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Fingerprinting Compound Profile of Tammate (*Lannea coromandelica* (Hout) Merr.) Bark Extract Using Chemometric and FTIR Analysis

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Abstract: *Lannea coromandelica* is a traditional medicinal plant rich in alkaloids, flavonoids, saponins, and tannins with reported antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and antirheumatic activities. Previous studies have primarily emphasized phytochemical screening and pharmacological evaluation, whereas metabolite fingerprint characterization using spectroscopic and chemometric approaches remains limited. This study aimed to characterize the metabolite fingerprint profile of *L. coromandelica* stem bark extracts using Fourier Transform Infrared (FTIR) spectroscopy and chemometric analysis. Twelve extracts were prepared using maceration, Reflux and sonication with distilled water, ethanol, n-hexane, and ethyl acetate to obtain metabolites with different polarities. FTIR spectroscopy was employed due to its rapid, non-destructive, and reproducible detection of functional groups, while chemometric analysis enhanced extract discrimination and pattern recognition. FTIR spectra revealed O–H, N–H, C–H, C=C, C=O, and C–C functional groups associated with phenolic, aromatic, and alkaloidal compounds related to antioxidant and anti-inflammatory activities. Principal Component Analysis (PCA) showed clear clustering patterns influenced by extraction method and solvent polarity, while Cluster Analysis (CA) demonstrated similarity values of 35.54–99.82% among extracts. These findings confirm that FTIR combined with chemometrics is effective for metabolite fingerprint characterization and quality standardization of *L. coromandelica* extracts.

Keywords: Chemometric; Fingerprinting compound; FTIR; *Lannea coromandelica* (Houtt.) Merr.

Introduction

Herbal medicines have been widely utilized worldwide due to their therapeutic potential and cultural acceptance. However, the chemical complexity and variability of herbal materials present major challenges for standardization and authentication (X. Wang et al., 2021). The Tammate plant (*Lannea coromandelica*) belongs to the *Anacardiaceae* family and commonly found in tropical regions, including Central Sulawesi, Indonesia. Locally, it is referred to as "kayu tammate" in Makassarese and "aju jawa" in Buginese. Traditionally, it is used as fencing material and firewood (Putra & Rahman, 2020). Previous studies have reported that Lannea coromandelica contains diverse secondary metabolites, including alkaloids, saponins, tannins, carbohydrates, steroids, cardiac glycosides, terpenoids, and flavonoids (Ulandari & Sani, 2023).

This plant is widely utilized in traditional medicine for treating internal and external ailments such as diarrhea, toothache, bruises, and burns. Despite its increasing use, the quality of traditional medicines must

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be improved. One major challenge is the complexity of chemical constituents in medicinal plants, which complicates quality control and increases the risk of adulteration, especially when raw material supply is limited (Laksono & Hayati, 2021). Furthermore, the quality control of natural products is particularly challenging because their chemical fingerprints are highly dynamic and may vary depending on plant species, plant organs, environmental factors, and extraction methodologies (Khair et al., 2017; Noviana et al., 2022). Adulteration often involves mixing with morphologically similar or closely related species.

Therefore, a reliable quality control method is needed to identify specific characteristics of medicinal plants, ensuring consistent quality, efficacy, and safety (Rafi et al., 2017). Two common approaches for quality control include active compound analysis and multicomponent fingerprinting. The latter provides more realistic and accurate information (Maruzy et al., 2020).

Chemical fingerprinting has been widely recognized as a comprehensive strategy for the holistic quality assessment of herbal materials because it provides integrated information regarding complex chemical matrices. Among various analytical techniques, Fourier Transform Infrared (FTIR) spectroscopy has emerged as a powerful platform for metabolite fingerprinting due to its rapid, non-destructive, cost-effective, and environmentally friendly characteristics, while requiring only minimal sample preparation (Liu et al., 2025; Socaciu et al., 2020; Y. Y. Wang et al., 2019). In addition, FTIR spectroscopy generates multidimensional spectral information that reflects the chemical characteristics of a sample (Agung et al., 2024; Dhea et al., 2023). However, FTIR spectra derived from complex plant extracts frequently contain overlapping absorption bands and high-dimensional data, making direct spectral interpretation challenging (Mahmuda et al., 2020). Therefore, FTIR analysis is commonly integrated with chemometric approaches to improve pattern recognition and sample discrimination. Furthermore, variations in extraction methods and solvent polarity may significantly influence metabolite recovery, thereby affecting chemical fingerprint profiles and complicating extract characterization and standardization (Syahrir et al., 2025).

