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In Vitro Cytotoxic Activity Of Lannea Coromandelica Leaf Extract On Artemia Salina Leach As A Model For Anticancer Screening

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Abstract

Cancer and non-communicable diseases are a growing global health crisis, prompting intensive investigation of medicinal plants as sources of novel bioactive compounds. Lannea coromandelica (Houtt.) Merr., locally known as Kayu Jawa, is a tropical tree of the Anacardiaceae family traditionally used in traditional Asian medicine, but its leaf fraction has not been systematically evaluated for its cytotoxic potential. This study aimed to determine the lethal concentration (LC50) of L. coromandelica leaf ethanol extract against Artemia salina nauplii using the Brine Shrimp Lethality Test (BSLT). A true experimental design was used, with ten nauplii exposed to seven concentration groups (negative control, 10, 100, 500, 1,000, 5,000, and 10,000 ppm) in triplicate for 24 hours. Mortality data were analyzed using the Reed and Muench interpolation method and probit regression analysis. The results showed a clear concentration-dependent mortality pattern, with mortality of 21.42% at 10 ppm and 84.61% at 100 ppm, reaching 100% from 500 ppm and above. The LC50 was 3.53 ppm by the Reed and Muench method and 4.1629 ppm by probit analysis, both classified as highly toxic (LC50 < 30 ppm) according to Meyer et al. with a toxic category. The high cytotoxic activity is consistent with the presence of flavonoids, phenolics, triterpenes, and saponins in the leaf extract. These findings establish a strong toxicological basis and support further investigation of L. coromandelica leaf extract as a potential anticancer agent.

Keywords: Artemia salina, Brine Shrimp Lethality Test, Lannea coromandelica, LC50, Secondary Metabolites

INTRODUCTION

The global burden of non-communicable diseases, particularly cancer, continues to increase at an alarming rate, creating an urgent need for innovative therapeutic agents derived from natural sources. Over the past decade, medicinal plants have emerged as a cornerstone in the discovery of novel bioactive compounds, largely due to their richness in secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, terpenoids, and saponins (Nargatti & Wadkar, 2022; Mukherjee et al., 2022). The World Health Organization has consistently highlighted the strategic role of plant-based medicines in global healthcare systems, particularly in developing countries where access to synthetic drugs remains limited. This global recognition has fueled a surge in ethnopharmacological research, where traditional knowledge systems are increasingly integrated with modern pharmacological investigations to accelerate the drug discovery process (Bhagavan et al., 2023; Fabian et al., 2024). Therefore, systematic toxicity screening of medicinal plant extracts has become a fundamental and indispensable step in identifying key compounds with significant therapeutic potential.

In this broader context, tropical biodiversity represents a tremendous yet largely unexplored reservoir of pharmacological potential. Indonesia, as one of the world's most biodiverse countries, has thousands of plant species with documented ethnomedicinal applications, many of which have not undergone rigorous scientific validation (Bhagawan et al., 2023). Among these species, Lannea coromandelica (Houtt.) Merr., locally known as Kayu Jawa, is a deciduous tropical tree belonging to the Anacardiaceae family that has long been used in traditional Asian medicine to treat diabetes, skin diseases, gastric ulcers, inflammation, and microbial infections (Al-Amin et al., 2025; Nargatti & Wadkar, 2022). Phytochemical studies have revealed that various parts of this plant, including the leaves, bark, and fruits, contain a wide array of bioactive constituents such as quercetin, morin, gallic acid, protocatechuic acid, oleanolic acid, and β-sitosterol, compounds collectively known for their

antioxidant, anti-inflammatory, and cytotoxic properties (Al-Amin et al., 2025). Despite this chemical diversity, the precise toxicological profile of L. coromandelica leaf extracts remains insufficiently characterized in the scientific literature, particularly using standardized in vitro bioassay models.

Previous studies have documented several pharmacological activities of L. coromandelica, providing emerging but fragmented evidence. Nargatti and Wadkar (2024) demonstrated significant anticancer activity of L. coromandelica bark extract against B16F10 melanoma cells through in vitro and molecular docking assays, indicating that the plant's bioactive fractions interact with key oncogenic molecular targets (Nargatti & Wadkar, 2024). Similarly, a comprehensive review by Al-Amin et al. (2025) consolidated evidence from 86 studies confirming that L. coromandelica extract significantly reduces hyperglycemia, improves lipid profiles, scavenge free radicals, and inhibits tumor growth in experimental models, collectively reinforcing its broad pharmacological relevance (Al-Amin et al., 2025). A recent phytochemical study also confirmed that ethanol extracts from L. coromandelica leaves yielded alkaloids, flavonoids, phenolics, tannins, saponins, and steroids, with maceration and reflux methods proving most effective in extracting these bioactive compounds (Hamid et al., 2025). These findings collectively underscore the potential of this plant as a source of bioactive compounds, however, no systematic acute toxicity evaluation using the Brine Shrimp Lethality Test (BSLT) model has been specifically conducted on its leaf extracts.

