



Phytochemical Profile and Preliminary Toxicity of Cumin Leaf (*Plectranthus amboinicus*) Infusion: UV-Vis Quantification and Brine Shrimp Lethality Test

Rahma Diyan Martha^{*1}, Sulastris¹, Defi Wulandari¹, and Danar²

¹Department of Pharmacy, STIKes Karya Putra Bangsa, Tulungagung, 66291, Indonesia

²Department of Chemistry, Faculty of Mathematics and Science, Universitas Negeri Malang, Malang 65145, Indonesia

*Corresponding Author: rahmadiyan@stikes-kartrasa.ac.id

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ABSTRACT

Cancer research increasingly explores natural products because plant secondary metabolites may possess diverse biological activities. This study evaluated the phytochemical profile, total levels of selected metabolite groups, and preliminary toxicity of a water infusion of cumin leaf (*Plectranthus amboinicus*) against *Artemia salina* larvae. Dried cumin leaves were infused in distilled water at 90 °C for 15 min, and the resulting filtrate was concentrated. Qualitative phytochemical screening was performed for alkaloids, flavonoids, saponins, and tannins. Quantitative determination was conducted using UV-Vis spectrophotometry with reference standards and calibration curves. BSLT was performed at concentrations of 100–1000 ppm for 24 h, with three replicates per concentration. The infusion showed group-level UV-Vis responses corresponding to alkaloids, flavonoids, saponins, and tannins at 7.90%, 62.81%, 22.20%, and 6.70%, respectively. These values represent estimates based on the respective reference standards and should not be interpreted as the percentages of pure compounds. Mortality increased from 6.7% at 100 ppm to 86.7% at 1000 ppm. Probit regression yielded the equation $y = 2.7423x - 2.3304$ ($R = 0.9579$), corresponding to an LC_{50} of 467.73 ppm. The aqueous infusion therefore showed measurable preliminary toxicity toward *A. salina* under the tested conditions.

Keywords: *Artemia salina*, BSLT, Cumin Leaf, Phytochemicals, *Plectranthus amboinicus*

ABSTRAK

Penelitian bahan alam untuk pengembangan kandidat antikanker terus dilakukan karena metabolit sekunder tumbuhan dapat memiliki aktivitas biologis yang potensial. Penelitian ini bertujuan mengevaluasi profil fitokimia, kadar kelompok metabolit tertentu, dan toksisitas awal infusa daun jinten (*Plectranthus amboinicus*) terhadap larva *Artemia salina*. Siplisia daun jinten diekstraksi dengan metode infudasi menggunakan aquadest pada suhu 90 °C selama 15 menit, kemudian filtrat dipekatkan. Skrining fitokimia dilakukan terhadap alkaloid, flavonoid, saponin, dan tanin. Penetapan kadar dilakukan secara kuantitatif menggunakan spektrofotometri UV-Vis dengan panjang gelombang spesifik dan kurva kalibrasi. Uji BSLT dilakukan pada konsentrasi 100–1000 ppm selama 24 jam dengan tiga kali pengulangan pada setiap konsentrasi. Hasil pengukuran UV-Vis menunjukkan respons kelompok alkaloid, flavonoid, saponin, dan tanin masing-masing sebesar 7,90%, 62,81%, 22,20%, dan 6,70%. Nilai tersebut merupakan estimasi berdasarkan standar pembandingan yang digunakan dan tidak menunjukkan persentase senyawa murni dalam sampel. Mortalitas larva meningkat dari 6,7% pada 100 ppm menjadi 86,7% pada 1000 ppm. Persamaan regresi probit diperoleh $y = 2,7423x - 2,3304$ dengan $R = 0,9579$ dan nilai LC_{50} sebesar 467,73 ppm. Hasil ini menunjukkan adanya toksisitas awal infusa terhadap *A. salina* pada kondisi pengujian.