Changes in peak positions and intensities in FTIR spectra are directly related to the chemical composition of a plant, allowing differentiation even when the exact compound composition is unknown. Previous studies have successfully used FTIR for plant identification and authentication (Umar et al., 2016; Yasir et al., 2021). FTIR coupled with PCA and Cluster Analysis has been successfully applied to classify Garcinia mangostana extracts based on solvent type and plant part (Yasir et al., 2025). Similar approaches integrating FTIR, UV–Vis spectroscopy, and PCA were also employed to classify Impatiens balsamina extracts according to extraction solvent with different polarities (Triawan et al., 2025). Fourier Transform Infrared (FTIR) spectroscopy combined with chemometric analysis is not only effective for differentiating extracts based on solvent type, but also reliable for evaluating chemical profile variations resulting from different extraction techniques. Conventional extraction methods such as maceration generally require prolonged extraction times and rely primarily on passive diffusion processes. In contrast, ultrasound-assisted extraction (sonication) generates acoustic cavitation capable of enhancing cell wall disruption and mass transfer efficiency, thereby promoting the release of intracellular metabolites into the extraction solvent (Patle et al., 2025). Due to the complexity of FTIR data interpretation, chemometric techniques such as multivariate analysis are essential. However, studies concerning FTIR-based chemometric fingerprinting of Lannea coromandelica stem bark extracts, particularly regarding the influence of extraction methods and solvent polarity on spectral classification patterns, remain very limited. Therefore, FTIR spectroscopy combined with multivariate chemometric approaches was employed to investigate the spectral fingerprint characteristics of Lannea coromandelica stem bark extracts and to assess the performance of chemometric models in identifying clustering patterns and chemical profile variations among the extracts.

Method

Materials

The materials used included bark of *L. coromandelica*, distilled water, chloroform, 70% ethanol, ethyl acetate, n-hexane, and KBr.

The equipment used in this study included a stirring rod, blender, funnel (HERMA®), Erlenmeyer flask (IWAKI®), beaker glass (IWAKI®), measuring cylinder (IWAKI®), heating mantle, filter paper, clamps, laptop (ASUS VivoBook 14), condenser, round-bottom flask (IWAKI®), volumetric flask (IWAKI®), drying oven, Minitab®21 software, sonicator (Branson®1800), FTIR spectrophotometer (SHIMADZU®), stand and clamp, thermometer, analytical balance (Mettler Toledo®), maceration container (glass jar), and water bath.

Sample Preparation

Freshly collected samples were wet-sorted and washed under running water. They were then dried in an oven at 55°C for three days. Once dried, the simplicia were dry-sorted, ground into powder, sieved using mesh no. 18, and stored in airtight containers (AR et al., 2023).

Extraction Procedures

Maceration Technique

A total of 100 grams of powdered simplicia was placed into four maceration vessels and pre-wetted with each solvent (distilled water, 70% ethanol, ethyl acetate, and n-hexane). Each solvent was added in a volume of 750 mL (1:7.5 ratio). The extraction was carried out for three days with occasional stirring. The mixture was filtered to obtain the first filtrate, and the residue was re-extracted for two more days. The second filtrate was combined with the first and concentrated using a water bath at temperatures below 60°C (AR et al., 2023).

Reflux Technique

A total of 100 grams of powdered simplicia was placed in a round-bottom flask and pre-wetted with each solvent. Each solvent was added in a volume of 750 mL (1:7.5 ratio). The extraction was conducted for two hours. The extract was filtered and concentrated using a water bath at temperatures below 60°C. The yield percentage of each extract was then calculated (AR et al., 2023).

Sonication Technique

A total of 100 grams of powdered simplicia was placed in a 250 mL Erlenmeyer flask and pre-wetted with each solvent. Each solvent was added in a volume of 187.5 mL. The extraction was performed for 30 minutes and repeated four times, resulting in a total solvent volume of 750 mL (1:7.5 ratio). The extract was filtered and concentrated using a water bath at temperatures below 60°C. The yield percentage of each extract was then calculated (AR et al., 2023).

FTIR Spectrophotometric Analysis

A mixture of 0.02 mg extract and 0.2 mg KBr (1:10 ratio) was ground using a mortar and pestle, then pressed into a thin transparent pellet using a hand press at 20 tons of pressure. The sample was analyzed using an FTIR spectrophotometer. The resulting spectra were processed and interpreted to obtain fingerprint profiles, followed by chemometric statistical analysis (Yasir et al., 2021).