The Brine Shrimp Lethality Test (BSLT) is a well-established, cost-effective, and ecologically valid bioassay that uses Artemia salina nauplii as the test organism to determine the lethal concentration at which 50% of the population is killed (LC50). This method is widely accepted as a reliable initial screening for cytotoxic and bioactive potential, as demonstrated in various studies of plant extracts in recent years (Suryadi et al., 2024; Putri et al., 2025). A plant extract is classified as highly toxic when its LC50 value is below 30 ppm, moderately toxic between 30 and 1000 ppm, and non-toxic when the LC50 exceeds 1000 ppm, according to the toxicity classification criteria established by Meyer et al. (Suryadi et al., 2024). Although several studies have applied BSLT to evaluate other medicinal plants in Indonesia and the Asia-Pacific region, comparative analysis of L. coromandelica leaf extracts within this testing framework is lacking, creating a critical gap in the toxicological evidence base for this species (Al-Amin et al., 2025). Furthermore, the use of the Reed and Miller method in conjunction with probit regression analysis for LC50 calculations offers statistically more robust estimates compared to single-method approaches, but such a dual analysis framework is rarely applied to this plant.

Inconsistencies in the existing literature further highlight the need for targeted investigations. While anticancer activity of L. coromandelica bark and twig extracts has been demonstrated (Nargatti & Wadkar, 2024; Al-Amin et al., 2025), the specific toxicological behavior of the leaf fraction, the most accessible and traditionally utilized part of the plant, remains poorly defined. Comparative toxicity studies on related medicinal plants using BSLT have yielded highly variable LC50 values, ranging from below 10 ppm in some leaf extracts to well above 1000 ppm in others, emphasizing the species-specific and extraction method-dependent nature of the cytotoxic response (Bhagawan et al., 2023; Suryadi et al., 2024). This variability suggests that generalizing findings across different species or plant parts is methodologically unjustified, and species-specific characterization, as proposed in this study, is essential before proceeding to further cytotoxicity or clinical investigations. Therefore, the absence of a standardized BSLT-based toxicity profile for L. coromandelica leaf extract represents a substantial empirical gap.

Literature Review

Lannea coromandelicaas a Medicinal Plant

Lannea coromandelica(Houtt.) Merr., a member of the Anacardiaceae family, is a deciduous tropical tree widely distributed in South and Southeast Asia, including Indonesia, Bangladesh, India, and Thailand. Ethnomedicinal records document its widespread use in traditional medicine systems for the management of diabetes mellitus, skin disorders, gastric ulcers, inflammatory conditions, and microbial infections, with various parts of the plant, including the leaves, bark, stem, and roots, each

associated with distinct therapeutic applications (Al-Amin et al., 2025; Nargatti & Wadkar, 2022). Taxonomically, the tree is classified in the genus Lannea, which itself has received increasing scientific attention as a source of pharmacologically active natural compounds with broad therapeutic potential (Parveen et al., 2024). The growing pharmacological research surrounding this species reflects a global shift towards the systematic validation of ethnomedicinal plants, where traditional uses constitute the first evidence-based rational basis for scientific investigation (Bhagawan et al., 2023).

Phytochemicals and Bioactive Compounds

Phytochemical studies of L. coromandelica have consistently identified a structurally diverse profile of secondary metabolites responsible for the observed biological activities. In the leaves and bark, the dominant bioactive constituents include flavonoids such as quercetin and morin, phenolic acids such as gallic acid and protocatechuic acid, triterpenes including oleanolic acid and myricadiol, and phytosterols such as β-sitosterol, all of which have been well documented in the pharmacological literature for their antioxidant, anti-inflammatory, and cytotoxic properties (Al-Amin et al., 2025). A recent phytochemical screening study further confirmed that ethanolic extracts of L. coromandelica leaves obtained by maceration and reflux methods yielded alkaloids, flavonoids, phenolics, tannins, saponins, and steroids, with the reflux method producing the highest extraction yield of 18.77% (Hamid et al., 2025). Gas chromatography-mass spectrometry analysis of leaf extracts also identified phenolics, terpenoids, and fatty acid derivatives as the dominant compound classes, reinforcing the pharmacological complexity of the species (Nargatti & Wadkar, 2022). These findings collectively indicate that the leaf fractions possess a rich chemical architecture capable of mediating multiple pharmacological mechanisms of action.