Kata Kunci: Antikanker, *Artemia salina*, BSLT, Daun Jinten, Metabolit Sekunder



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1. Introduction

Cancer is characterized by uncontrolled and abnormal cellular growth and remains an important target for the development of new therapeutic agents. Although conventional anticancer treatments can be effective, treatment-related toxicity and other limitations continue to encourage the search for complementary approaches based on natural products. Plant-derived bioactive compounds have attracted considerable attention because secondary metabolites can interact with multiple cellular pathways involved in proliferation, apoptosis, oxidative stress, and other processes associated with cancer development [1,2].

Medicinal plants have long been used in Indonesian communities, and the continued use of traditional preparations provides a basis for investigating plants that may contain pharmacologically relevant compounds. Cumin leaf, locally known as *daun jinten* or *bangun-bangun*, belongs to the Lamiaceae family and is distributed throughout tropical regions. *Plectranthus amboinicus* has traditionally been used as both a food and medicinal plant, while phytochemical studies have reported flavonoids, alkaloids, saponins, phenolic compounds, and essential oils in its leaves [2,3].

Previous studies have strengthened the relevance of *P. amboinicus* for bioactivity-oriented research. Rachutami et al. reported an LC_{50} of 697.99 ppm for an ethanol extract of cumin leaf against *Artemia salina* using BSLT, indicating preliminary biological activity [4]. In a more specific chemical investigation, Martha et al. identified several flavonoid compounds in cumin leaves using liquid chromatography–mass spectrometry (LC–MS) and discussed their potential relevance to anticancer research [3]. These findings suggest that the biological activity of *P. amboinicus* may be associated with multiple secondary metabolites rather than a single constituent.

The extraction procedure is an important determinant of the compounds recovered from plant material. Infusion is a simple hot-water extraction technique that is particularly relevant to water-soluble and relatively polar constituents. It also represents a preparation method that is closer to traditional aqueous use than extraction with organic solvents [5,6]. In the present study, dried cumin leaves were therefore processed as an infusion using distilled water under controlled heating conditions.

Phytochemical screening provides preliminary evidence of the major secondary-metabolite groups present in an extract. UV–Vis spectrophotometry can subsequently be used to estimate the total level of selected metabolite groups when appropriate reference standards and calibration curves are available. The technique measures the absorption of electromagnetic radiation at selected wavelengths, allowing the relationship between concentration and absorbance to be evaluated through calibration and linear regression [7].

Preliminary toxicity screening is also useful for assessing the biological activity of natural-product preparations. The Brine Shrimp Lethality Test (BSLT) is a simple in vitro assay that uses *A. salina* larvae to evaluate acute toxicity and is commonly employed as an initial screening method for bioactive natural products. Its advantages include simple procedures, rapid assessment, and relatively low cost. LC_{50} values below 1000 ppm are commonly used as an indication of preliminary biological activity in screening studies [8,9]. However, BSLT does not establish anticancer efficacy because it does not directly evaluate cancer cell selectivity or molecular mechanisms.

The present study integrated three levels of evaluation: qualitative phytochemical screening, quantitative determination of selected metabolite groups by UV–Vis spectrophotometry, and acute toxicity screening using BSLT. The objectives were to identify alkaloids, flavonoids, saponins, and tannins in *P. amboinicus* infusion, determine their total levels, and estimate the LC_{50} against *A. salina* larvae. Particular emphasis was placed on the aqueous infusion, which differs from previous studies that mainly investigated organic extracts and therefore provides complementary information on the chemical and biological characteristics of water-soluble constituents.

2. Material and Method

2.1. Plant Material and Infusion Preparation

This study employed an experimental laboratory design. Dried cumin leaves (*Plectranthus amboinicus*) were obtained from the Herbal Laboratory Unit, Materia Medica Batu, East Java, Indonesia. Plant determination confirmed the material as *P. amboinicus*, with *Coleus amboinicus* Lour. recorded as a synonym in the determination report.

For infusion preparation, 500 g of powdered dried leaves was mixed with 1500 mL of distilled water. The mixture was heated at 90 °C for 15 min, cooled, filtered, and concentrated using a rotary evaporator at 40 °C. The extraction yield was calculated as the mass of concentrated infusion relative to the initial mass of dried material. The resulting concentrated infusion was used for phytochemical screening, quantitative analysis, and BSLT.

