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## Antioxidant and Cytotoxic Activities of Postpartum Herbal Remedies Use in Aceh, Indonesia

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### Abstract

Traditional medicinal plants continue to play an important role in postpartum care in Aceh, Indonesia, yet their pharmacological properties remain insufficiently documented. This study examined the phytochemical composition, antioxidant capacity, and cytotoxic profile of five commonly used postpartum plants: *Glochidion zeylanicum* var. *zeylanicum*, *Glochidion obscurum*, *Mesua ferrea*, *Vitex pinnata*, and *Salix babylonica*. Ethanolic leaf extracts were prepared by macerating the leaves and analyzed for major secondary metabolites. Antioxidant activity was evaluated using the DPPH radical scavenging assay, while cytotoxicity was assessed through the brine shrimp lethality test (BSLT). All extracts contained flavonoids, phenolics, tannins, and terpenoids, with alkaloids detected in most species. *G. obscurum* showed the strongest antioxidant activity ( $IC_{50} = 2.30 \mu\text{g/mL}$ ), followed by *M. ferrea*, *V. pinnata*, and *G. zeylanicum*, whereas *S. babylonica* exhibited moderate activity. BSLT results indicated high cytotoxicity in *M. ferrea*, *V. pinnata*, and *S. babylonica* ( $LC_{50} < 10 \mu\text{g/mL}$ ), while *G. obscurum* and *G. zeylanicum* demonstrated moderate toxicity. These findings provide scientific support for the traditional postpartum use of these plants, particularly regarding antioxidant and restorative functions. However, the strong cytotoxicity observed in several species highlights the need for cautious dosage and further toxicological validation. Additional studies are recommended to isolate active constituents and clarify their safety and therapeutic relevance.



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### 1. Introduction

Medicinal plants have historically been among the principal therapeutic resources in traditional medical systems across diverse cultural settings, particularly for

the care of women during and after childbirth [1]. In many regions of Southeast Asia, including Indonesia, the administration of herbal preparations during the postpartum period is regarded as essential for restoring physical vitality, regulating internal physiological balance,

and preventing complications associated with childbirth [2, 3]. Such treatments aim to promote uterine involution, enhance lactation, reduce postpartum pain and inflammation, and support overall maternal recovery. In the province of Aceh, Indonesia, these practices are deeply rooted in cultural identity and have been preserved through longstanding oral transmission among traditional birth attendants (*Mak bidan*), family matriarchs, and local healers [4]. The continued use of these remedies reflects both the cultural practices of postpartum phytotherapy in Aceh and, therefore, reflects both cultural continuity and the strength of plant-based therapies.

Despite their enduring ethnomedical importance, the scientific understanding of many postpartum medicinal plants used in Aceh remains limited. Numerous plant species are traditionally used for postpartum health, yet the biochemical and pharmacological bases underlying their therapeutic actions have not been systematically examined [5, 6]. This gap is noteworthy because postpartum recovery involves substantial hormonal fluctuations, metabolic stress, and immune modulation, all of which increase vulnerability to oxidative stress. Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the capacity of endogenous antioxidants to counteract them [7]. Excessive ROS can induce inflammation, impair tissue regeneration, and delay wound healing, thereby contributing to potential postpartum complications [8]. Previous research has demonstrated that plant-derived secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids, play indispensable roles in neutralizing ROS via hydrogen-atom and electron-transfer mechanisms, thereby restoring redox homeostasis [9, 10]. These observations underscore the importance of investigating the antioxidant potential of postpartum medicinal plants.

In addition to evaluating antioxidant properties, there is a pressing need to assess the safety profiles of traditionally used medicinal plants. Phytochemicals that confer therapeutic benefits may also exert cytotoxic effects, particularly when consumed as concentrated extracts or without proper dosing. The Brine Shrimp Lethality Test (BSLT) has therefore become a widely accepted preliminary assay for assessing general cytotoxicity and for identifying extracts that contain potent bioactive constituents, including compounds with potential therapeutic value [11–13]. However, any cytotoxic activity detected through BSLT must be interpreted cautiously in the context of postpartum use, where inappropriate dosing or preparation could pose safety concerns for mothers and infants.

This study focuses on five plant species traditionally employed in postpartum care in Aceh: *Glochidion zeylanicum* var. *zeylanicum*, *Glochidion obscurum*, *Mesua ferrea*, *Vitex pinnata*, and *Salix babylonica*. Information regarding the traditional use of these plants was obtained through interviews with traditional healers. Although many species were identified, these five were prioritized because they are considered important in postpartum practice yet remain among the least investigated in scientific literature. Their inclusion, therefore, enhances the research's novelty while addressing a substantial gap in current knowledge. Although these plants are commonly administered as decoctions, herbal baths, and topical preparations, empirical evidence supporting their phytochemical composition and biological activities remains scarce.