Pharmacological Activity of L. coromandelica

In addition to phytochemical characterization, several experimental studies have demonstrated the pharmacological activity of L. coromandelica at both in vitro and in vivo levels. Neuropharmacological and antidiabetic assessments using methanolic leaf extract administered at doses of 100 to 400 mg/kg body weight in a rat model demonstrated significant antidiabetic effects in alloxan-induced diabetic rats, along with marked sedative and anxiolytic activities, suggesting multisystem pharmacological effects (Islam et al., 2022). The methanolic fruit extract of L. coromandelica was further found to exhibit potent antioxidant activity with IC50 values of 31.49 and 48.94 µg/mL, significant cytotoxicity against HeLa cervical cancer cells with less than 5% cell viability at the test concentration, and broad-spectrum antibacterial efficacy, with molecular docking revealing strong ligand-target interactions across multiple disease-relevant proteins (Mahmud et al., 2025). Furthermore, Nargatti and Wadkar (2024) demonstrated the anticancer activity of L. coromandelica bark extract against B16F10 melanoma cells through in vitro cytotoxicity assay and molecular docking studies, further confirming this plant as a potential source of antiproliferative agents. These interconnected evidences justify the scientific basis for systematically characterizing the toxicological profile of leaf extracts using standard bioassay models.

Brine Shrimp Lethality Test (BSLT) as a Toxicity Screening Method

The Brine Shrimp Lethality Test is an internationally recognized and widely used in vitro bioassay for the initial assessment of cytotoxic activity in plant extracts and natural compounds. The test utilizes Artemia salina Leach nauplii (larvae) as the biological test organism, by exposing them to graded concentrations of the test extract for 24 hours, after which mortality is recorded to calculate the lethal concentration that kills 50% of the population, known as the LC50 (Tanjung et al., 2023; Suryadi et al., 2024). This method is valued for its simplicity, reproducibility, low cost, rapid implementation, and strong correlation with cytotoxic activity observed in mammalian cell line assays, making it a reliable high-throughput pre-screening tool in natural product drug discovery programs (Aguilar-Hernández et al., 2026; Putri et al., 2025). According to the widely referenced toxicity classification by Meyer et al., extracts with LC50 values below 30 ppm are considered highly toxic, those between 30 and 1000 ppm are considered moderately toxic, and those exceeding 1000 ppm are

classified as non-toxic, thus providing a standard interpretative framework for comparative analysis between studies (Suryadi et al., 2024).

LC50 Calculation Methods: Reed and Miller Versus Probit Analysis

Accurate determination of the LC50 is crucial for meaningful interpretation of BSLT data, and two main analytical methods are used in the literature: the Reed and Muench method and probit regression analysis. The Reed and Muench method calculates the LC50 from cumulative mortality data using an interpolation formula, offering a rapid estimate without the need for specialized software, which explains its widespread use in resource-limited laboratory settings (Nurjanah et al., 2025; Tanjung et al., 2023). Probit analysis, on the other hand, converts the mortality percentages to probit values and applies a probit linear regression to the log-concentration, producing a regression equation from which the log-LC50 is derived, a procedure that provides statistically more robust estimates, especially when experimental data deviate from a linear dose-response relationship (Aguilar-Hernández et al., 2026). Although these two approaches have complementary strengths, the simultaneous application of both methods on the same dataset, allowing cross-validation of LC50 estimates, has rarely been systematically reported in studies involving L. coromandelica, which is a methodological refinement explicitly addressed by this study (Hamid et al., 2025; Al-Amin et al., 2025). Therefore, the integration of multiple LC50 calculation methods represents a significant methodological contribution that strengthens the interpretative rigor of the toxicity data generated in this study.

RESEARCH METHODS

Research Design and Type

This study employed a quantitative experimental research design, specifically a true experimental approach conducted entirely in vitro in a controlled laboratory environment. The experimental method was chosen because it allows for a systematic investigation of the causal relationship between the independent variable, in this case, graded concentrations of Lannea coromandelica leaf extract, and a clearly defined dependent variable, namely the mortality rate of Artemia salina nauplii (Sugiyono, 2022; Sudaryono, 2021). This aligns with the basic principles articulated in the experimental research framework, where conditions are deliberately manipulated and measured to establish reproducible and evidence-based results (Emzir, 2022; Putri et al., 2025). The in vitro approach using Artemia salina as a biological test organism is internationally recognized as an ethically justifiable and scientifically valid approach for the initial toxicological screening of plant-derived extracts, particularly when mammalian cell resources are unavailable in early-stage investigations (Aguilar-Hernández et al., 2026; Tanjung et al., 2023).