Chemometric Analysis

The wavenumber values derived from FTIR spectroscopy measurements were processed using chemometric analysis. The chemometric methods utilized in this study were Principal Component Analysis (PCA) and Cluster Analysis (CA), performed using Minitab® 21 software (Syahrir et al., 2025).

Result and Discussion

Extraction Analysis

The analysis of metabolite compounds in *Lannea coromandelica* (Houtt) Merr. was conducted using Fourier-transform infrared (FTIR) spectrophotometry and subsequently analyzed through chemometric techniques. Extracts of the stem bark of *L. coromandelica* were prepared employing maceration, reflux, and sonication extraction methods, utilizing four different solvents: distilled water (aquadest), ethanol, ethyl acetate, and n-hexane. The extraction process was performed using a simplicia-to-solvent ratio of 1:7.5. This ratio was selected based on the principle that an increased volume of solvent facilitates the extraction of a greater quantity of compounds (Dmitrienko et al., 2024). The application of three distinct extraction methods aimed to evaluate the influence of temperature on the extraction efficiency. Cold extraction techniques, such as maceration, are advantageous for isolating heat-sensitive secondary metabolites, whereas hot extraction methods, such as reflux, leverage thermal energy to enhance the solvent's capacity to dissolve compounds, thereby optimizing extraction yield (Ananda et al., 2025; Chaves et al., 2020; Idawati et al., 2019; Mardin, 2025).

This study employed solvents with varying degrees of polarity, namely n-hexane, ethyl acetate, and ethanol. The rationale for using four different solvents was to investigate the functional group characteristics of the extracts obtained with solvents of differing polarity (Ma et al., 2018). Solvents serve as the medium in the extraction process to attract or separate bioactive compounds. The primary factors in selecting an extraction solvent include selectivity, ease of handling, compatibility with the extraction process, cost-effectiveness, environmental friendliness, and safety (Chaves et al., 2020; Tampang et al., 2024).

Fingerprint Compound Analysis

The FTIR spectra of the samples are presented in Figure 1. The FTIR spectra of the *Lannea coromandelica* stem bark extracts obtained through various extraction methods and solvents exhibit similar spectral patterns, with differences primarily in the intensity of transmittance. This suggests the presence of several common chemical compounds across the different extraction techniques and solvents.

Analysis of the FTIR measurements revealed several absorption peaks indicative of functional groups. The observed absorption peaks at wavenumbers 1026.13 cm^{-1}, 1284.59 cm^{-1}, 1058.92 cm^{-1}, 1163.08 cm^{-1}, 1273.02 cm^{-1}, 1112.93 cm^{-1}, 1037.7 cm^{-1}, 1282.66 cm^{-1}, 1060.85 cm^{-1}, 1024.2 cm^{-1}, 1238.3 cm^{-1}, and 1255.66 cm^{-1} correspond to C–O streching vibration functional groups (Wong, 2015). These absorption bands are commonly associated with alcohols, ethers, esters, glycosides, and phenolic compounds, suggesting the presence of oxygenated secondary metabolites that may contribute significantly to the characteristic chemical fingerprint of the extracts (Hssaini et al., 2021). Peaks at 1575.84 cm⁻¹, 1448.54 cm⁻¹, 1678.07 cm⁻¹, 1514.12 cm⁻¹, 1411.89 cm⁻¹, 1521.84 cm⁻¹, 1620.21 cm⁻¹, 1670.35 cm⁻¹, 1460.11 cm⁻¹, 1560.41 cm⁻¹, 1568.13 cm⁻¹, 1614.42 cm⁻¹, 1658.78 cm⁻¹, and 1512.19 cm⁻¹ were attributed predominantly to aromatic and conjugated C=C stretching vibrations, which are commonly associated with phenolic compounds, flavonoids, and other aromatic phytochemicals (Ma et al., 2018). The strong presence of these aromatic-associated bands suggests that phenolic-derived metabolites constitute major fingerprint markers contributing to the discrimination of the extracts. Peaks detected at 1870.95 cm⁻¹, 1695.43 cm⁻¹, 1869.02 cm⁻¹, 1739.79 cm⁻¹, 1867.09 cm⁻¹, 1699.29 cm⁻¹, 1662.64 cm⁻¹, 1670.35 cm⁻¹, 1741.72 cm⁻¹, 1614.42 cm⁻¹, 1830.45 cm⁻¹, and 1822.73 cm⁻¹ correspond to characteristic of C=O stretching vibrations of carbonyl-containing phytochemicals (Wong, 2015). These bands may indicate the presence of aldehydes, ketones, esters, carboxylic acids, and quinone-type compounds, which are frequently involved in defining the unique metabolite fingerprint profile of medicinal plant extracts.

