at 20°C). However, it had a similar specific gravity value with *L. elliptica* essential oil (0.83 at 20°C) (Aryani et al., 2023). This discrepancy suggested that *L. firma* essential oil extracted from leaf parts exhibited a higher water content than *L. cubeba* and *L. angulata*.

The refractive index represents the ratio between the sine of the angle of incidence and the sine of the angle of refraction when a beam of light of a specific wavelength transitions from air to oil at a particular angle (Guenther, 1987). According to a study by Erliyanti et al. (2020), the refractive index exhibits a direct relationship with density, implying that higher density values correspond to higher refractive index values. This correlation arises from the influence of molar refractivity on density. The refractive index represents the behaviour of light waves, where a higher density in a material leads to a more excellent refractive index value, indicating slower light movement.

The measurement of the refractive index of *L. firma* essential oil revealed no significantly different values across the leaves, stems, and roots, ranging from 1.43 to 1.44 (Table 1). In comparison, the reported refractive index of *L. cubeba* at 20°C ranges from 1.47 to 1.49. Likewise, the refractive index of *L. firma* essential oil falls short of the quality standard set by *L. cubeba*. It is important to note that the refractive index indicates water content in essential oils, with higher water content leading to a lower refractive index. This phenomenon arises from the refractive properties of water, which can negatively impact the quality of essential oils (Guenther, 1987). Hence, the refractive index value serves as an indicator of the essential oil's purity level. A higher refractive index value indicates lower water content and higher compound density.

In order to decrease the water content in essential oils, purification can be achieved by adding anhydrous $Na_2SO_4$. The anhydrous $Na_2SO_4$ should be activated by heating it in an oven for 4 hours at 80°C before applied to essential oil (Marfina et al., 2019). This activation process ensures that $Na_2SO_4$ delivers optimal outcomes in binding water molecules present in essential oils. Hence, in terms of the refractive index, although *L. firma* essential oil has not yet attained the quality standard of *L. cubeba*, there remains potential for enhancing its quality through purification techniques and reducing water content.

Essential oils possess the ability to exhibit optical activity, which refers to the capacity of compounds within the oil to alter the polarization direction of light as it traverses a specific medium. The optical rotation angle in essential oils can be measured using a polarimeter instrument, which quantifies the rotation angle in degrees. The optical rotation in essential oils can vary depending on the composition of compounds within the oil. Optical rotation is a significant physical characteristic in analyzing and identifying essential oils, providing insights into the structural and optical properties of the essential oil compounds.

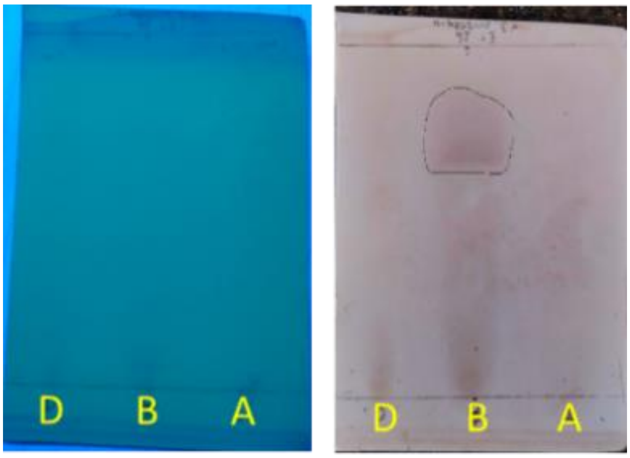
Among the *L. firma* essential oils, stem essential oil exhibited the bigger optical rotation angle with +16.8°. Meanwhile, the roots and leaves essential oils showed similar optical rotation angle values, specifically +15.8° and +15.3°, respectively (Table 1). Nevertheless, all optical rotation values exhibited positive values. A positive (+) optical rotation signifies that the essential oil compound components can rotate the polarization plane to the right. Such compounds are characterized by asymmetric carbon atoms, bonding the carbon atom to four distinct groups within its structure (Lasapo et al., 2016). This result added to a previous report that *L. firma* leaves and stems essential oils possessed chiral compounds (Jamal, 2009).

