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Phytochemical Profile, Antioxidant Activity, and Brine Shrimp Lethality Test of *Thyrsostachys siamensis* (Siamese Bamboo) Leaf Extracts

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Abstract

Cancer and oxidative-stress–related disorders continue to rise globally, driving interest in plant-based antioxidants with concurrent cytotoxic potential. This study aimed to characterize the phytochemical profile, antioxidant activity, and brine shrimp (*Artemia salina*) lethality of *Thyrsostachys siamensis* (Siamese bamboo) leaf extracts obtained with solvents of increasing polarity (n-hexane, chloroform, acetone, ethyl acetate, methanol, ethanol, and water). Ethanol appears as the most effective solvent for *T. siamensis*, combining a solid extraction yield (7.78%) with the richest phytochemical profile (positive for alkaloids, flavonoids, saponins, tannins, and phenolics). This broad chemical recovery corresponds to the strongest bioactivity, shown by its lowest IC_{50} (91.48 ± 0.12 µg/mL) and LC_{50} (80.78 ± 0.62 µg/mL). Acetone (6.42%) and ethyl acetate (5.11%) offer moderate yields and selective enrichment of semi-polar constituents, reflected in intermediate IC_{50} values (144–150 µg/mL) and LC_{50} values (273–350 µg/mL). In contrast, hexane despite providing the highest mass recovery (11.34%) contains mainly non-phenolic components and shows weak biological activity (IC_{50} 200.52 ± 0.38 µg/mL, LC_{50} 620.06 ± 0.76 µg/mL). Chloroform, with limited phytochemical content, yields the poorest performance (IC_{50} 238.30 ± 1.56 µg/mL, LC_{50} 926.40 ± 4.27 µg/mL).

Keywords: antioxidant activity; Artemia salina; brine shrimp lethality; phytochemical profile; *Thyrsostachys siamensis*.

Abbreviations: BSLT (Brine Shrimp Lethality Test)

INTRODUCTION

Cancer is widely perceived as a frightening disease by the public because it is characterised by uncontrolled cell proliferation and abnormal spread of cells that can be fatal if not treated promptly (National Cancer Institute, 2025). Globally, cancer is one of the leading causes of death and is currently the second most common cause of mortality after cardiovascular disease (American Cancer Society, 2024; World Health Organization, 2024). Estimates from the International Agency for Research on Cancer reported around 7.6 million cancer deaths worldwide in 2012, and more recent global data indicate approximately 20 million new cases and 9.7 million deaths in 2022, meaning that about one in five people will develop cancer in their lifetime (Bray et al., 2024). The World Health Organization predicts that annual cancer deaths will continue to rise significantly and that the burden will increasingly fall on low- and middle-income countries (World Health Organization, 2024).

In Indonesia, Basic Health Research (Riskesdas) data show that the prevalence of tumours/cancer increased by about 28%, from 1.4 per 1,000 population in 2013 to approximately 1.79–1.8 per 1,000 population in 2018, which corresponds to nearly 480,000 people (S. Gondhowiardjo et al., 2021; S. A. Gondhowiardjo et al., 2021). An unbalanced lifestyle involving smoking, alcohol consumption, unhealthy diet, obesity, low physical activity, and exposure to environmental pollutants is recognised as an important factor in the development of cancer (World Health Organization, 2024). Standard cancer treatments such as surgery, radiotherapy, and cytostatic chemotherapy are designed to kill cancer cells but often cause serious side effects, including hair loss, skin hyperpigmentation, organ toxicity, and deterioration of quality of life. These limitations highlight the need for alternative or complementary approaches using natural ingredients that are more targeted, effective, and associated with fewer adverse effects (Situmorang et al., 2024).

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the capacity of endogenous antioxidant systems, is now recognised as a key mechanism linking chronic

inflammation to cancer development and progression (Jomova et al., 2025; Sorriento, 2024; Sung et al., 2021). Persistent oxidative stress can induce DNA damage, genomic instability, and dysregulation of signalling pathways that promote tumour initiation, angiogenesis, and metastasis (Jomova et al., 2025; Sorriento, 2024). Targeting ROS-related redox and inflammatory pathways has therefore emerged as a promising strategy for cancer prevention and therapy because modulation of these pathways can inhibit tumour growth and alter the tumour microenvironment (Sorriento, 2024). Concerns about the long-term safety of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ), which have shown cytotoxic, genotoxic, or organ-specific toxicity at high doses, further strengthen the interest in safer natural antioxidants (Ren et al., 2025).