Accordingly, this study aims to (i) characterize the phytochemical constituents of these five postpartum medicinal plants, (ii) evaluate their antioxidant activity using the DPPH radical scavenging assay, and (iii) determine their cytotoxic potential through the Brine Shrimp Lethality Test. By linking ethnobotanical relevance with laboratory-based evaluation, this research seeks to provide an evidence-based foundation for traditional therapeutic use, identify potential safety considerations, and support the development of phytopharmaceutical candidates derived from postpartum medicinal plants of Aceh.

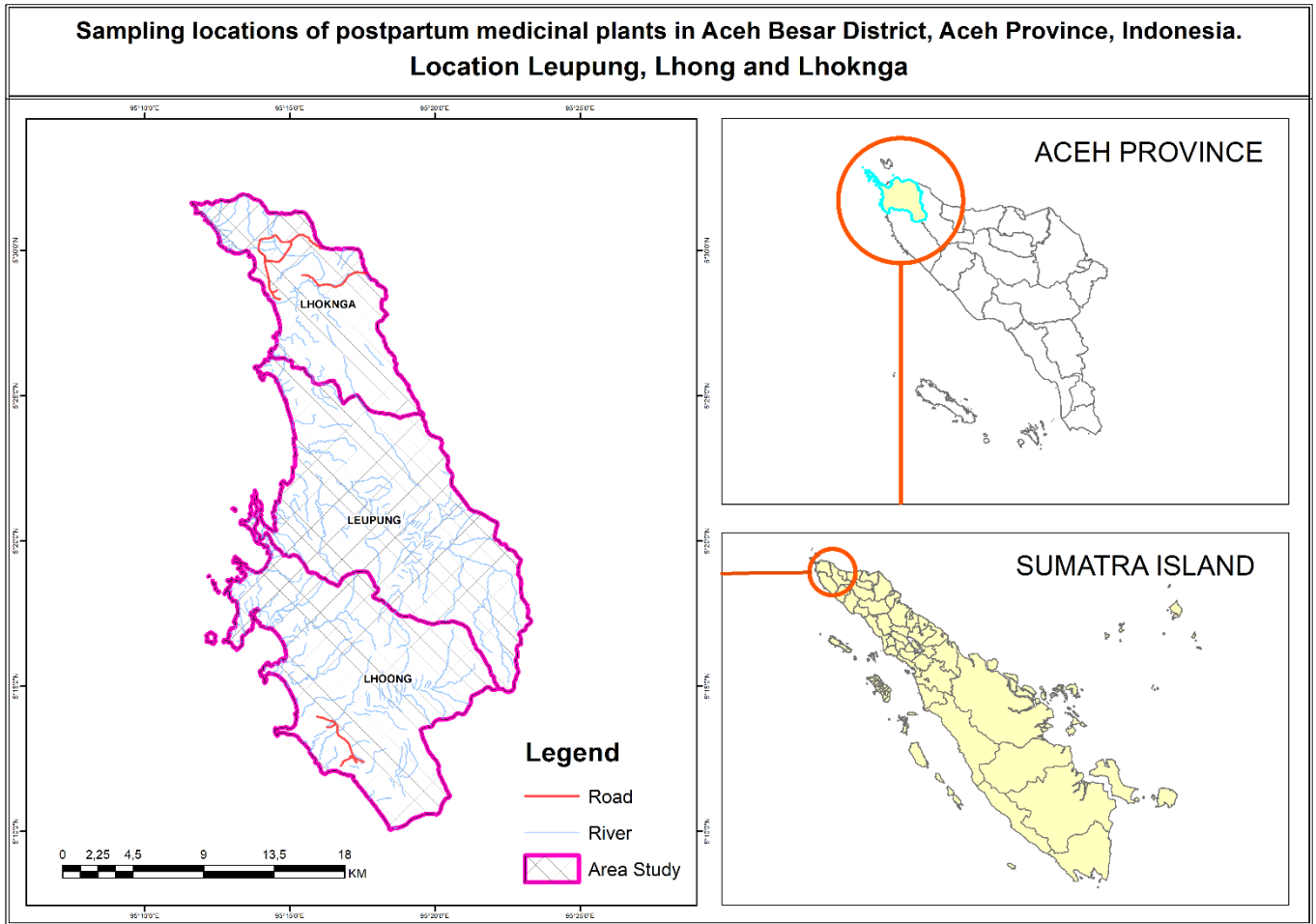
## 2. Materials and Methods

### 2.1. Study Area and Sample Collection

Plant samples were collected from three locations in Aceh Besar District, Aceh Province, Indonesia: Lhong, Lhoknga, and Leupung (Figure 1). These areas were selected because postpartum herbal practices remain strongly preserved, and traditional healers (*mak bidan*) continue to play central roles in transmitting ethnomedical knowledge. Sampling sites are presented in Fig. 1, which illustrates the geographic distribution of the collected postpartum medicinal plants in Aceh Besar. For each species, fresh leaves were collected from 3 different plants to ensure representation of natural variation and to avoid extraction from a single individual. The species collected were *Glochidion zeylanicum* var. *zeylanicum*, *Glochidion obscurum*, *Mesua ferrea*, *Vitex pinnata*, and *Salix babylonica*. Leaves were cleaned, air-dried for 7–10 days at room temperature, and ground into coarse powder before extraction.

