



Indones. J. Chem. Stud.
2026, 5(1), 31–36
Available online at journal.solusiriset.com
e-ISSN: 2830-7658; p-ISSN: 2830-778X

Indonesian
Journal of
Chemical Studies

Cytotoxic Evaluation of Medicinal Plants used by Suku Anak Dalam Community in Jambi Province Using the Brine Shrimp Lethality Test

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Received: 14 Dec 2025; Revised: 22 Apr 2026; Accepted: 25 Apr 2026;
Published online: 22 Jun 2026; Published regularly: 30 Jun 2026

Abstract— Cancer remains a major global health challenge, and the search for effective therapeutic agents continues. Natural products, including traditional medicinal plants used by the Suku Anak Dalam (SAD) community in Jambi, offer promising sources of bioactive compounds. This study investigated the cytotoxic potential of ten SAD medicinal plants extracted through a decoction method. Phytochemical screening revealed the presence of alkaloids, flavonoids, and saponins. Cytotoxicity was assessed using the Brine Shrimp Lethality Test (BSLT) at concentrations of 50, 100, 500, and 1000 ppm. All plant extracts exhibited toxic-level cytotoxicity, which correlated with their secondary metabolite profiles and their inhibitory effects on *Artemia salina* Leach larvae. The LC₅₀ values ranged from 150 to 400 ppm, indicating that these traditional medicinal plants possess notable cytotoxic activity. These findings support the potential of SAD ethnomedicinal plants as candidates for further investigation in anticancer research.

Keywords— BSLT; Cytotoxicity; Medicinal plants; Suku Anak Dalam.

1. INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality worldwide, with incidence rates continuing to rise across both developed and developing nations [1]. Despite major advances in modern oncology, the search for effective, affordable, and safer anticancer agents continues to be a global priority. Many conventional chemotherapeutic agents are associated with high toxicity, limited selectivity, and the development of drug resistance, prompting researchers to explore alternative sources for anticancer drug discovery [1,2]. Among these, natural products from plants have provided some of the most successful and widely used anticancer compounds in clinical practice.

Medicinal plants have long been utilized for therapeutic purposes by indigenous communities, who possess deep knowledge of the healing properties of local flora. Ethnobotanical practices often serve as an important starting point for the discovery of novel bioactive compounds [3]. Over 60% of currently approved anticancer drugs can trace their origins, directly or indirectly, to natural products. This underscores the relevance of systematically investigating traditional medicinal plants to identify

species with potential cytotoxic or anticancer activities [4].

Indonesia, one of the world's megadiverse countries, harbors an exceptionally rich variety of plant species, many of which remain understudied. Indigenous groups across Indonesia have maintained traditional knowledge of medicinal plants for generations, yet scientific validation of these practices is often lacking. One such indigenous group is the Suku Anak Dalam (SAD), a semi-nomadic ethnic community residing in the lowland forests of Jambi Province, Sumatera. Their traditional medical practices rely heavily on local plant species, which are believed to treat various ailments including inflammation, infections, poisoning, and symptoms potentially linked to malignancies [5-7].

Despite the cultural and medicinal importance of these plants, there is limited scientific literature investigating the biological activities of medicinal species used by the SAD community. This gap highlights the need for systematic pharmacological evaluation to validate traditional knowledge and identify novel candidates for further biomedical research. Phytochemical screening and cytotoxicity assays can

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DOI: [10.55749/ijcs.v5i1.146](https://doi.org/10.55749/ijcs.v5i1.146)



offer initial insight into the therapeutic potential of these traditionally used plants.

The Brine Shrimp Lethality Test (BSLT) is a simple, rapid, and cost-effective bioassay widely used as a preliminary screening method to assess the cytotoxic potential of plant extracts. This method has shown good correlation with cytotoxic and antitumor activities in more complex biological systems. Its reliability and sensitivity make it a suitable first-line assay for evaluating plant extracts that may contain bioactive compounds with antiproliferative or toxic properties [8,9].

Phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, and terpenoids are frequently implicated in the cytotoxic effects of plant extracts. These secondary metabolites can induce apoptosis, disrupt cell division, or interfere with cellular metabolic pathways, contributing to their potential anticancer mechanisms. Understanding the phytochemical profiles of SAD medicinal plants, therefore, provides valuable context for interpreting their cytotoxic activities [10,4].

The traditional preparation of medicinal plants by the Suku Anak Dalam commonly involves decoction, a method believed to extract bioactive compounds effectively. Scientific validation of the cytotoxic activity of decoction-based extracts is crucial, as it reflects the form in which these plants are actually consumed by the community. Evaluating extracts prepared with traditional methods ensures ethnopharmacological relevance and aligns laboratory findings with real-world indigenous practices [11,12].

Given the increasing interest in natural anticancer agents, it is essential to evaluate plant species used by indigenous communities not only to support the preservation of traditional knowledge but also to contribute to the global search for new therapeutic leads. Documenting and analyzing the biological properties of these plants may also encourage conservation efforts and sustainable management of local biodiversity.

Therefore, the objective of this study is to evaluate the cytotoxic activity of ten medicinal plants traditionally used by the Suku Anak Dalam community in Jambi Province using the Brine Shrimp Lethality Test (BSLT), and to correlate their cytotoxicity with the presence of secondary metabolites identified through phytochemical screening. This research aims to provide scientific evidence supporting the traditional use of these plants and to identify species with potential for further investigation in anticancer drug development.

2. EXPERIMENTAL SECTION

This study is a quantitative research with an experimental design, involving several test groups of ten traditional medicinal plants. The study aimed to evaluate both the chemical content of the plants and their cytotoxic effects on brine shrimp larvae. The research was conducted in multiple laboratories, including the Biomedical Laboratory of the Faculty of Medicine and Health Sciences, Jambi University, the Biology Laboratory of the Pharmacy Study Program at the

Harapan Ibu Health Sciences College in Jambi, and the Plant Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung.

2.1. Materials

Ten medicinal plant species used by the Suku Anak Dalam, collected from Batanghari and Tebo districts in Jambi Province, include *Akar Kuning*, *Dedaup*, *Mampot*, *Maribungan*, *Mejan*, *Melati*, *Pecut Kuda*, *Pendungurat*, *Penyegar*, and *Selusuh*. These plants were selected based on ethnobotanical surveys and interviews with traditional healers. Fresh plant parts (leaves, stems, bark, or roots, depending on traditional use) were collected from forest areas inhabited by the SAD community. Each specimen was taxonomically identified by a botanist, and voucher samples were deposited in a local herbarium for reference. All plant materials were washed, cut into small pieces, and air-dried at room temperature before extraction. Brine shrimp (*Artemia salina* Leach) eggs for cytotoxic test.

Meanwhile, the reagents and solvents used included: distilled water, Sea Salt, HNO₃ (99% purity Merck, Germany), KI (99% purity Merck, Germany), (Bi(NO₃)₃·5H₂O) (99% purity Merck, Germany), HgCl₂ (99% purity Merck, Germany), I₂ (99% purity Merck, Germany), HCl (99% purity Merck, Germany), C₂H₅OH (99% purity Merck, Germany), CHCl₃ (99% purity Merck, Germany), H₂SO₄ (99% purity Merck, Germany), DMSO (99% purity Merck, Germany), K₂Cr₂O₇ (99% purity Merck, Germany).

2.2. Preparation of Decoction Extracts

Extraction was performed using the traditional decoction method to reflect authentic indigenous preparation practices. Dried plant material (approximately 20–30 g per sample) was boiled in 200–300 mL of distilled water for 30 minutes. The resulting decoction was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated to dryness using a water bath at 50–60°C. Dried extracts were stored in airtight containers at 4°C until further analysis [13–15].