Plant-based antioxidants that are rich in polyphenols and other phenolic compounds are intensively explored because they are able to scavenge free radicals, enhance endogenous antioxidant enzymes, and interfere with pro-carcinogenic signalling pathways, thereby contributing to chemopreventive effects (Ahmed et al., 2025; Jameel et al., 2025). Natural products with high antioxidant content from medicinal plants frequently show selective cytotoxicity towards cancer cells while exhibiting lower toxicity towards normal cells, which makes them attractive candidates for anticancer drug discovery and adjuvant therapy (Situmorang et al., 2024). In many modern phytochemical studies, total phenolic content and total flavonoid content are determined together with in vitro antioxidant capacity using standardised DPPH and ABTS radical-scavenging assays to relate chemical composition to biological activity (Baliyan et al., 2022; Yoo & Keum, 2019). This combined approach allows researchers to characterise plant extracts not only in terms of their phytochemical profile and antioxidant capacity but also in relation to their potential cytotoxic effects against cancer-related models (Ahmed et al., 2025; Baliyan et al., 2022)

The Poaceae family includes numerous food, forage, and bamboo species. It is one of the most economically important plant groups and has long been used in traditional medicine in many countries (Sasu et al., 2023). Recent reviews indicate that many Poaceae species contain phenolic acids, flavonoids, terpenoids, and other secondary metabolites with antioxidants, anti-inflammatory, antidiabetic, antimicrobial, and cytotoxic properties, supporting their use in chronic disease prevention and management (Fatima et al., 2022). Studies on Poaceae weeds have shown that leaf extracts can possess appreciable level of phenolic and flavonoid contents, strong DPPH radical-scavenging activity, and inhibitory effects against pathogenic bacteria, suggesting that underutilised grasses can serve as inexpensive sources of bioactive compounds (Swami et al., 2024). At the food level, cereals such as barley (*Hordeum vulgare* L.) contain bioactive constituents with significant free-radical-scavenging capacity that contribute to the health benefits associated with cereal consumption. The members of the Poaceae family are regarded as important reservoirs of natural antioxidants that may be developed as raw materials for functional foods, dietary supplements, and phytopharmaceutical products (Noreen et al., 2025).

Studies of the Poaceae family include several species evaluated for preliminary anticancer activity using the brine shrimp lethality test (BSLT) with *Artemia salina* larvae as a simple cytotoxicity model (Nurcholis et al., 2019; Prasticha, 2024). Extracts from several Poaceae species have also been reported to exhibit strong cytotoxic properties in the brine shrimp lethality test (BSLT). Essential oil from *Cymbopogon citratus* demonstrated potent toxicity toward *Artemia salina* larvae, with LC_{50} values ranging from 1.21 to 1.28 µg/mL, indicating remarkable lethality in this assay (Valdés & Martins, 2019). Similarly, essential oil from *Cymbopogon winterianus* showed high cytotoxic activity, producing LC_{50} values of 5.29 µg/mL (Chaves Girão Neto et al., 2025). Crude extracts of *Imperata cylindrica* also exhibited notable toxicity, where root extracts yielded LC_{50} values between 342 and 1530 µg/mL, and leaf extracts showed LC_{50} values ranging from 490 to 1067 µg/mL, depending on the solvent used (Konan et al., 2022). Young shoot extracts from several cereal species within Poaceae (*Zea mays*, *Triticum aestivum*, and *Hordeum vulgare*) also displayed cytotoxicity in the BSLT, with LC_{50} values of 326.41 µg/mL, 473.61 µg/mL, and 6768.75 µg/mL, respectively (Kalauni et al., 2024). Collectively, these findings reinforced that members of the Poaceae family possess diverse yet significant cytotoxic potential in bioassays involving *Artemia salina*.

The chemotaxonomic approach states that plants from the same genus or family tend to contain similar classes of compounds, so members of Poaceae are expected to share related phytochemical profiles and potential bioactivities (Amin & Park, 2025). For this reason, initial screening of anticancer activity is often conducted by testing the toxicity of plant extracts against *Artemia salina* shrimp larvae using the BSLT as a rapid, low-cost, and sensitive bioassay (Amin & Park, 2025; Konan et al., 2022). In this context, extracts that are toxic to A. salina at relatively low concentrations can be described as having potential anticancer activity and are prioritised for further evaluation in more specific in vitro or in vivo models (Konan et al., 2022; Nurcholis et al., 2019). Based on this rationale, previous work on Poaceae species has combined BSLT-based toxicity testing with phytochemical analysis to explore the anticancer potential of grass-derived extracts.

Bamboo, a major group within Poaceae, is recognised not only as a renewable material and food source but also

as a provider of nutraceutical and medicinal products due to its rich content of polyphenols and other bioactive constituents (Narzary et al., 2025; Shen et al., 2024). Bamboo leaves from several species have been reported to contain high levels of phenolic and flavonoid compounds and to exhibit strong antioxidant activity in DPPH, ABTS, and related assays, with some extracts also showing antifungal and anti-inflammatory properties. In Thailand, phytochemical profiling of bamboo leaves from different genotypes identified chlorogenic acid, caffeic acid, orientin, isoorientin, vitexin, isovitexin, and p-coumaric acid as major constituents contributing to antioxidant and antimelanogenic activities (Kasemsukphaisan & Maksup, 2022). Dietary studies have shown that bamboo leaf flavonoids can enhance in vivo antioxidant capacity, modulate gut microbiota, and improve health parameters in animal models, which supports the potential of bamboo leaves as functional ingredients for managing oxidative-stress-related conditions (Cao et al., 2022).