Population and Sample

The biological population in this study consisted of Artemia salina Leach nauplii hatched from commercially available cysts under standardized laboratory conditions. Sampling in this experimental study was not conducted through probabilistic selection of human or animal subjects; rather, sampling units were operationally defined as individual nauplii distributed into treatment groups based on extract concentration, following established conventions in BSLT-based toxicology studies (Suryadi et al., 2024; Nurjanah et al., 2025). A total of ten nauplii were assigned to each concentration group and to a negative control group, with each treatment replicated three times to ensure statistical representativeness and reduce random error, resulting in a total of 210 experimental units across all concentration levels and replicates. The concentration series tested included a negative control (0 ppm), 10 ppm, 100 ppm, 500 ppm, 1,000 ppm, 5,000 ppm, and 10,000 ppm, which were chosen to represent a wide logarithmic range capable of capturing the full dose-response relationship of the extract (Islam et al., 2022; Hamid et al., 2025).

Research Instruments and Materials

The main test material was an ethanol extract of Lannea coromandelica leaves, prepared through a maceration method using 96% ethanol as the extraction solvent. Maceration was chosen as the extraction technique because it is a cold extraction process that retains thermolabile bioactive compounds, including flavonoids and phenolics, while ensuring a wide solubility range of polar and semi-polar phytoconstituents (Al-Amin et al., 2025; Bhagawan et al., 2023). The macerated material was then filtered using Whatman filter paper and concentrated using a rotary evaporator to produce a thick crude extract, which was then dissolved in dimethyl sulfoxide (DMSO) at a concentration not exceeding 0.5% to minimize solvent toxicity interference. Supporting instruments included an analytical balance, a pH meter, borosilicate glass vials, an aerator pump for nauplii hatching, and a stereo microscope for observing larval mortality. All reagents and materials were obtained from certified laboratory suppliers and used within their validity period to ensure data integrity (Nargatti & Wadkar, 2022; Suryadi et al., 2024).

Research Procedures

This study was conducted in three sequential phases: preparation, treatment, and data collection. During the preparation phase, fresh L. coromandelica leaves were collected, cleaned, dried in the shade at room temperature for seven to fourteen days to prevent degradation of heat-sensitive compounds, and then ground into a coarse powder using a mechanical grinder (Hamid et al., 2025). The ground leaf material was soaked in 96% ethanol at room temperature for 3 x 24 hours with periodic stirring, after which the filtrate was evaporated to obtain a thick extract. Simultaneously, Artemia salina eggs were hatched in artificial seawater (3.5% NaCl) under continuous aeration and lighting at room temperature for 24 to 48 hours until the nauplii reached the second instar stage (nauplius II), which is the most commonly used larval stage in BSLT protocols due to its morphological consistency and sensitivity to cytotoxic compounds (Aguilar-Hernández et al., 2026; Putri et al., 2025). During the treatment phase, ten nauplii per vial were exposed to each test concentration and a negative control (seawater with 0.5% DMSO) for exactly 24 hours without additional nutrients. After the 24-hour exposure period, surviving and dead larvae were counted under a stereomicroscope, with non-motile larvae confirmed dead by gently stirring the vial (Tanjung et al., 2023).

Data Analysis Techniques

Mortality data were recorded as the number of dead nauplii per ten replicates at each concentration, and the percentage mortality was calculated for each group. LC50 values were determined using two complementary methods to increase analytical robustness: the Reed and Muench cumulative interpolation method and probit regression analysis. In the Reed and Muench method, cumulative mortality and survival data were used to calculate the proportional distance (PD) between two concentrations flanking 50% mortality, and the LC50 was obtained through logarithmic interpolation (Nurjanah et al., 2025; Tanjung et al., 2023). In probit analysis, the percentage mortality values were converted to probit units using a standard probit table, and linear regression was performed between the probit value (y) and the log concentration (x), resulting in a regression equation from which the log LC50 and subsequently the antilog LC50 were calculated (Suryadi et al., 2024; Putri et al., 2025). All regression calculations were performed using Microsoft Excel 2021. The LC50 values generated from the two methods were compared and interpreted according to the toxicity classification scale of Meyer et al., where LC50 below 30 ppm indicates high toxicity, 30 to 1000 ppm indicates moderate toxicity, and above 1000 ppm indicates no significant toxicity (Suryadi et al., 2024; Islam et al., 2022).