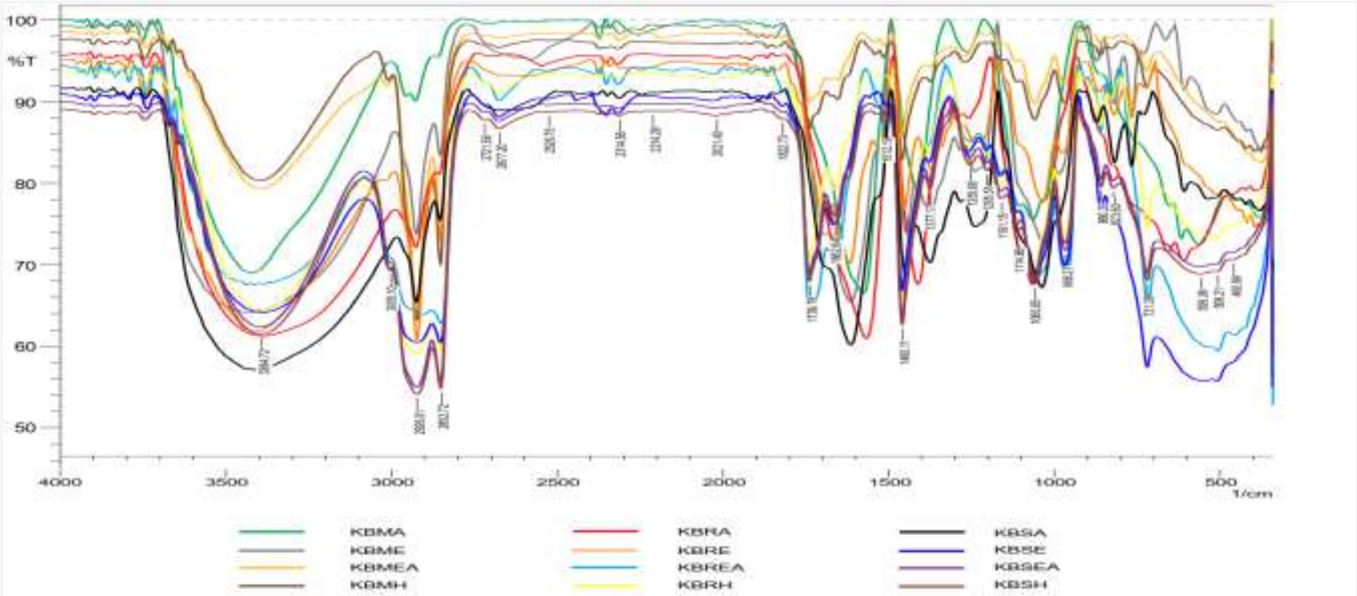

Figure 1. FTIR spectrum of lannea coromandelica extract using maceration, reflux and sonication extraction techniques with aquades, 70% ethanol, ethyl acetate and n-hexane as solvents

Additionally, absorption bands at 2709.99 cm⁻¹, 2862.36 cm⁻¹, 2927.94 cm⁻¹, 2956.87 cm⁻¹, 2854.65 cm⁻¹, 2926.01 cm⁻¹, 2924.09 cm⁻¹, 2852.72 cm⁻¹, 2858.58 cm⁻¹, and 2850.79 cm⁻¹ were attributed to aliphatic C–H stretching vibrations, indicating the presence of methyl and methylene groups (Mutmainnah et al., 2017; Wong, 2015). These functional groups are commonly related to terpenoids, steroids, lipids, and hydrocarbon chains that may contribute to the hydrophobic fingerprint characteristics of the extracts. Peaks observed at 3421.72 cm⁻¹, 3446.79 cm⁻¹, 3388.93 cm⁻¹, 3394.72 cm⁻¹, 3385.07 cm⁻¹, 3419.79 cm⁻¹, 3747.69 cm⁻¹, 3020.53 cm⁻¹, 3388.93 cm⁻¹, 3442.94 cm⁻¹, 3387 cm⁻¹, and 2445.74 cm⁻¹ were attributed primarily to O–H and possibly N–H stretching vibrations, indicating the presence of hydroxylated and nitrogen, containing phytochemicals such as phenolics, flavonoids, alkaloids, and amino compounds (Juariah et al., 2025; Marwulan et al., 2023; Wong, 2015). Overall, the presence and variation of these functional groups across the extracts indicate that the FTIR spectra successfully captured the characteristic chemical fingerprint profiles of Lannea coromandelica stem bark extracts. The observed spectral differences may reflect variations in metabolite composition resulting from differences in extraction methods and solvent polarity, thereby supporting the applicability of FTIR fingerprinting combined with chemometric analysis for extract discrimination and quality evaluation.