Thyrsostachys siamensis Gamble (Siamese bamboo) is a tropical bamboo that is widely cultivated in Southeast Asia, especially in Thailand, and in Indonesia it is commonly planted as an ornamental hedge or barrier along walls and roadsides, where its dense foliage helps absorb air pollution and reduce noise. Although the culms and shoots of *T. siamensis* are used as construction material and vegetables (Techamahasaranont et al., 2025), many of the leaves are still discarded as organic waste despite their potential as a source of valuable bioactive compounds (Hidayah et al., 2022).

To date, information on the detailed phytochemical composition, antioxidant activity, and preliminary cytotoxicity of *T. siamensis* leaves remains very limited. No published study has systematically evaluated *T. siamensis* leaf extracts using a combination of in vitro antioxidant assays and the brine shrimp lethality test (BSLT), so the antioxidant profile and basic toxicity of this plant part are still largely unknown. Therefore, the present study aims to conduct qualitative phytochemical analysis, evaluate the in vitro antioxidant activity, and assess the brine shrimp lethality of *Thyrsostachys siamensis* leaf extracts as an integrated first step toward exploring their potential as natural antioxidant and anticancer candidates within the Poaceae family.

MATERIALS AND METHODS

Materials

The plant material used in this study consisted of Siamese bamboo (*Thyrsostachys siamensis* Gamble) leaves collected from Depok, Sleman, Special Region of Yogyakarta, Indonesia, and authenticated at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta. All solvents used for extraction and analysis were pro analysis (p.a.) grade and purchased from Merck, including ethanol 96%, methanol, n-hexane, chloroform, acetone, and ethyl acetate.

Reagents for qualitative phytochemical screening, such as concentrated hydrochloric acid (HCl), ammonia solution (NH_4OH), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$) 1%, magnesium turnings, Dragendorff's reagent, Mayer's reagent, acetic anhydride, and concentrated sulfuric acid (H_2SO_4), were also of analytical grade and obtained from Merck. For the antioxidant assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH, ≥95% purity) and L-ascorbic acid (≥99% purity) were used as the free radical source and reference antioxidant, respectively, and were purchased from Merck. For the brine shrimp lethality test, *Artemia salina* eggs were obtained from a commercial supplier, and artificial seawater was prepared from non-iodized commercial sea salt and distilled water.

All chemicals and reagents were of analytical (p.a.) grade and were used without further purification unless otherwise stated.

Instrumentations

UV–Vis spectrophotometer (Shimadzu UV-1800), analytical balance (Shimadzu AUW220D), rotary evaporator with water bath (BUCHI R-300), drying oven, a set of glassware (Pyrex beakers, Erlenmeyer and volumetric flasks, measuring cylinders, funnels, test tubes, Pasteur pipettes, glass rods), hatching chamber for *Artemia salina* with aquarium aerator, continuous light source (fluorescent/LED lamp), magnifying glass or dissecting microscope, and small glass vials.

Preparation of Extracts

Dried powdered leaves of *Thyrsostachys siamensis* were extracted by cold maceration using n-hexane, chloroform, acetone, ethyl acetate, methanol, and distilled water at a plant material to solvent ratio of 1:10 (w/v). The powder was immersed in each solvent for 7 days, and the solvent was renewed every 48 h; filtrates from each renewal were combined and filtered, then concentrated under reduced pressure using a rotary evaporator at 40–50 °C (for organic solvents) or dried at low temperature (for the aqueous extract) to obtain crude n-hexane, chloroform, acetone, ethyl acetate, methanol, and water extracts, which were stored at 4 °C until analysis.

Qualitative Phytochemical Screening

Qualitative phytochemical screening of *Thyrsostachys siamensis* leaf extracts was performed using standard color reactions for major metabolite classes (Maheshwaran et al., 2024). Flavonoids were identified by Shinoda's test, in which 0.5 g of extract was dissolved in methanol, followed by the addition of magnesium powder and concentrated HCl; the development of a yellow to reddish color indicated a positive reaction for flavonoids (Lazuardi et al., 2022). Alkaloids were detected by dissolving 0.5 g of extract in chloroform and

ammonia, filtering, acidifying the filtrate with HCl, and adding Dragendorff's and Mayer's reagents; formation of orange–brown or creamy precipitates was interpreted as evidence of alkaloids (Raal et al., 2020). Saponins were tested using the foam test, where 0.5 g of extract was shaken vigorously with hot distilled water, and the presence of stable, persistent foam indicated the presence of saponins (Góral & Wojciechowski, 2020). Tannins were identified by mixing 0.5 g of extract with distilled water, heating, and adding 1% $FeCl_3$ solution: a dark blue or greenish-black color signified tannins (Abdelfatah et al., 2021). Steroids and triterpenoids were screened using the Liebermann–Burchard reaction by treating an aliquot of the extract solution with acetic anhydride and concentrated H_2SO_4; a blue–green color indicated steroids and a reddish to brownish color indicated triterpenoids (Adu et al., 2019). All concentrated acids and acetic anhydride were handled in a fume hood with appropriate PPE.