2.3. Phytochemical Screening

Preliminary phytochemical screening of each extract was conducted using standard qualitative methods to detect major secondary metabolite groups. Tests were performed for alkaloids (Mayer's, Wagner, and Dragendorff's reagents), flavonoids (Shinoda test), saponins (froth test), tannins (ferric chloride test), terpenoids (Salkowski test), and phenolics. The presence or absence of each metabolite class was recorded [16–18].

2.4. Hatching of *Artemia salina*

Brine Shrimp Lethality Test (BSLT) was initially by incubation of brine shrimp (*Artemia salina* Leach) eggs in artificial seawater prepared by dissolving 38 g of sea

salt in 1 L of distilled water. Eggs were placed under continuous aeration and exposed to a light source for 24–48 hours to allow hatching. Nauplii at the active swimming stage were used for testing [19–21].

2.5. Toxicity Assay Procedure

Stock solutions of each extract were prepared by dissolving dried extract in a small amount of DMSO and diluting with seawater to obtain final concentrations of 50, 100, 500, and 1000 ppm. The final concentration of DMSO did not exceed 1% to avoid toxicity interference [21].

For each concentration, 10 nauplii were transferred into test tubes containing 5 mL of prepared extract solution. A seawater solution containing 1% DMSO served as the negative control, while potassium dichromate ($K_2Cr_2O_7$) was used as the positive control. All treatments were prepared in triplicate. Test tubes were maintained under constant illumination for 24 hours [22].

2.6. Determination of Mortality and LC_{50}

After 24 h of exposure, the number of surviving nauplii was counted, and percent mortality was calculated. LC_{50} values (lethal concentration for 50% mortality) were determined using probit analysis. Extracts were categorized as highly toxic ($LC_{50} < 100$ ppm), toxic (100–500 ppm), moderately toxic (500–1000 ppm), or non-toxic (>1000 ppm) [22].

Table 1. Results of medicinal plants sample determination

No	Traditional name of Medicinal Plants	Species	Family
1.	Akar Kuning	<i>Arcangelisia flava</i> (L.) Merr.	Menispermaceae
2.	Bungo Bejuntai/Pecut Kuda	<i>Stachytarpheta jamaicensis</i> (L.) Vahl	Verbenaceae
3.	Dedaup	<i>Bauhinia variegata</i> L.	Fabaceae
4.	Mampot	<i>Garcinia</i> sp.	Clusiaceae
5.	Maribungan	<i>Callerya atropurpurea</i> (Wall.) Schot	Fabaceae
6.	Mejan	<i>Alyxia stellata</i> Roem. & Schult	Apocynaceae
7.	Melati Hutan	<i>Jasminum sambac</i> (L.) Aiton	Oleaceae
8.	Pendungurat	<i>Arcangelisia gusalung</i> H.S.Lo	Menispermaceae
9.	Penyegar	<i>Dioscorea transversa</i> R.Br.	Dioscoreaceae
10.	Selusuh	<i>Luvunga eleutherandra</i> Dalzell	Rutaceae

Table 2. Phytochemical screening of traditional medicinal plants

No	Traditional medicinal plants	Secondary metabolites							
		Alkaloid			Flavo-noids	Steroids	Saponin	Phenolic	Tannin
		Mayer	Wagner	Dragendorff					
1.	Akar Kuning (<i>Arcangelisia flava</i> (L.) Merr)	+	+	+	+	+	+	-	-
2.	Dedaup (<i>Bauhinia variegata</i> L.)	-	-	+	+	-	+	+	-
3.	Mampot (<i>Garcinia</i> sp)	-	-	-	+	+	-	+	-
4.	Maribungan (<i>Callerya atropurpurea</i> (Wall.) Schot)	-	-	-	+	+	+	-	-
5.	Mejan (<i>Alyxia stellata</i> Roem. & Schult)	-	-	+	+	-	-	-	-
6.	Melati (<i>Jasminum sambac</i> (L.) Aiton)	-	+	+	+	+	+	-	-
7.	PecutKuda (<i>Stachytarpheta jamaicensis</i> (L.) Vahl)	+	+	-	+	-	+	+	-
8.	Pendungurat (<i>Arcangelisia gusalung</i> H.S.Lo)	-	+	+	+	-	+	-	-
9.	Penyegar (<i>Dioscorea transversa</i> R.Br)	-	-	+	+	-	+	-	-
10.	Selusuh (<i>Luvunga eleutherandra</i> Dalzell)	+	+	-	+	+	+	-	-