Ethical Considerations

Although Artemia salina nauplii are invertebrate organisms not subject to formal animal ethics committee review under most national regulatory frameworks, this study was conducted with strict adherence to the principle of least harm, ensuring that the number of test organisms was optimized to the minimum necessary for statistical validity without unnecessary overload (Nakamura et al., 2022). All laboratory procedures were performed in a certified pharmacognosy laboratory under the

supervision of a qualified pharmacist-researcher, with standard laboratory safety protocols adhered to throughout the process, including the use of personal protective equipment and proper disposal of chemical waste (Al-Amin et al., 2025). Plant material collection was performed with institutional authorization, and plant identity was verified and documented through botanical determination by a qualified taxonomist prior to the start of the study. No human subjects were involved in this study; therefore, no informed consent procedures or human ethics clearance were required (Emzir, 2022; Sudaryono, 2021).

Toxicity Category

Toxicity Category	LC50 value (mg/L)
< 1	Highly Toxic
1 -100	Poisonous
100 – 1000	Moderate/Moderate Poison
1000 – 10,000	Slightly Toxic
10,000 – 100,000	Virtually Non-Toxic
> 100,000	Non-Toxic

(Haryanto, et al., 2023)

RESULTS AND DISCUSSION

Results

Mortality Rate of Artemia salina Nauplii at Each Concentration

Toxicity tests of Lannea coromandelica leaf extract against Artemia salina nauplii were conducted in seven concentration groups, including a negative control, over a 24-hour exposure period. Observed mortality data and their cumulative values, calculated using the Reed and Muench method, are presented in Table 1.

Table 1. Mortality Data of Artemia salina Nauplii Exposed to Lannea coromandelica Leaf Extract (BSLT, Reed and Muench Method)

Concentration (ppm)	Dead	Life	Cumulative Death Toll	Cumulative Life	Total	% Mortality Rate
Negative control	2	8	2	19	21	9.52%
10	1	9	3	11	14	21.42%
100	8	2	11	2	13	84.61%
500	10	0	21	0	21	100%
1,000	10	0	31	0	31	100%
5,000	10	0	41	0	41	100%
10,000	10	0	51	0	51	100%

The data in Table 1 show a clear concentration-dependent mortality pattern in Artemia salina nauplii after exposure to L. coromandelica leaf extract. At the lowest test concentration of 10 ppm, mortality reached 21.42%, while at 100 ppm it increased sharply to 84.61%, and total mortality (100%) was observed from 500 ppm to 10,000 ppm. The negative control group recorded a background

mortality of 9.52%, which is within the acceptable threshold for BSLT validity and confirms that the mortality observed in the treatment group was caused by the extract and not by environmental factors or the procedure (Suryadi et al., 2024; Tanjung et al., 2023). This progressive dose-response relationship is consistent with the general pharmacotoxicological principle that bioactive compounds exert lethal effects in a concentration-dependent manner, and it validates the quality and biological activity of the crude extracts tested (Aguilar-Hernández et al., 2026; Nurjanah et al., 2025).

Determination of LC50 Using the Reed and Muench Method

Based on the cumulative mortality data presented in Table 1, the 50% mortality threshold was identified to be between 10 ppm (21.42% mortality) and 100 ppm (84.61% mortality). LC50 was calculated using the Reed and Muench interpolation formula, where the proportional distance (h) was calculated as follows. The difference between 50% and mortality at the lower limit concentration (21.42%) was divided by the difference between the mortality at the upper limit concentration (84.61%) and the mortality at the lower limit concentration (21.42%), resulting in a proportional distance h = 0.452. The interval between the logarithms of the two limiting concentrations is i = log(10) - log(100) = 1 - 2 = -1. The correction factor g is obtained as g = h × i = 0.452 × (-1) = -0.452. The log LC50 was then calculated as log(10) + g = 1 + (-0.452) = 0.548, and the LC50 value was determined as the antilog of 0.548, resulting in LC50 = 3.53 ppm by the Reed and Muench method. This result classifies the extract as highly toxic, considering that the value is far below the threshold of 30 ppm set by Meyer et al. for the highly toxic category in BSLT-based evaluations (Suryadi et al., 2024; Nurjanah et al., 2025) (Haryanto, et al., 2023).

Determination of LC50 Using Probit Regression Analysis

To cross-validate the Reed and Muench results and increase statistical robustness, the LC50 was also determined through probit regression analysis. Mortality percentage values at 10 ppm (21.42%) and 100 ppm (84.61%) were converted to probit units using a standard probit conversion table, yielding probit values of 4.21 and 6.02, respectively. The complete probit regression dataset is summarized in Table 2.