The results of each test were recorded qualitatively as negative (−), weakly positive (+), or strongly positive (++), providing an overview of the phytochemical profile of *T. siamensis* leaf extracts in line with the earlier study on Siamese bamboo (Hidayah et al., 2022).

Antioxidant Activity: DPPH Radical-Scavenging Assay

The in vitro antioxidant activity of *Thyrsostachys siamensis* leaf extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical-scavenging assay, following widely used procedures with minor modifications (Fikroh & Hidayah, 2025; Gulcin & Alwasel, 2023).

A 0.1 mM DPPH solution was freshly prepared in methanol, protected from light, and used on the same day to ensure radical stability (Gulcin & Alwasel, 2023). Crude extracts were dissolved in ethanol or methanol to obtain a stock solution of 1 mg/mL, then serially diluted to prepare test solutions with final concentrations in the range of 40–200 µg/mL. Ascorbic acid prepared in the same solvent was used as a reference antioxidant standard at concentrations of 2–10 µg/mL. For each measurement, 3.8 mL of the DPPH solution was mixed with 0.2 mL of extract or standard solution in a test tube, giving a total reaction volume of 4.0 mL, while the control contained 3.8 mL of DPPH solution plus 0.2 mL of solvent only. The reaction mixtures were vortexed briefly, incubated in the dark at room temperature for 5 minutes, and the decrease in absorbance was recorded at 515 nm using a UV–Vis spectrophotometer (Fikroh and Hidayah, 2025). A sample blank without DPPH was prepared for each extract concentration to correct for any intrinsic absorbance of the extract at the measurement wavelength. The percentage of DPPH radical scavenging was calculated using the equation:

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the DPPH solution with solvent only and $A_{\text{sample}}$is the absorbance of the DPPH solution in the presence of the extract or standard. All measurements were performed in triplicate, and results were expressed as mean ± standard deviation.

The IC_{50} value (concentration providing 50% inhibition) was obtained by plotting percentage inhibition against the logarithm of concentration and applying linear regression; lower IC_{50} values were interpreted as indicating stronger antioxidant activity (Sridhar & Charles, 2019).

Brine Shrimp Lethality Test (BSLT)

Preliminary toxicity of *Thyrsostachys siamensis* leaf extracts was evaluated using the brine shrimp lethality test (BSLT) with *Artemia salina* nauplii as a simple in vivo bioassay for general cytotoxicity, following recent protocols with minor modifications (Konan et al., 2022; Ramli et al., 2024; Waghulde et al., 2019). Artificial seawater was prepared by dissolving commercial sea salt in distilled water to a salinity of approximately 38 g/L and filtering the solution to remove undissolved particles (Valentin Bashige Chiribagula et al., 2020). *A. salina* cysts were hydrated and incubated in aerated artificial seawater under continuous illumination for about 48 h until free-swimming nauplii hatched and accumulated near the light source; active nauplii were collected with a Pasteur pipette and transferred into fresh artificial seawater for the assay (Andini et al., 2020).

Stock solutions of the *T. siamensis* extracts were prepared by dissolving an appropriate amount of each crude extract in a small volume of dimethyl sulfoxide (DMSO) and diluting with artificial seawater to obtain a series of test concentrations (1, 10, 100, 250, 500, 1000 µg/mL) , ensuring that the final concentration of organic solvent did not exceed 1% (v/v), a level reported as non-toxic to *A. salina* nauplii (Vázquez-Torres et al., 2025; Waghulde et al., 2019). For each concentration, an aliquot of the test solution was transferred into a small vial and mixed with a suspension of nauplii to give a final volume of 1.0 mL containing approximately 30 larvae per vial; each concentration was tested in triplicate together with a seawater blank and a solvent control containing ≤1% DMSO (Álvarez-Alarcón et al., 2021; Ramli et al., 2024). All vials were kept at room temperature under constant illumination for 24 h without feeding, after which the number of surviving nauplii in each vial was counted under a magnifying lens, and larvae showing no movement for several seconds, even after gentle agitation, were recorded as dead.

Percentage mortality was calculated for each extract concentration after correction for any mortality in the solvent control, and the median lethal concentration (LC_{50}) with 95% confidence limits was estimated by

plotting percentage mortality against the logarithm of concentration and fitting a probit or similar dose–response model. In line with commonly used criteria in recent plant-toxicity studies, extracts with LC_{50} values below 1000 µg/mL in the BSLT were interpreted as bioactive and considered to have potential cytotoxic or anticancer properties warranting further investigation in more specific cell-based assays (Ramli et al., 2024; Waghulde et al., 2019).