In comparison to *L. cubeba* leaves essential oil, its optical rotation angle was recorded at +20.3°, while its stem essential oil's optical rotation angle was recorded at -18.3°. This comparison result could indicate that the chemical composition of *L. firma* essential oils might differ from that of *L. cubeba* essential oils.

**Phytochemical analysis**

In order to identify the presence of terpenes in *L. firma* essential oils, TLC plates were employed with an eluents system consisting of *n*-hexane and ethyl acetate (97:3) and a terpenes spotting reagent. TLC chromatogram showed one purple spot on TLC for essential oil from stems, with a retention factor (Rf) value of 0.7 after adding the spotting reagent (Figure 2). although the spot colours were not as bright as the stems' essential oil, similar identifiable purple spots were detected for essential oil from leaves and roots. This might be due to less concentration of terpenes contained in the latter essential oils. This analysis of the leaves strengthened this suggestion and stem essential oil's chemical composition previously reported (Jamal, 2009), which showed that detected terpenes in leaves essential oil were slightly less amount than in stems. Thus, since similar TLC spots are shown by roots essential oil with one in leaves essential oil, the chemical composition in roots essential oil might be closely identic to leaves essential oil. Nevertheless, these results confirmed that essential oils from all parts of *L. firma* contained terpenes.

Terpenes are applied to numerous facets of everyday life and human health. They are utilized in developing pharmaceutical products, nutraceuticals, food and beverage formulations, cosmetics, perfumes, synthetic chemicals, aroma and flavour additives, rubber products, and even the biofuel industry. Hence, terpene compounds assume a broad and diverse range of roles across various sectors that directly impact human existence (Masyita et al., 2022).




(a) (b)

**Figure 3.** TLC chromatogram profile of *L. firma* essential oil from leaves (D), stems (B), and roots (A) (*n*-hexane and ethyl acetate (97:3)) after heated and sprayed with a spotting reagent of $H_2SO_4$ (10%), (a) under UV light at 265 nm and (b) under visible light.

**Anti-termite analysis**

The local community has reported the potential of *L. firma* wood to act as an insect repellent. Anti-termite evaluation on essential oil from *L. firma* leaves towards *Coptotermes curvignathus* Holmgren proved that the essential oil exhibited very strong anti-termite activity (Table 1.) with 100% termite death. The Percentage of paper weight loss was below 50%, which suggests that the essential oil possessed feeding deterrent activity (Adfa et al., 2017). It was supported by an absolute coefficient percentage value of 58%, which was classified as strong antifeedant (Dungani et al., 2012).

**Table 2**. Anti-termite data of essential oil from *L. firma* (Blume) Hook F.

| Sample | Mortality (%)[a] | Paper weight loss (%)[a] | Antifeedant Absolute Coefficient (%)[a] |
|---|---|---|---|
| Leaves | 100,00 ± 0,00 | 11,59 ± 4,72 | 58,60 ± 18,49 |
| Stems | n.d | n.d | n.d |
| Roots | n.d | n.d | n.d |

[a]as a mean with *n*=3 and SD
[b]not determined

## CONCLUSIONS

This study concluded that *L.firma* produced essential oil more from its leaves. Regardless of their lower quantity compared to *L. cubeba* essential oil, the quality of all *L. firma* leaves, stems, and roots essential oils were similar. Additionally, terpenoid compound was detected in all *L. firma* essential oils. Hence, it can be suggested that an effective and efficient extraction method be found to produce essential oil with more yield. This study successfully added more value to essential oil from *L.firma* leaves, in which the essential oil exhibited pronounced anti-termite and antifeedant activity. Thus, *L. firma* leaves essential oil possessed significant potential as a natural termite control. Therefore, further research is needed to investigate the toxicity of *L. firma* essential oils and its other biological activities.

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***Authors' Contributions:*** All authors designed the study. Febrilia Gerina carried out the laboratory work. Rini Muharini analyzed the data. Rini Muharini and Febrilia Gerina wrote the manuscript. All authors read and approved the final version of the manuscript.

***Competing Interests:*** The Authors declare that there are no conflicts of interest. The authors reported no potential conflict of interest.

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