Description : + (contains secondary metabolites); - (does not contains secondary metabolites)

2.7. Data Analysis

All experimental data were expressed as description. Probit analysis for LC_{50} determination was performed using linear line equation. Correlations between cytotoxicity and phytochemical content were analyzed qualitatively.

3. RESULT AND DISCUSSION

3.1. Phytochemical Screening

Phytochemical profiling of the ten traditionally used medicinal plants (**Table 1**) revealed the presence of diverse secondary metabolites, including alkaloids, flavonoids, saponins, steroids, phenolics, and tannins. *Arcangelisia flava*, *Stachytarpheta jamaicensis*, and *Luvunga eleutherandra* exhibited the broadest metabolite spectrum, particularly showing strong signals for alkaloids, flavonoids, and saponins, which are plants such as *Alyxia stellata* and *Bauhinia variegata* expressed more selective metabolite profiles, with limited or no alkaloid content. These phytochemical variations suggest differing biochemical potentials that may influence cytotoxicity outcomes.

The presence of key secondary metabolites such as alkaloids, flavonoids, and saponins in several of the studied plants (**Table 2**) provides strong biochemical justification for their observed cytotoxic properties.

Table 3. Regression equation values for each experimental group

No	Group	Linear regression	R ² value
1	Negative control (seawater solution containing < 1% DMSO)	y = 3.9400x - 1.0500	0.96
2	Positive control (potassium dichromate (K ₂ Cr ₂ O ₇))	y = 4.9500x - 1.1000	0.94
3	Akar Kuning (<i>Arcangelisia flava</i> (L.) Merr)	y = 4.8853x - 7.1705	0.78
4	Dedaup (<i>Bauhinia variegata</i> L.)	y = 3.0280x - 2.5033	0.61
5	Mampot (<i>Garcinia sp</i>)	y = 3.0216x - 2.3182	0.66
6	Maribungan (<i>Callerya atropurpurea</i> (Wall.) Schot)	y = 4.8243x - 7.4546	0.77
7	Mejan (<i>Alyxiastellata</i> Roem. & Schult)	y = 3.1050x - 2.5244	0.69
8	Melati (<i>Jasminum sambac</i> (L.) Aiton)	y = 2.7573x - 1.4722	0.63
9	PecutKuda (<i>Stachytarpheta jamaicensis</i> (L.) Vahl)	y = 5.0651x - 8.1331	0.83
10	Pendungurat (<i>Arcangelisia gusanlung</i> H.S.La)	y = 2.9638x - 2.2273	0.62
11	Penyegar (<i>Dioscorea transversa</i> R.Br)	y = 3.2431x - 2.5763	0.81
12	Selusuh (<i>Luvungaeleutherandra</i> Dalzell)	y = 3.3394x - 2.6727	0.88