RESULTS AND DISCUSSION

Plant material of Siamese bamboo was collected from Depok District, Sleman Regency, Special Region of Yogyakarta, Indonesia, and its taxonomic determination was conducted by the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada (UGM). The identification confirmed the specimen as Thyrsostachys siamensis Gamble (family Poaceae), as stated in the official determination certificate attached as an appendix (SK No. 65.1.03UN1/FFA.2/BF/PT/2023, dated 1 March 2023, registration number 15, issued by the Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM).

Extraction Yields

Extraction yields across solvents ranged from 3.47% to 11.34% and the downstream IC_{50} (DPPH) and LC_{50} (BSLT) patterns confirm that mass recovery and bioactivity are not interchangeable endpoints (Mungwari et al., 2025). The maceration-style workflow with repeated solvent replacement is expected to keep the concentration gradient high and sustain diffusion-driven extraction, which can elevate yield without necessarily enriching the same metabolite classes across solvents (Tourabi et al., 2025). In addition, low-temperature concentration for organic extracts typically preserves more labile constituents than harsher heating, but it still does not prevent co-extraction of nonfunctional bulk matrices when solvent selectivity is low (Palos-Hernández et al., 2025). Therefore, yield is treated here as a process performance parameter, while IC_{50} and LC_{50} are treated as functional readouts tied to redox chemistry and general cytotoxicity, respectively (Shahidi & Samarasinghe, 2025).

Tabel 1. The Percentage Yield Value of Each Extracts.

Solvents	%Yield
Hexane	11.34
Methanol	10.03
Ethanol	9.95
Acetone	8.34
Ethyl Acetate	7.78
Chloroform	6.83
Water	3.47

Hexane produced the highest yield (11.34%), followed by methanol (10.03%) and ethanol (9.95%), whereas water yielded 3.47%, establishing a wide solvent-dependent mass spread. This ordering is consistent with the principle that nonpolar solvents readily solubilize lipophilic constituents that contribute strongly to recovered mass, including waxy or lipid-like fractions that inflate gravimetric yield (Mungwari et al., 2025). However, a higher hexane yields alongside weaker performance in DPPH and BSLT indicates that much of the recovered mass likely contains fewer hydrogen or electron donating antioxidants and fewer broadly cytotoxic secondary metabolites at equivalent mass dosing (Shahidi & Samarasinghe, 2025). This disconnect is expected because phenolics and many flavonoids partition preferentially into polar or intermediate-polarity solvents, so nonpolar mass recovery can be compositionally diluted with redox-inert matrices (Xiang et al., 2024). Accordingly, the intermediate yields obtained with acetone (8.34%), ethyl acetate (7.78%), and chloroform (6.83%) are better interpreted as selectivity-limited recoveries rather than indicators of weaker extraction, because each solvent targets different chemical space (Table 1).

Phytochemical Screening

Table 2. Phytochemical Screening Results of Extracts in Various Solvents.

Extracts	Phytochemical Screening Results				
	Flavonoids	Alkaloids	Saponins	Tannins	Steroids/Triterpenoids
Hexane	+	-	-	-	-
Methanol	++	++	++	+	$+^{T}$
Ethanol	++	++	+	+	$++^{T}$
Acetone	++	+	++	++	$+^{T}$
Ethyl Acetate	+	-	-	-	-
Chloroform	-	+	-	-	-
Water	+	+	+	-	-

++: Very Strong Result; +: Strong Result; -: Negative; ^{T}: Triterpenoids; ^{S}: Steroids

Qualitative screening supports polarity-driven enrichment, with methanol and ethanol showing very strong flavonoid and alkaloid signals, while acetone shows very strong flavonoids, saponins, and tannins, indicating broad recovery of multiple bioactive classes (Hernandez-Fuentes et al., 2024). Methanol presents very strong responses for flavonoids, alkaloids, and saponins with a strong tannin signal, which aligns with methanol's strong affinity for polar and moderately polar secondary metabolites (Metrouh-Amir et al., 2015). Ethanol similarly shows very strong flavonoids and alkaloids with strong saponins and tannins plus very strong triterpenoid-associated response, supporting a chemically diverse extract likely to express both radical scavenging and bioactivity in a general toxicity screen (Shourove et al., 2025). Acetone's pattern very strong flavonoids, saponins, and tannins with strong alkaloids fits reports that intermediate-polarity solvents can pull condensed phenolics and other mid-polar constituents that often drive antioxidant signals in DPPH-type assays (Palos-Hernández et al., 2024). In contrast, ethyl acetate and hexane show narrow positivity profiles, which is consistent with reduced affinity for hydrophilic phenolic pools and helps explain weaker functional outputs despite acceptable mass yields.