2.2. Moisture and Ash Determination

Moisture content was determined gravimetrically as a quality parameter of the dried plant material. Total ash content was determined to estimate the inorganic residue remaining after incineration. The results were compared with the relevant criteria in the Indonesian Herbal Pharmacopoeia and were used as preliminary indicators of raw-material quality [10].

2.3. Qualitative Phytochemical Screening

Qualitative phytochemical screening was performed for alkaloids, flavonoids, saponins, and tannins. Alkaloids were screened using Dragendorff's reagent, with the formation of an orange precipitate considered a positive reaction. Flavonoids were tested by heating the aqueous sample followed by the addition of magnesium powder and concentrated hydrochloric acid; the development of a red, yellow, or orange color was considered positive. Tannins were detected by adding 5% ferric chloride to the aqueous extract, with a dark-green or blue-black color indicating a positive reaction. Saponins were evaluated by vigorous shaking of the aqueous sample and observation of stable foam for at least 5 min. The procedures followed the qualitative principles reported in previous phytochemical studies [11–13].

2.4. UV–Vis Spectrophotometric Determination

Quantitative determination was performed using UV–Vis spectrophotometry. Reference standards were prepared at concentrations of 1, 5, 10, 25, and 50 ppm. Measurements were performed at 273 nm for alkaloids, 431 nm for flavonoids, 756 nm for tannins, and 466 nm for saponins. Calibration curves were constructed by plotting concentration against absorbance using the linear equation (Equation 1).

$$Y = ax + b \quad (1)$$

The correlation coefficient (r) was used to evaluate the linearity of the calibration curves. Sample concentrations were calculated from the corresponding calibration equations and expressed as total percentages according to the study calculations [7,14].

2.5. Brine Shrimp Lethality Test

For the BSLT assay, artificial seawater was prepared by dissolving 15 g of sodium chloride in 1 L of distilled water and adjusting the solution to approximately pH 8–9 with sodium bicarbonate. *A. salina* eggs were incubated under aeration until active larvae were obtained. Approximately 48 h old larvae were transferred to test vials containing artificial seawater.

A 1000 ppm stock solution was prepared by dissolving 100 mg of concentrated infusion in a small amount of 2% DMSO and diluting it to 100 mL with artificial seawater. Serial concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 ppm were prepared.

Each treatment concentration and the negative control consisted of 10 larvae per vial with three replicates. In total, 330 larvae were used in 33 vials. After 24 h of exposure, larval movement was observed. A larva was considered dead when no movement was observed during the observation period. Mortality was expressed as the percentage of dead larvae relative to the total number of larvae tested. The experimental arrangement followed the general BSLT procedure reported in previous studies [8,9,15].

2.6. LC₅₀ Determination

The LC₅₀ was estimated using probit analysis. The logarithm of concentration was plotted against the probit-transformed mortality, followed by linear regression. The concentration corresponding to probit 5 was taken as the LC₅₀ and converted from the log₁₀ concentration scale to the original concentration scale. For interpretation, the toxicity categories used in the study were applied, in which LC₅₀ values of 100–500 ppm were considered moderately toxic and values below 1000 ppm were considered indicative of preliminary biological activity [9].

3. Results and Discussion

3.1. Plant Identification and Quality Characteristics

Plant determination confirmed that the sample used in this study was cumin leaf, *Plectranthus amboinicus*. The determination report placed the plant in the family Lamiaceae and recorded *Coleus amboinicus* Lour. as a synonym. Correct botanical identification is essential because the chemical profile and biological activity of plant material depend strongly on species identity [2].

The dried material had a moisture content of 9.48%. This value was below the 10% criterion used for comparison in this study [10]. Low moisture content is relevant to raw-material stability because excessive moisture may promote microbial growth and affect the stability of plant constituents during storage.

The total ash content was 15.85%. This relatively high value indicates a substantial inorganic residue and should be considered when evaluating the quality of the starting material. Total ash represents the mineral and inorganic fraction remaining after incineration, and an elevated value may result from naturally high mineral content or contamination by soil, sand, dust, or other inorganic materials [10].