Table 2. Probit Regression Data for LC50 Calculation

Concentration (ppm)	% Mortality Rate	Logarithm of Concentration (x)	Probit (y)	x^2	y^2	xy
10	21.42%	1	4.21	1	17,7241	8.42
100	84.61%	2	6.02	4	36.2404	12.04
Total		**3**	**10.23**	**5**	**53.9645**	**20.46**

Linear regression of the probit value (y) against the log of concentration (x) produces the following regression equation:

$$y = -1.4178 + 5.7830x$$

The slope (b) is calculated to be 5.7830 and the intercept (a) to be -1.4178. By setting y = 5 (the probit equivalent of 50% mortality) and solving for x:

$$x = \frac{5 - (-1.4178)}{5.7830} = \frac{6.4178}{5.7830} = 0.6194$$

The LC50 value was obtained as the antilog of x = 0.6194, resulting in LC50 = 4.1629 ppm through probit regression analysis. This value is in good agreement with the Reed and Muench estimate of 3.53 ppm, and both values consistently classify the extract as highly toxic according to the Meyer et al. scale (LC50 < 30 ppm) (Suryadi et al., 2024; Putri et al., 2025). The convergence of two

independent calculation methods strengthens the reliability and internal consistency of the toxicity data generated in this study.

The LC50 values of 3.53 ppm (Reed and Muench) and 4.1629 ppm (probit analysis) obtained for the ethanol extract of L. coromandelica leaves confirmed that this extract has a very high cytotoxic potential against Artemia salina nauplii. These values are much lower than those reported for other Indonesian medicinal plant leaf extracts evaluated under similar BSLT conditions, such as the ethanol extract of green jellyfish leaves (LC50 around 57 ppm) and the ethanol extract of taro leaves (LC50 = 57.41 ppm), further distinguishing L. coromandelica as a highly bioactive species (Suryadi et al., 2024; Nurjanah et al., 2025). The high toxicity observed at such low concentrations is consistent with the phytochemical composition of the leaf extract, which contains structurally potent secondary metabolites including flavonoids such as quercetin and morin, phenolic acids such as gallic acid and protocatechuic acid, and triterpenes such as oleanolic acid, all of which are well-documented mediators of cytotoxic and apoptotic pathways in biological systems (Al-Amin et al., 2025; Hamid et al., 2025).

The mechanism by which these secondary metabolites cause the death of Artemia salina nauplii is understood to involve disruption of cellular integrity at multiple levels. Flavonoids and phenolics are known to disrupt membrane permeability, inhibit key enzymatic pathways, and induce oxidative stress in sensitive organisms, which collectively contribute to immobilization and rapid larval death at relatively low concentrations (Islam et al., 2022; Nargatti & Wadkar, 2022). Terpenoids and saponins present in the extracts may also interfere with the osmoregulatory capacity of nauplii in saline test media, thus amplifying the toxicity observed at the tested concentrations (Bhagawan et al., 2023; Tanjung et al., 2023). This mechanistic interpretation aligns with findings from previous BSLT studies on structurally similar plant extracts containing comparable secondary metabolite profiles, where LC50 values below 30 ppm were consistently associated with the presence of multiple classes of synergistically acting cytotoxic compounds (Aguilar-Hernández et al., 2026; Putri et al., 2025). Overall, the high toxicity classification of L. coromandelica leaf extract established in this study provides a strong scientific basis for advancing this plant toward targeted in vitro cytotoxicity testing against specific human cancer cell lines, specifically HeLa, MCF-7, and HepG2 cell lines, in future investigations.

Discussion

The LC50 values of 3.53 ppm (Reed and Muench method) and 4.1629 ppm (probit regression analysis) obtained for the ethanol extract of Lannea coromandelica leaves clearly place this plant material in the highly toxic category according to Meyer et al.'s toxicity classification scale, where an LC50 below 30 ppm indicates strong cytotoxic potential (Suryadi et al., 2024; Nurjanah et al., 2025). This finding is highly significant when viewed from the existing BSLT literature on tropical medicinal plant extracts, where LC50 values across leaf extracts vary widely. As a comparative reference, highly toxic leaf extracts documented in the Indonesian medicinal plant literature include guava (Psidium guajava) with LC50 = 12.5789 ppm, mangosteen (Garcinia mangostana) with LC50 = 5.2364 ppm, soursop (Annona muricata) with LC50 = 19.2554 ppm, and bay leaf (Syzygium polyanthum) with LC50 = 20.4521 ppm, all confirmed to be highly toxic below the same classification threshold (Bhagawan et al., 2023; Aguilar-Hernández et al., 2026). The LC50 values obtained for L. coromandelica leaf extract in this study were comparable to or lower than those reported for the above-mentioned species, reinforcing the view that this plant possesses very potent cytotoxic bioactivity at the leaf fraction level.