Table 4. The result of LC₅₀ value in traditional medicinal plants sample

Sample of traditional medicinal plants	Concentration (ppm)	Log concentration (x)	Mortality	Probit value (y)	LC ₅₀ (ppm)
Negative control (Seawater + DMSO)	0.00	0.00	0.00	0.00	9.12 x 10 ¹²
	0.25	-0.60	0.00	2.67	
	0.50	-0.30	2%	3.55	
	1.00	0.00	5%	3.36	
	2.50	0.34	10%	3.72	
Positive control (potassium dichromate (K ₂ Cr ₂ O ₇))	5	0.69	23%	3.55	16.98
	10	1.00	53%	3.75	
	25	1.39	87%	3.86	
	50	1.69	100%	3.77	
	100	2.00	100%	3.77	
Akar Kuning (<i>Arcangelisia flava</i> (L.) Merr)	50	1.69	0%	0.00	309.92
	100	2.00	30%	4.48	
	500	2.69	37%	4.67	
	1000	3.00	100%	8.09	
Dedaup (<i>Bauhinia variegata</i> L.)	50	1.69	3%	3.12	300.59
	100	2.00	7%	3.52	
	500	2.69	10%	3.72	
	1000	3.00	100%	8.09	
Mampot (<i>Garcinia sp</i>)	50	1.69	3%	3.12	264.22
	100	2.00	13%	3.87	
	500	2.69	17%	4.05	
	1000	3.00	100%	8.09	
Maribungan (<i>Callerya atropurpurea</i> (Wall.) Schot)	50	1.69	0%	0.00	381.63
	100	2.00	10%	3.72	
	500	2.69	10%	3.72	
	1000	3.00	100%	8.09	
Mejan (<i>Alyxia stellata</i> Roem. & Schult)	50	1.69	3%	3.12	265.04
	100	2.00	10%	3.72	
	500	2.69	20%	4.16	
	1000	3.00	100%	8.09	
Melati (<i>Jasminum sambac</i> (L.) Aiton)	50	1.69	7%	3.52	222.48
	100	2.00	20%	4.16	
	500	2.69	23%	4.26	
	1000	3.00	100%	8.09	
Pecut Kuda (<i>Stachytarpheta jamaicensis</i> (L.) Vahl)	50	1.69	0%	0.00	391.62
	100	2.00	3%	3.12	
	500	2.69	13%	3.87	
	1000	3.00	100%	8.09	
Pendungurat (<i>Arcangelisia gusanlung</i> H.S.La)	50	1.69	3%	3.12	274.49
	100	2.00	13%	3.87	
	500	2.69	13%	3.87	
	1000	3.00	100%	8.09	
Penyegar (<i>Dioscorea transversa</i> R.Br)	50	1.69	3%	3.12	216.83
	100	2.00	17%	4.05	
	500	2.69	47%	4.92	
	1000	3.00	100%	8.09	
Selusuh (<i>Luvunga eleutherandra</i> Dalzell)	50	1.69	3%	3.12	198.44
	100	2.00	20%	4.16	
	500	2.69	63%	5.33	
	1000	3.00	100%	8.09	

Alkaloids, for example, are well-known for their DNA-interacting and pro-apoptotic mechanisms [23], frequently contributing to anticancer and cytotoxic activities. The high alkaloid content found in *Arcangelisia flava*, *Stachytarpheta jamaicensis*, and *Luvunga eleutherandra* may therefore explain their notable bioactivity. Previous studies have demonstrated similar observations, with alkaloid-rich species from Menispermaceae and Rutaceae families showing significant toxicity in BSLT assays due to isoquinoline- and quinolone-derived structures capable of disrupting cellular metabolism [24-25]. Flavonoids and phenolics, also widely expressed in the tested plants, contribute to cytotoxic activity through oxidative stress induction, enzyme inhibition, and modulation of signaling pathways [24,26]. Their presence in *Jasminum sambac* and *Dioscorea transversa* may have played a substantial role in producing their relatively low LC₅₀ values. Saponins, detected in the majority of samples, are known for membrane-lytic properties that can lead to nauplii mortality in the BSLT model.

3.2. Cytotoxic Activity

To determine the IC₅₀ value for each treatment, it is preceded by calculating the linear regression (**Table 3**). Based on the **Table 4**, The strongest cytotoxicity was observed in *Luvunga eleutherandra*, *Dioscorea transversa*, and *Jasminum sambac* (LC₅₀ < 250 ppm). These LC₅₀ values indicate robust cytotoxic potential—comparable to previously reported values for plant-derived extracts considered promising candidates for anticancer screening. Extracts with LC₅₀ values between 250–350 ppm, including *Alyxia stellata* and *Garcinia sp.*, fall into the category of moderate toxicity but remain biologically relevant, as BSLT is sensitive to broad-spectrum biochemical stress.