DPPH Antioxidant Activity (IC_{50})

Table 3. IC_{50} (µg/mL) and LC_{50} (µg/mL) results.

Extracts	IC_{50} (µg/mL)	LC_{50} (µg/mL)
Methanol	148.59 ± 0.73^{a}	143.11 ± 0.36
Ethanol	91.48 ± 0.12^{b}	80.78 ± 0.62
Acetone	144.57 ± 0.85^{c}	273.74 ± 1.17
Hexane	200.52 ± 0.38^{d}	620.06 ± 0.76
Ethyl Acetate	153.54 ± 0.22^{e}	359.04 ± 1.10
Chloroform	238.30 ± 1.56^{f}	926.40 ± 4.27
Water	170.21 ± 1.07^{g}	630.29 ± 1.35
Ascorbic Acid	22.84 ± 0.06	-

In the DPPH assay, ethanol produced the lowest IC_{50} at 91.48 ± 0.12 µg/mL, followed by acetone (144.57 ± 0.85 µg/mL) and methanol (148.59 ± 0.73 µg/mL), while chloroform showed the weakest activity (238.30 ± 1.56 µg/mL). The qualitative profile supports this alignment because ethanol shows very strong flavonoids and alkaloids plus additional triterpenoid-associated positivity, collectively plausible contributors to stronger DPPH quenching at lower concentration (Fikroh & Hidayah, 2025; Shourove et al., 2025).

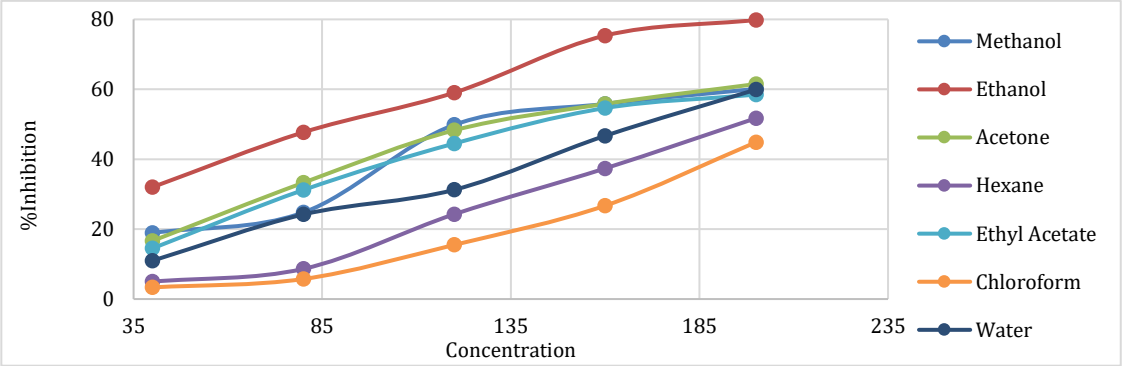

Figure 1. %Inhibition curve of the different extracts.

Hexane delivered the highest gravimetric recovery (11.34%) yet showed weak DPPH scavenging potency with an IC_{50} of 200.52 ± 0.38 µg/mL, indicating that extracted mass was not enriched in effective DPPH-reactive hydrogen/electron donors. Nonpolar maceration commonly solubilizes bulk lipophilic matrices (neutral lipids, waxes, pigments, and hydrophobic structural components) that can inflate yield while diluting phenolic density and overall radical-quenching capacity per unit mass (Tuhanioglu & Ubeyitogullari, 2022). Such yield–activity decoupling is repeatedly observed in polarity-fractionation work where hexane fractions contain markedly lower total phenolics and weaker DPPH performance than ethyl acetate or alcoholic fractions, even when crude mass recovery is high (Taviano et al., 2024). Ethyl acetate (153.54 ± 0.22 µg/mL) sits in the mid-range, consistent with limited recovery of polar antioxidants despite being able to solubilize some semi-polar constituents (Lohvina et al., 2021; Shourove et al., 2025). Ascorbic acid was employed as the standard reference in this study because of its well-known and strong antioxidant activity.

The IC_{50} dataset meets standard parametric assumptions closely enough to justify one-way ANOVA followed by Tukey HSD, based on homogeneity and normality checks. Levene's test indicates no strong evidence of heteroscedasticity for IC_{50} ($p = 0.074$), which supports equal-variance modeling across solvent groups. Shapiro–Wilk p-values for IC_{50} are acceptable across groups (all shown $p > 0.05$), supporting approximate normality at the group level despite small n. The ANOVA indicates a decisive solvent effect on IC_{50} ($F =$

8973.589, $p < 0.001$), consistent with large between-group separation relative to within-group variance. Post-hoc separation labels a–g across all extracts, indicating that each solvent's IC_{50} differs detectably from the others under Tukey control of familywise error. The extremely small within-group variance implied by the ANOVA table suggests high instrumental and procedural repeatability for the DPPH workflow, consistent with tightly controlled radical preparation, light protection, and absorbance timing.