The concentrated infusion obtained from 500 g of dried leaves weighed 103.6 g, corresponding to a yield of 20.72%. The yield reflects the total material recovered after concentration and should not be interpreted as a direct measure of secondary-metabolite content or biological potency. Differences in extraction solvent, temperature, extraction time, particle size, plant condition, and concentration procedure may affect the yield.

The dried material had a moisture content of 9.48%. This value was below 10%, the criterion cited in the Indonesian Herbal Pharmacopoeia, and therefore indicated acceptable moisture control for the tested material. Low moisture is important because excessive water can promote microbial growth and may reduce the stability of plant material during storage. The result also supports the use of the material for subsequent extraction because excessive residual moisture can influence extraction efficiency and reproducibility.

The total ash content was 15.85%. In contrast to the moisture result, this value was interpreted in the thesis as not meeting the expected quality criterion and suggested a relatively high inorganic residue. Total ash represents the mineral and inorganic fraction remaining after incineration; an elevated value can reflect naturally high mineral content or contamination by soil, sand, dust, or other inorganic materials [10]. Thus, the relatively high ash value should be considered when interpreting the overall quality of the starting material and should be controlled in future studies through improved raw-material handling.

The concentrated infusion obtained from 500 g of dried leaves was 103.6 g, corresponding to a yield of 20.72%. This yield was higher than the 10% yield reported for an ethanol extract in the previous study by Rachutami et al. [4]. The difference may be related to the extraction solvent, temperature, extraction time, particle size, plant condition, and the concentration procedure. The 90 °C hot water process can facilitate the release of polar constituents into the aqueous phase, while concentration of the filtrate preserves the extracted solids in the final preparation.

Table 1. Quality characteristics of dried cumin leaves and the concentrated infusion.

Parameter	Sample	Result	Interpretation
Moisture content	Dried cumin leaf powder	9.48%	Below 10%; acceptable
Total ash	Dried cumin leaf powder	15.85%	Relatively high inorganic residue
Infusion yield	500 g dried leaves	20.72%	Above 10% benchmark cited in thesis

3.2. Qualitative Phytochemical Screening

All four metabolite groups examined were detected in the concentrated infusion. Alkaloids produced an orange precipitate after the addition of Dragendorff's reagent, flavonoids produced a brick-red color following the magnesium–hydrochloric acid reaction, saponins produced stable foam, and tannins produced a dark-green color after treatment with ferric chloride.

Table 2. Qualitative phytochemical screening of *P. amboinicus* infusion.

Metabolite group	Reagent	Observed reaction	Result
Alkaloid	Dragendorff	Orange precipitate	+
Flavonoid	Mg + concentrated HCl	Brick-red color	+
Saponin	Hot water; shaking	Stable foam	+
Tannin	5% FeCl ₃	Dark-green color	+

The positive alkaloid reaction is consistent with the chemical behavior of nitrogen-containing alkaloids, which can interact with Dragendorff's reagent to produce characteristic precipitates [16]. Alkaloids are pharmacologically diverse, and several members of this class have been investigated for anticancer activity through mechanisms involving cell proliferation, cell-cycle regulation, and apoptosis [17]. In the present study, however, the qualitative reaction should be regarded as evidence of the presence of the alkaloid group rather than identification of a specific alkaloid.

The positive flavonoid reaction, indicated by the formation of a brick-red color, is consistent with the presence of polyphenolic compounds. Flavonoids commonly occur in plants in glycosylated forms, and under acidic conditions, magnesium can participate in reactions that produce characteristic colored complexes. Flavonoid-containing plant extracts have been widely investigated for antioxidant and anticancer properties, including effects on oxidative stress, proliferation, apoptosis, and cell-cycle regulation [18].