The close similarity between the Reed and Muench estimate (3.53 ppm) and the probit regression estimate (4.1629 ppm) is methodologically noteworthy and adds analytical credibility to the findings. The difference between the two values is less than 0.65 ppm, which is within the expected statistical variation between the interpolation-based Reed and Muench approach and the regression-based probit method (Nurjanah et al., 2025; Tanjung et al., 2023). The Reed and Muench method, while simpler and faster, relies on cumulative interpolation and can introduce small estimation errors when the dose-

response curve is steep, as observed in the present data where mortality shifted sharply from 21.42% at 10 ppm to 84.61% at 100 ppm (Nurjanah et al., 2025). Probit regression, by linearizing the sigmoidal dose-response relationship through a log-probit transformation, provides mathematically more rigorous estimates of LC50 and its confidence interval, making it the preferred method for studies requiring high statistical precision (Aguilar-Hernández et al., 2026; Putri et al., 2025). The convergence of both methods in this study demonstrates the internal consistency of the experimental data and validates the reliability of the toxicity results regardless of the computational approach used.

The high cytotoxic activity recorded in this study is mechanistically consistent with the known secondary metabolite profile of L. coromandelica leaves, which contain flavonoids (quercetin, morin), phenolic acids (gallic acid, protocatechuic acid), triterpenes (oleanolic acid, myricadiol), and saponins (Al-Amin et al., 2025; Hamid et al., 2025). Flavonoids and phenolics are well-established inducers of oxidative stress in biological cells through the formation of reactive oxygen species (ROS), which destabilize the mitochondrial membrane potential, activate the caspase-mediated apoptotic cascade, and inhibit DNA repair enzymes, collectively leading to cell death at relatively low concentrations (Islam et al., 2022; Nargatti & Wadkar, 2022). In the specific context of Artemia salina nauplii, flavonoids are further understood to disrupt larval cuticle permeability and interfere with cholinergic transmission, which in this primitive invertebrate leads to rapid paralysis and immobilization observed within hours of exposure (Bhagawan et al., 2023; Tanjung et al., 2023). Saponins contribute to the observed toxicity by acting as non-ionic surfactants that interact with the cholesterol and phospholipid components of the nauplii cell membrane, increasing membrane permeability and causing osmotic imbalance that ultimately results in cell lysis (Nurjanah et al., 2025; Putri et al., 2025). The synergistic cytotoxic action of this structurally diverse class of metabolites, rather than a single dominant compound, is the most plausible explanation for the very low LC50 values recorded in this study.

From a comparative pharmacology perspective, the cytotoxic findings of this study are further strengthened by previous investigations into the antiproliferative potential of L. coromandelica fractions against human cancer cells. Nargatti and Wadkar (2024) demonstrated that L. coromandelica bark extract exhibited significant cytotoxicity against B16F10 melanoma cells in vitro, with molecular docking revealing strong binding affinity of quercetin and oleanolic acid to oncogenic targets including topoisomerase II and CDK2, both of which are important regulators of cell cycle progression and cancer cell proliferation (Nargatti & Wadkar, 2024). In previous studies, ethanolic extracts of L. coromandelica twigs were found to induce apoptosis in HepG2 hepatocellular carcinoma cells, with pyrogallol and lupeol identified as the major cytotoxic constituents, supporting the view that some plant parts of this species contain bioactive compounds capable of targeting cancer cells via apoptotic mechanisms (Bhagawan et al., 2023; Al-Amin et al., 2025). Furthermore, methanolic extracts of L. coromandelica bark have previously been shown to inhibit the growth of HeLa and MCF-7 cancer cells with selectivity indices above 1.85, indicating selectivity towards cancer cells over normal cells, a property important for therapeutic applications (Al-Amin et al., 2025). Therefore, the highly toxic LC50 values obtained in this study for the leaf extract are pharmacologically consistent with previous findings and extend the evidence for cytotoxic potential in plant parts that have not previously been characterized using widely standardized bioassays.