BSLT Toxicity (LC_{50})

In the BSLT (Table 3), ethanol again shows the strongest activity with LC_{50} = 80.78 ± 0.62 µg/mL, followed by methanol (143.11 ± 0.36 µg/mL) and acetone (273.74 ± 1.17 µg/mL), while chloroform shows the weakest LC_{50} at 926.40 ± 4.27 µg/mL. Toxicologically, a smaller LC_{50} means that lethal effects occur at lower concentrations, so ethanol shows the strongest toxic activity in the Artemia model compared to other extracts in the same protocol (Araya et al., 2024; Nguyen et al., 2024). Current interpretation categories also generally place LC_{50} < 1000 µg/mL as an indication of bioactivity/toxicity in BSLT that warrants follow-up to cell-based cytotoxicity assays, noting that BSLT is a general screening and not specific evidence of anticancer activity (Nguyen et al., 2024; Djafarou et al., 2025).

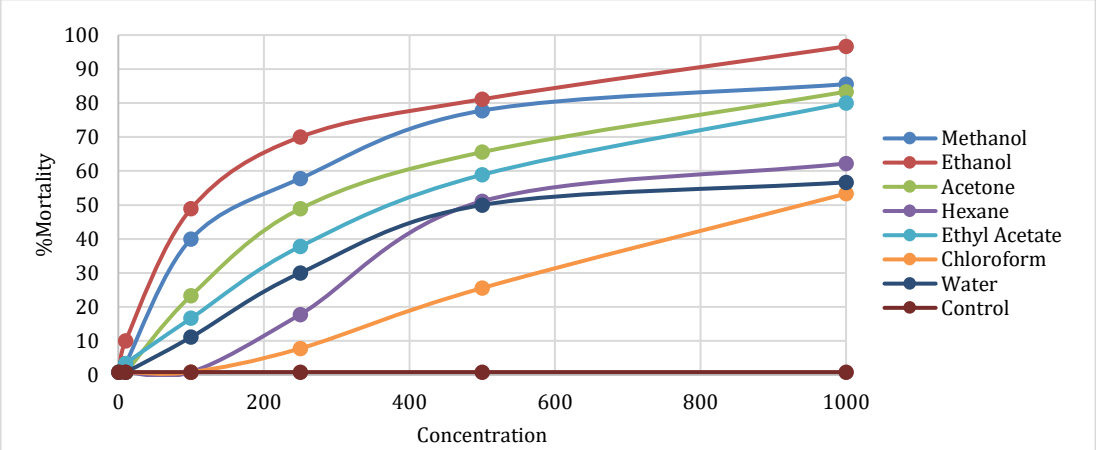

Figure 2. Dosage-response curve of the different extracts against *Artemia salina.*

Because BSLT is a general acute-toxicity screen in Artemia nauplii over a fixed exposure period, the LC_{50} pattern is typically driven by combined effects of membrane interaction, neurotoxicity, and broad metabolic disruption rather than any single target mechanism. The ethanol extract's strong phytochemical breadth offers a chemically plausible basis for stronger bioactivity in BSLT, since multiple metabolite classes can contribute additively or synergistically to lethality in a whole-organism model. Chloroform's near-threshold LC_{50} combined with narrow qualitative positivity suggests limited recovery of the polar and mid-polar classes that frequently underlie cytotoxic and redox-linked bioactivities in crude plant extracts. The use of a ≤1% DMSO vehicle is consistent with common BSLT practice intended to avoid confounding solvent toxicity while still enabling dissolution of less polar extracts (Nerdy et al., 2021). Finally, when zero mortality occurs at low doses (or complete mortality at high doses), probit workflows commonly apply Finney-style corrections of 0% mortality = 100(0.25/n) and 100% mortality = 100(n − 0.25)/n prior to probit conversion, which corresponds to 0.83% and 99.17% when n = 30 (Adeshina et al., 2024; Paray et al., 2025; Tadee et al., 2023).

Normality testing for LC_{50} indicates substantial deviation from normality in multiple groups (Shapiro–Wilk $p = 0.000$ is reported for several extracts), which justifies a nonparametric approach for group comparison. Under those conditions, the Kruskal–Wallis H test is an appropriate omnibus test for group differences based on ranks rather than means, especially when distributional assumptions are violated. The Kruskal–Wallis result rejects equality of medians ($p = 0.0031$), confirming that at least one solvent group differs in LC_{50} distribution. Dunn's pairwise post-hoc testing with multiplicity adjustment is a standard follow-up to localize differences after a significant Kruskal–Wallis result (Maheshwaran et al., 2024). After adjustment, only chloroform versus ethanol remains significant (adjusted $p = 0.008$), which is consistent with the largest observed separation between the weakest and strongest LC_{50} values. The lack of additional significant pairs after correction is interpretable as a combined consequence of small per-group replication and the conservative nature of familywise error control under multiple comparisons.