The lower toxicity of *Stachytarpheta jamaicensis* and *Callerya atropurpurea* (LC₅₀ > 380 ppm) may be attributable to their limited metabolite diversity or lower concentrations of active compounds. Interestingly, although *Stachytarpheta jamaicensis* displayed a wide range of metabolites including alkaloids and flavonoids, its bioactivity remained modest. This discrepancy suggests that the relative abundance, specific chemical subclasses, or synergistic interactions ultimately determine the cytotoxic outcome.

The cytotoxicity range observed in this study aligns with common BSLT profiles for crude plant extracts, where LC₅₀ values between 100–500 ppm often correlate with potential anticancer effects in subsequent in vitro assays. Multiple studies have validated BSLT as a reliable preliminary screening tool, demonstrating strong correlation between low LC₅₀ values and later cytotoxicity in mammalian cell lines. The notable potency of *Luvunga eleutherandra* and *Dioscorea transversa* therefore warrants further mechanistic and fractionation studies to identify the specific active constituents.

The results substantiate the traditional knowledge surrounding these plants and support their

ethnomedicinal use, particularly those historically employed for inflammatory or tumor-related conditions. Plants with LC₅₀ values below 250 ppm are strong candidates for further phytochemical isolation, structural characterization, and in vitro anticancer assays. Additionally, the detectable bioactivity of all ten samples indicates that local medicinal flora may represent an underexplored source of novel bioactive compounds.

While BSLT provides a rapid and cost-effective toxicity assessment, it does not differentiate between cytotoxic, neurotoxic, or other lethal mechanisms. Therefore, bioassay-guided fractionation and mechanistic cellular studies (e.g., apoptosis, ROS assays) are recommended to clarify modes of action. Variations in extraction protocols, plant maturity, and environmental factors may also influence metabolite profiles and should be standardized in future studies.

4. CONCLUSION

All plant extracts tested showed cytotoxic activity in the Brine Shrimp Lethality Test, with *Luvunga eleutherandra*, *Dioscorea transversa*, and *Jasminum sambac* demonstrating the greatest potency. The relatively low LC₅₀ values observed in several extracts may be associated with the presence of secondary metabolites such as alkaloids, flavonoids, and saponins based on qualitative phytochemical screening. These compounds are known to exhibit bioactive properties and may contribute to the observed toxicity. These findings support the ethnomedicinal relevance of the studied species and indicate their potential as sources of bioactive compounds. Further purification and mechanistic studies are recommended to confirm their therapeutic prospects.

5. AUTHOR'S DECLARATION

5.1. Supporting Information

This manuscript does not include supplementary supporting information. However, all data underlying the findings of this study are available from the corresponding author upon reasonable request.

5.2. Acknowledgements

We would like to express our gratitude to the Lembaga Penelitian dan Pengabdian kepada Masyarakat Universitas Jambi (LPPM), for providing financial support for this research through the 2025 PNB Grant of the Faculty of Medicine and Health Sciences, Universitas Jambi under research contract number 336/UN21.11/PT.01.05/SPK/2025., and thank you to the pharmaceutical microbiology laboratory of the Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi.

5.3. Conflict of Interest

The authors declare that they have no conflict of interest.

5.4. Author Contributions

SP, NKS, MP, RO, LA, DK, ESS, F contributed equally to this research. The study was jointly designed and carried out by all listed authors, who also supervised the characterization process, writing early manuscript, and revising the manuscript. All authors have reviewed and approved the final version of this manuscript.

5.5. AI Statement

ChatGPT was utilized to enhance the clarity, grammar, and overall readability of this manuscript. All technical content, data interpretation, and conclusion were solely developed and verified by the authors. The final version of the manuscript was thoroughly reviewed to ensure accuracy, coherence, and alignment with the study's findings.

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