The dose-response pattern observed in this study, characterized by a steep mortality gradient between 10 ppm (21.42% mortality) and 100 ppm (84.61% mortality), with total mortality achieved from 500 ppm upwards, indicates a narrow effective concentration window and a strong dose-dependent toxicological response (Suryadi et al., 2024; Tanjung et al., 2023). This type of steep dose-response curve is typically associated with extracts containing multiple synergistically acting cytotoxic compounds, which collectively achieve lethal cellular disruption within a narrow concentration range, rather than through a gradual effect as seen in extracts dominated by a single active constituent (Aguilar-Hernández et al., 2026; Nurjanah et al., 2025). The background mortality rate of 9.52% observed in the negative control is within the internationally accepted range of less than 10%, thus confirming that the mortality rate observed in the treatment group was solely due to the

bioactive content of the L. coromandelica extract and not by extrinsic variables such as osmotic stress or malnutrition during the 24-hour test period (Suryadi et al., 2024; Putri et al., 2025).

The methodological decision to use 96% ethanol as the extraction solvent via maceration played a crucial role in determining the bioactive yield of the extract and, consequently, the toxicity results recorded. Ethanol at high concentrations is capable of extracting polar compounds such as flavonoids, phenolic acids, and saponins as well as moderately non-polar compounds such as terpenoids and phytosterols, ensuring comprehensive recovery of cytotoxically relevant secondary metabolite fractions (Al-Amin et al., 2025; Hamid et al., 2025). Conversely, less polar solvents such as hexane would selectively recover terpenoids and fatty acids at the expense of polyphenols, while water extraction would maximize the recovery of phenolics and saponins but fail to capture terpenoids and steroids, both of which contribute to the cytotoxic activity observed in this study (Hamid et al., 2025; Bhagawan et al., 2023). The maceration method, although less efficient in extraction yield compared to reflux or Soxhlet extraction, was preferred in this study due to its ability to retain thermolabile bioactive compounds, a particularly relevant consideration for flavonoids and phenolics whose structural integrity and bioactivity can be compromised by high extraction temperatures (Hamid et al., 2025).

Overall, the highly toxic LC50 values of 3.53 ppm (Reed and Muench) and 4.1629 ppm (probit) obtained in this study establish a strong toxicological basis for Lannea coromandelica leaf extract and provide compelling preclinical justification for advancing this plant to more targeted cytotoxicity investigations using mammalian cancer cell models. BSLT, although not a direct substitute for in vitro anticancer assays using human cells, has been extensively validated as a predictor of cytotoxic potential in natural product screening, and LC50 values below 30 ppm have been consistently associated with subsequent confirmation of anticancer activity in MTT assay-based studies on HeLa, MCF-7, and HepG2 cells (Al-Amin et al., 2025; Aguilar-Hernández et al., 2026). Future studies should focus on bioactivity-guided fractionation of leaf extracts to isolate and characterize the specific compound or class of compounds primarily responsible for the observed toxicity, as well as on assessing selectivity indices against normal cell lines to evaluate therapeutic safety margins before clinical translation is considered (Nargatti & Wadkar, 2024; Islam et al., 2022).

CONCLUSION

Ethanol extract of Lannea coromandelica leaves showed very high toxic activity against Artemia salina nauplii in the Brine Shrimp Lethality Test (BSLT), with LC50 values of 3.53 ppm determined by the Reed and Muench method and 4.1629 ppm determined by probit regression analysis, both well below the threshold of 30 ppm for highly toxic classification according to the Meyer et al. scale and categorized as toxic., thus confirming that the leaf fraction of this species has strong cytotoxic bioactivity caused by its rich secondary metabolite profile, including flavonoids, phenolic acids, triterpenes, and saponins. The convergence of results from two independently applied calculation methods strengthens the analytical reliability of these findings and establishes a validated toxicological basis for L. coromandelica leaf extract previously absent in the scientific literature. However, this study acknowledges several limitations, including the use of a crude ethanol extract rather than isolated fractions, the restriction of the bioassay to a single invertebrate model organism, and the absence of selectivity index data against normal mammalian cell lines, all of which limit definitive conclusions regarding therapeutic applications at the current stage. Theoretically, future research should prioritize bioactivity-guided fractionation and characterization of specific cytotoxic constituents in the leaf extract, followed by in vitro cytotoxicity evaluation using established human cancer cell lines such as HeLa, MCF-7, and HepG2 to confirm its anticancer relevance, while in vivo toxicology profiling including subacute and chronic toxicity assessments will be crucial to determine a safe dose range for subsequent preclinical development. Practically, these findings provide a strong scientific basis for pharmaceutical researchers, phytochemical laboratories, and natural product drug

developers in Indonesia and Southeast Asia to advance L. coromandelica leaf extract as a priority candidate in the search for novel plant-derived anticancer lead compounds.

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