The combined IC_{50}–LC_{50} pattern places ethanol as the most consistent primary extract in maximizing functional activity while demonstrating strong bioactivity in toxicity

screening, while hexane is more rationally positioned as a defatting/lipophilic step due to its high yield but relatively weak DPPH activity and toxicity (Lima-Pereira et al., 2025; Nurcholis et al., 2023).

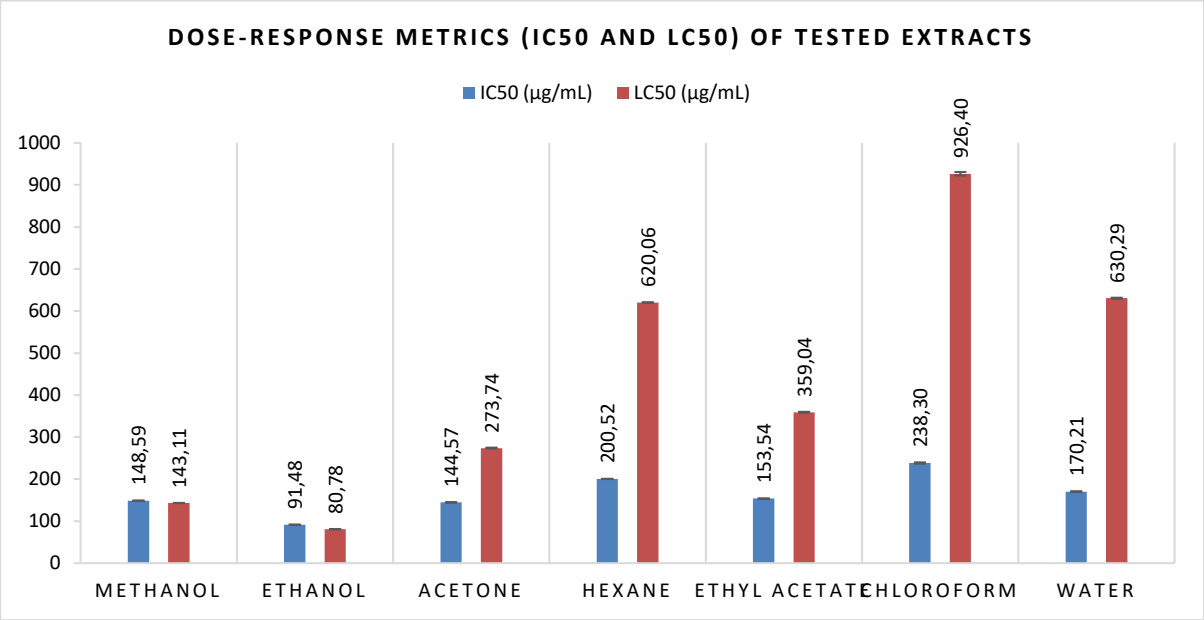

Figure 3. Dosage-response metrics (IC_{50} and LC_{50}) of tested extracts.

Acetone and ethyl acetate remain strategic as orthogonal solvents for further partitioning because they tend to narrow chemical complexity and can help separate fast-reactive fractions against DPPH from less active bulk fractions, although the potential of the crude extract is not always maximized (Barbouchi et al., 2024; Džarić et al., 2025). With statistically significant LC_{50} differences primarily between chloroform and ethanol, the most scientifically defensible next step is to proceed with activity-guided fractionation of the ethanol extract (and orthogonal acetone/ethyl acetate candidates), followed by bioactivity validation in relevant cell models, as BSLT is a general screening tool and does not replace specific mechanistic or cytotoxic assays.

CONCLUSIONS

Ethanol appears as the most effective solvent for *T. siamensis*, combining a solid extraction yield (7.78%) with the richest phytochemical profile (positive for alkaloids, flavonoids, saponins, tannins, and phenolics). This broad chemical recovery corresponds to the strongest bioactivity, shown by its lowest IC_{50} (91.48 ± 0.12 µg/mL) and LC_{50} (80.78 ± 0.62 µg/mL). Acetone (6.42%) and ethyl acetate (5.11%) offer moderate yields and selective enrichment of semi-polar constituents, reflected in intermediate IC_{50} values (144–150 µg/mL) and LC_{50} values (273–350 µg/mL). In contrast, hexane despite providing the highest mass recovery (11.34%) contains mainly non-phenolic components and shows weak biological activity (IC_{50} 200.52 ± 0.38 µg/mL, LC_{50} 620.06 ± 0.76 µg/mL). Chloroform, with limited phytochemical content, yields the poorest performance (IC_{50} 238.30 ± 1.56 µg/mL, LC_{50} 926.40 ± 4.27 µg/mL). Collectively, these data confirm ethanol as the most reliable solvent for maximizing functional activity and justify its use in future bioactivity-guided isolation

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