The formation of stable foam in the saponin test is associated with the amphiphilic structure of saponins, which contains polar sugar groups and non-polar aglycone components. Saponins have also been investigated for biological activities related to membrane interactions and apoptosis [19]. The dark-green color observed in the tannin test following ferric chloride treatment is consistent with complex formation between ferric ions and phenolic hydroxyl groups. Tannins are polyphenolic constituents that have also been investigated for potential cytotoxic and anticancer effects [20]. Overall, the qualitative screening confirms the presence of four secondary-metabolite groups in the aqueous infusion. These findings provide a preliminary chemical profile and support subsequent quantitative determination by UV–Vis spectrophotometry.

3.3. UV–Vis Quantitative Determination

The quantitative analysis showed that flavonoids were the predominant measured metabolite group, with a total level of 62.81%, followed by saponins (22.20%), alkaloids (7.90%), and tannins (6.70%). The calibration curves showed strong linear relationships, with correlation coefficients of 0.9897 for alkaloids, 0.9961 for flavonoids, 0.9828 for saponins, and 0.9805 for tannins.

The predominance of flavonoids may be related to the use of hot water as the extraction medium. Many flavonoid glycosides are relatively polar and can therefore be extracted into aqueous media, while heating may enhance mass transfer from plant tissues into the solvent. The measured flavonoid level was higher than the total flavonoid level of 23.93% reported for cumin leaf in the previous study cited in the thesis [4]. However, such differences should not be attributed solely to biological variation because extraction solvent, temperature, extraction time, plant material, standardization procedures, and spectrophotometric calculations can all influence the measured total content.

The high flavonoid value should also be interpreted cautiously because UV–Vis spectrophotometry provides a group-level estimate rather than definitive structural identification. A colorimetric response may reflect multiple compounds with similar chromophoric properties. Thus, the present result represents a quantitative estimate of the total flavonoid-related response under the analytical conditions used rather than evidence for the presence of a specific flavonoid compound. LC–MS or other chromatographic techniques would be appropriate for subsequent compound-level characterization [3].

The saponin level of 22.20% was the second highest among the four metabolite groups. The presence of saponins is consistent with previous phytochemical reports on *P. amboinicus* and is relevant because saponins may exhibit membrane-interacting and apoptosis-related biological effects [19]. Alkaloids and tannins were measured at lower levels, at 7.90% and 6.70%, respectively. Although present at lower levels, these metabolite groups may contribute to the overall biological activity of the infusion.

Table 3. Calibration linearity and total secondary-metabolite levels determined by UV–Vis spectrophotometry.

Metabolite group	Correlation coefficient (r)	Total level (%)
Alkaloid	0.9897	7.90
Flavonoid	0.9961	62.81
Saponin	0.9828	22.20
Tannin	0.9805	6.70

The quantitative results demonstrate that the four metabolite groups occurred at different levels in the infusion. Flavonoids showed the highest measured content at 62.81%, followed by saponins at 22.20%, alkaloids at 7.90%, and tannins at 6.70%. This distribution indicates that flavonoid-related compounds represented the dominant group among the metabolites measured by the selected UV–Vis procedures. The calibration curves also showed strong linear relationships. The correlation coefficients were 0.9897 for alkaloids, 0.9961 for flavonoids, 0.9828 for saponins, and 0.9805 for tannins. These values indicate that the analytical responses increased consistently with the standard concentrations used in the calibration. The results therefore provide a quantitative description of the relative abundance of the four metabolite groups in the concentrated infusion.

The high flavonoid content is consistent with the discussion in the thesis that hot-water extraction may facilitate the recovery of flavonoid glycosides and other polar constituents. The saponin content was the second highest and is consistent with the positive foam test obtained during qualitative screening. Alkaloids and tannins were measured at lower levels, but their presence remains relevant because the biological activity of a plant preparation cannot be interpreted from the most abundant metabolite group alone.

The UV–Vis values should be interpreted as group-level measurements. They do not establish the concentration of a particular flavonoid, alkaloid, saponin, or tannin molecule. Nevertheless, they are useful for describing the chemical characteristics of the infusion and for supporting comparison with the phytochemical screening results. The numerical results further demonstrate the difference in abundance among the metabolite groups. The flavonoid value of 62.81% was substantially higher than the saponin value of 22.20%, while alkaloids and tannins were measured at 7.90% and 6.70%, respectively. The order of the measured groups was therefore flavonoids > saponins > alkaloids > tannins.