Chemometric Analysis

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were employed to visualize extract clustering patterns based on variations in chemical and spectral profiles, thereby enabling objective discrimination among sample groups (Zahara et al., 2023; Zulfani et al., 2025). These multivariate approaches utilize dimensionality reduction to simplify the complexity of spectral variables into more informative principal components, allowing similarity and dissimilarity patterns among samples to be interpreted within a lower-dimensional space (Rafi et al., 2022).

A scree plot is a diagram that illustrates the trend of decreasing eigenvalues and is used subjectively to determine the number of factors to be retained. Based on the scree plot results shown in Figure 2, the eigenvalues can be observed, where values greater than two indicate more significant variation within the data. The larger the eigenvalue, the greater its contribution to explaining the variability in the dataset. In matrix analysis, eigenvalues greater than two are considered strong indicators of relevant structures or patterns within the data. The eigenvalues can be identified by a sharp downward slope on the scree plot, which in this case reveals two principal components: PC1 with an eigenvalue of 6.0605 and PC2 with an eigenvalue of 3.3994.

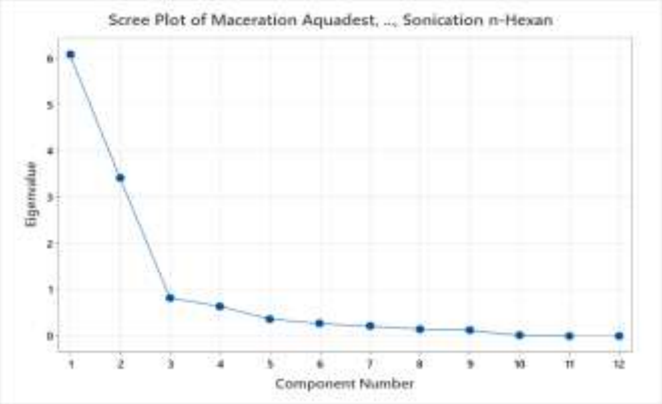

Figure 2. Scree plot of *L. coromandelica* extract using maceration, reflux and sonication extraction techniques with aquadejs, 70% ethanol, ethyl acetate and n-hexane as solvents

The loading plot is used to identify the functional group variables that contribute most significantly to the formation of principal component values. In PCA-based chemometric analysis, loading values are used to evaluate the relative contribution of each variable to the principal components and to identify the variables that play the most significant role in discriminating sample groups (Osman et al., 2022). The contribution of each functional group variable in the loading plot can be inferred from its distance from the origin. The farther a variable is from the origin (0,0), the greater its contribution to the principal component analysis (PCA) process (Umar et al., 2016).

Based on the loading plot results, the variables influencing the formation of the principal components can be determined through the eigenvector values of PC1 and PC2. The ethyl acetate sonication extract sample exhibited the highest eigenvector value of 0.375, indicating a significant influence on the formation of PC1. The distinct clustering pattern observed in the sonication–ethyl acetate extract may be attributed to the enhanced extraction efficiency of sonication and the selective solvation properties of ethyl acetate toward semi-polar metabolites, resulting in highly distinctive spectral fingerprint characteristics compared with the other extracts. Meanwhile, for PC2, the maceration aquadest sample showed an eigenvector value of 0.419, indicating its substantial contribution to the formation of PC2.