The calibration results support the quantitative measurements because all four correlation coefficients were close to one. The highest correlation was obtained for flavonoids at 0.9961, followed by alkaloids at 0.9897, saponins at 0.9828, and tannins at 0.9805. These results indicate a consistent relationship between the standard concentrations and the measured absorbance within the respective analytical procedures.

The high flavonoid content is also consistent with the positive qualitative flavonoid reaction. The thesis relates the result to the use of hot water as the extraction medium, particularly because polar flavonoid-related constituents can be recovered during the infusion process. The presence of saponins at the second-highest level is likewise consistent with the stable foam observed in qualitative testing. Although the measured levels differed considerably, each metabolite group remained detectable in the infusion. This finding is important for interpreting the BSLT result because the biological test was performed using the whole infusion rather than an isolated metabolite fraction. The biological response therefore represents the activity of the concentrated preparation containing the combination of detected metabolite groups.

3.4. BSLT Toxicity and LC_{50}

The BSLT assay showed an overall concentration dependent increase in larval mortality. Mortality increased from 6.7% at 100 ppm to 86.7% at 1000 ppm, while no mortality was observed in the negative control. The increasing mortality across the concentration range indicates that the concentration of the infusion influenced the acute toxic response of *A. salina* under the experimental conditions. The observed concentration response pattern allowed the LC_{50} to be estimated by probit analysis. The regression equation was presented in Equation 2.

$$y = 2.7423x - 2.3304 \quad (2)$$

with $R = 0.9579$. Setting $y = 5$, corresponding to 50% mortality, gave $x = 2.67$. The antilogarithm of this

value yielded an LC_{50} of 467.73 ppm.

Under the toxicity classification applied in the study, this value falls within the moderate-toxicity range and below the 1000 ppm threshold commonly used as an indicator of preliminary biological activity in BSLT screening [9,15]. However, the LC_{50} should be interpreted as preliminary toxicity rather than direct evidence of anticancer activity. BSLT is a general toxicity assay and does not reproduce the molecular environment of human cancer cells. A lower LC_{50} indicates that the infusion contains constituents capable of producing lethality in the test organism, but it does not establish cancer-cell selectivity, mechanism of action, therapeutic index, or in vivo efficacy.

The biological response may plausibly be associated with the combined presence of several secondary-metabolite groups. Flavonoids were the predominant measured group and have been reported to influence oxidative stress, apoptosis, and cell proliferation [18]. Alkaloids can affect cell cycle and apoptotic pathways [17], while saponins and tannins have also been associated with cytotoxic and apoptosis related effects [19,20]. Nevertheless, the present study cannot determine which constituent is responsible for the observed BSLT response. The activity should therefore be attributed to the whole infusion rather than to a specific metabolite group.

The practical significance of the findings is that a simple aqueous infusion was able to recover several secondary-metabolite groups while exhibiting measurable preliminary toxicity toward *A. salina*. This provides a basis for further natural product investigation, particularly through chemical profiling and bioactivity guided fractionation. However, the relatively high ash content of the starting material and the limitations of group-level UV-Vis quantification indicate that additional standardization is required before pharmacological applications can be considered.

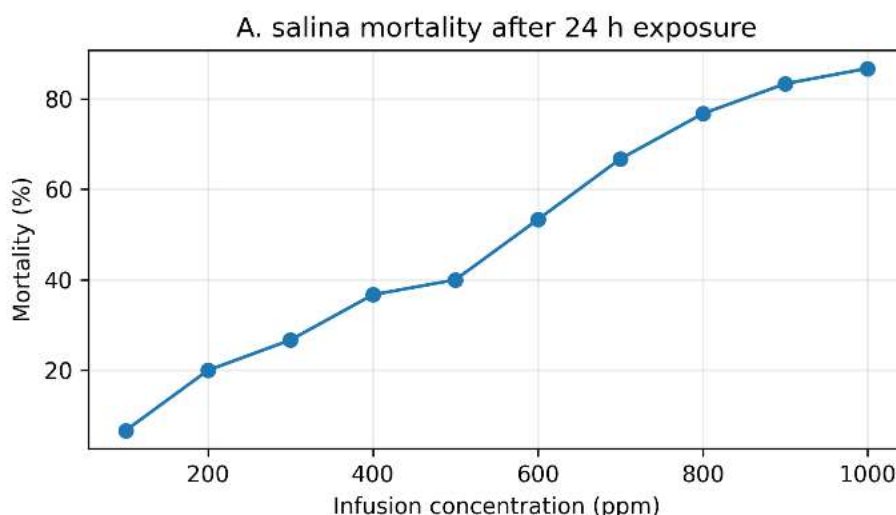


Figure 1. Concentration-dependent mortality of *A. salina* larvae after 24 h of exposure to *P. amboinicus* infusion.

Table 4. Mortality of *A. salina* larvae after exposure to *P. amboinicus* infusion.

Concentration (ppm)	Total larvae	Dead larvae	Mortality (%)
100	30	2	6.7
200	30	6	20.0
300	30	8	26.7
400	30	11	36.7
500	30	12	40.0
600	30	16	53.3
700	30	20	66.7
800	30	23	76.7
900	30	25	83.3
1000	30	26	86.7

The BSLT assay showed that mortality increased as the concentration of the infusion increased. Mortality was 6.7% at 100 ppm, 26.7% at 200 ppm, 30.0% at 300 ppm, 36.7% at 400 ppm, 40.0% at 500 ppm, 53.3% at 600 ppm, 66.7% at 700 ppm, 76.7% at 800 ppm, 83.3% at 900 ppm, and 86.7% at 1000 ppm. The control group showed no mortality. The increasing pattern indicates a concentration-dependent response under the conditions used in the experiment. The regression equation obtained from the probit analysis was $y = 2.7423x - 2.3304$, with $R = 0.9579$. The strong relationship between concentration and probit response supports the estimation of the LC₅₀ from the tested concentration series. Based on the calculation presented in the thesis, the LC₅₀ of the infusion was 467.73 ppm.

The LC₅₀ result indicates that the infusion produced measurable toxicity toward *A. salina* larvae. In the classification used in the study, an LC₅₀ below 1000 ppm indicates biological activity, while the value obtained in this study falls within the moderate-toxicity category. The result is therefore useful as preliminary evidence supporting further investigation of the infusion. The BSLT result should be interpreted together with the phytochemical findings. The infusion contained alkaloids, flavonoids, saponins, and tannins, and the combined presence of these groups provides a possible chemical basis for the observed biological response. However, the present experiment does not determine which particular compound or metabolite group is responsible for the mortality. The result is consequently considered preliminary evidence of cytotoxic potential rather than direct evidence of anticancer efficacy.

The mortality results provide a clear progression across the concentration series. The response remained below 50% mortality through 500 ppm and exceeded 50% beginning at 600 ppm. Mortality then continued to increase at 700, 800, 900, and 1000 ppm. This pattern allowed the LC₅₀ to be estimated from the relationship between concentration and probit response. The absence of mortality in the negative control is also relevant to the interpretation of the assay. It indicates that, under the experimental conditions described in the study, the control medium did not produce observable larval mortality. The mortality observed in the treatment groups can therefore be evaluated in relation to increasing concentrations of the infusion.

The use of three replicates for each concentration also provided repeated observations for the mortality response. Each vial contained 10 larvae, resulting in 30 larvae evaluated at each treatment concentration. Across the full experiment, 330 larvae were used. This arrangement allowed mortality to be expressed as a percentage for each concentration and subsequently used for probit analysis. The calculated LC₅₀ of 467.73 ppm represents the concentration associated with 50% mortality according to the regression analysis. The result is lower than the LC₅₀ reported for the ethanol extract in the previous study cited in the thesis. Within the limitations of comparing different preparations, this finding indicates that the aqueous infusion produced a measurable biological response in the BSLT model.