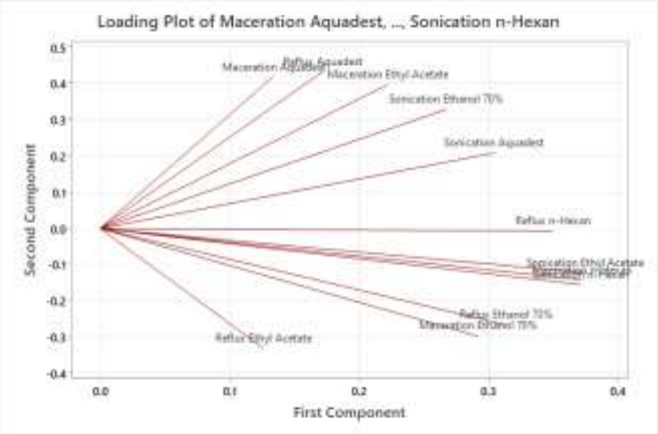

Figure 3. Loading plot of L. coromandelica extract using maceration, reflux and sonication extraction techniques with aquades, 70% ethanol, ethyl acetate and n-hexane as solvents.

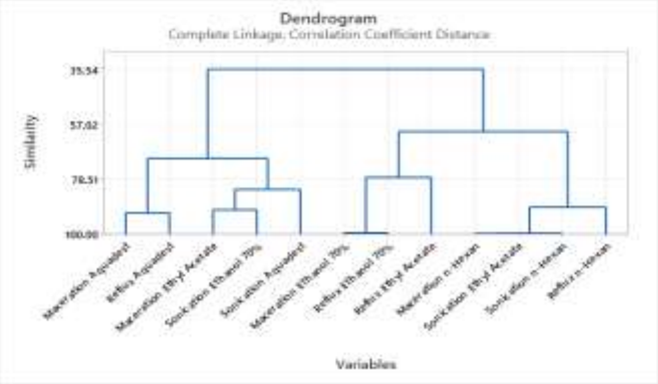

Figure 4. Dendrogram of *Lannea coromandelica* extracts obtained using maceration, reflux, and sonication extraction techniques with solvents including aquadest, 70% ethanol, ethyl acetate, and n-hexane

Cluster analysis is a multivariate statistical technique used to classify objects into groups based on the similarity of their characteristics, such that objects within the same cluster are more similar to each other than to those in different clusters (Farnsworth, 2016). In cluster analysis, a measure that describes the similarity or proximity between data points is used, with Euclidean distance being one of the most commonly employed metrics. The results of cluster analysis are typically presented in the form of a dendrogram, which illustrates the similarity between samples. In this diagram, the smaller the similarity value of the line connecting two samples, the greater the difference between those samples (Cao et al., 2017; Tulukcu et al., 2019; Umar et al., 2016). Based on the dendrogram shown in Figure 4, the twelve samples exhibit varying degrees of similarity, ranging from 35.54% to 99.82%. Samples are considered similar if the similarity value exceeds 80%. Among the twelve extracts, the highest similarity was observed between the n-hexane maceration and n-hexane sonication extracts at 99.82%, followed by the ethyl acetate maceration and 70% ethanol sonication extracts at 90.72%. These two clusters share similarities in extraction technique.

Similarly, the 70% ethanol maceration and 70% ethanol reflux extracts displayed a similarity of 99.66%, followed by the aquadest maceration and aquadest reflux extracts with a similarity of 96.86%. These clusters also share common extraction methods. The maceration ethyl acetate and sonication aquadest extracts, with a similarity value of 80.72%, indicate the presence of similar compounds despite differences in extraction techniques. Based on these dendrogram results, it can be concluded that the extraction process for *Lannea coromandelica* can effectively utilize solvents such as aquadest, ethanol, ethyl acetate, and n-hexane with extraction methods including maceration, reflux, and sonication to obtain the desired compounds.

Conclusion

Based on the results of the *Lannea coromandelica* extract analysis using FTIR spectrophotometry, it can be concluded that the twelve extracts obtained through variations of extraction methods—maceration, reflux, and sonication—and using four different solvents (aquadest, 70% ethanol, ethyl acetate, and n-hexane) share several common functional group components. Chemometric analysis revealed a high degree of similarity among the twelve extracts, with the highest similarity value reaching 99.82%.

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Author Contributions
Conceptualization, M. A. A. R.; methodology, M. A. A. R., M. S., M., and F. G. R.; formal analysis, M. A. A. R.; investigation, M. S., M., and F. G. R.; data curation, M. A. A. R.; writing—original draft preparation, M. A. A. R.; writing—review and editing, M. A. A. R.; visualization, M. A. A. R.; supervision, J. and H.; project administration, J. and H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
All authors declare that there are no conflicts of interest regarding this manuscript.

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