3.5. Comparison with Previous Studies and Research Implications

The present findings complement previous studies of *P. amboinicus*. Rachutami et al. reported an LC₅₀ of 697.99 ppm for an ethanol extract of cumin leaf, whereas the aqueous infusion examined in the present study produced an LC₅₀ of 467.73 ppm [4]. The lower LC₅₀ indicates a stronger toxicity response under the respective experimental conditions. However, direct comparison should be made cautiously because the two studies used different extraction systems and may have differed in plant processing and assay conditions.

Previous LC–MS research has demonstrated the presence of multiple flavonoid constituents in cumin leaves [3]. The high total flavonoid response measured by UV–Vis in the present study is consistent with this broader phytochemical profile but cannot identify the individual compounds reported in the LC–MS study. The two analytical approaches provide complementary information: UV–Vis enables rapid estimation of a metabolite class, whereas LC–MS provides more specific chemical characterization. Combining these approaches would strengthen future investigations by linking total metabolite levels with individual constituents.

The infusion procedure is also relevant to natural product development. The experimental preparation used a 1:3 ratio of dried material to water, followed by heating at 90 °C for 15 min and concentration of the filtrate. Hot-water extraction is relatively simple and avoids the use of large volumes of organic solvents. However, extraction efficiency depends on temperature, extraction time, particle size, solvent to material ratio, and the physicochemical properties of the target compounds. Future optimization should therefore evaluate whether the current conditions maximize recovery of biologically relevant constituents while minimizing degradation of heat sensitive compounds.

The calibration curves showed strong linear relationships between standard concentration and absorbance, with the highest correlation coefficient obtained for flavonoids (0.9961), followed by alkaloids (0.9897), saponins (0.9828), and tannins (0.9805). Although these values indicate strong linear associations within the tested ranges, complete analytical validation would require additional parameters, including accuracy, precision, selectivity, limit of detection, limit of quantification, and recovery. Such validation was outside the scope of the present study and should be incorporated into future quantitative analyses.

Taken together, the phytochemical and BSLT findings indicate that *P. amboinicus* infusion warrants further pharmacognostic and pharmacological investigation. However, the observed BSLT activity should not be interpreted as evidence of anticancer efficacy. Future studies should chemically characterize the infusion or its fractions and evaluate their activity against established cancer cell lines alongside non-malignant cells. Mechanistic studies could subsequently investigate apoptosis, cell cycle regulation, oxidative stress, and other relevant pathways.

3.6. Interpretation of the Extraction Process

This section can be substantially shortened because the rationale for aqueous extraction has already been addressed in the Introduction and Section 3.5. The extraction conditions used in this study are relevant to the resulting chemical profile. Infusion is a hot-water extraction process that favors constituents with sufficient polarity to enter the aqueous phase. Heating at 90 °C for 15 min may enhance diffusion and solubilization from plant tissues. However, extraction yield does not directly represent pharmacological potency because the recovered material may include both target and non-target constituents. The 15.85% total ash content also indicates the need for further raw-material standardization. Future studies should control cleaning, drying, milling, and storage procedures and evaluate foreign matter and mineral composition in greater detail. Because this discussion overlaps with the Introduction and Section 3.5, the section may be retained only if required by the journal; otherwise, it can be deleted to make the manuscript more concise.

4. Conclusion

The aqueous infusion of *Plectranthus amboinicus* contained four secondary-metabolite groups detected by qualitative phytochemical screening: alkaloids, flavonoids, saponins, and tannins. UV–Vis analysis indicated group-level responses corresponding to flavonoids (62.81%), saponins (22.20%), alkaloids (7.90%), and tannins (6.70%) under the analytical conditions used. These values represent estimates relative to the respective reference standards and should not be interpreted as percentages of purified compounds. Under the classification applied in this study, the result indicates preliminary toxicity toward *A. salina*. The findings provide a basis for further chemical characterization and direct cytotoxicity testing using cancer and non-cancer cell models.

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