### 2.2. Ethnobotanical Interviews and Plant Collection

Ethnobotanical information was obtained through structured interviews with five traditional healers (*mak*



**Figure 1.** Map showing the locations where postpartum medicinal plants were collected in Aceh Besar District, Aceh Province, Indonesia.

**Table 1.** Plant samples investigated in this study.

Scientific Name	Common Name	Part of Plant Analyzed
<i>Glochidion zeylanicum</i> var. <i>zeylanicum</i>	<i>On dolang</i>	Leaves
<i>Glochidion obscurum</i> (Roxb. Ex Willd.) Blume	<i>On dama beuso</i>	Leaves
<i>Mesua ferreai</i> L.	<i>Rancong buloh</i>	Leaves
<i>Vitex pinnata</i> L.	<i>On mane</i>	Leaves
<i>Salix babylonica</i> L.	<i>On jaloh</i>	Leaves

*bidan*) across different sub-districts in Aceh Besar. The interview guide included questions: (i) local plant names, (ii) parts used, (iii) preparation methods, and (iv) therapeutic indications. Species cited in interviews were subsequently collected and botanically authenticated at the Herbarium of Universitas Syiah Kuala. Taxonomic identification was confirmed under Ref. No. 1107/UN11.F8.4/TA.00.03/2024 based on morphological diagnostic characteristics, and all scientific names were validated using the accepted nomenclature listed in the GBIF global species database. The plant samples investigated in this study are shown in [Table 1](#).

### 2.3. Preparation of Plant Extracts

Fresh leaves were washed thoroughly with distilled water, air-dried at room temperature (25–28°C), and

pulverized into a fine powder. Approximately 200 g of powdered material from each species was extracted using 96% ethanol by maceration for 72 hours with intermittent agitation. The filtrates were collected and concentrated under reduced pressure at 40–45°C using a rotary evaporator, then dried in a desiccator to obtain crude extracts. Extraction yields for all species were calculated on a dry-weight basis and ranged from 0.08% to 0.12% (w/w). The macerates were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40–45°C. Extracting 200 g of dried plant material with 2 L of ethanol yielded 0.2 g of dried extract, corresponding to an extraction yield of 0.1%. The concentrated extracts were further dried in a desiccator to obtain crude extracts for analysis. For bioassays, crude

extracts were dissolved in methanol to prepare 10 mg/mL stock solutions, which were subsequently diluted to obtain the working concentrations for antioxidant and cytotoxicity assays.

#### 2.4. Phytochemical Screening

Qualitative phytochemical screening was performed following Tiwari et al. [14] to detect major secondary metabolites, including alkaloids, flavonoids, tannins, phenolics, saponins, and terpenoids/steroids. Alkaloids were detected using Dragendorff's, Mayer's, and Wagner's reagents; flavonoids were identified using the Shinoda and NaOH tests; tannins and phenolics were tested using FeCl<sub>3</sub>; saponins were confirmed through the foam test; and terpenoids/steroids were evaluated using the Liebermann–Burchard reaction.

#### 2.5. Antioxidant Activity (DPPH Assay)

The antioxidant activity of the plant extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, following the procedure described by Bello et al. [15] with slight modifications. The DPPH assay is based on the reduction of the purple DPPH radical to the yellow-colored diphenyl picrylhydrazine (DPPH-H) upon interaction with antioxidant compounds capable of donating hydrogen or electrons. This reaction is accompanied by a decrease in absorbance at 517 nm, indicating radical-scavenging capacity.

A 0.004% (w/v) DPPH solution was freshly prepared in methanol and stored in the dark before use. Each plant extract (*Glochidion zeylanicum* var. *zeylanicum*, *Glochidion obscurum*, *Mesua ferrea*, *Vitex pinnata*, and *Salix babylonica*) was dissolved in methanol to obtain concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. For each measurement, 1 mL of extract solution was mixed with 1 mL of the DPPH solution, and the final volume was adjusted to 5 mL with methanol. The mixtures were vortexed and incubated in the dark at 37°C for 30 minutes.

Ascorbic acid, prepared across the same concentration range, was used as the positive control, while a negative control was prepared by mixing 1 mL of DPPH solution with 4 mL of methanol. Both controls were processed under identical conditions, including incubation time, temperature, and sample-to-reagent volume ratio. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). All assays were performed in triplicate (n = 3). Radical scavenging activity was calculated using Equation (1):

$$\% \text{ Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad (1)$$

where  $A_{\text{control}}$  is the absorbance of the negative control, and  $A_{\text{sample}}$  is the absorbance of the test extract solution.

The IC<sub>50</sub> value, defined as the concentration required to inhibit 50% of DPPH radicals, was determined by linear regression of percentage inhibition versus extract concentration. All IC<sub>50</sub> calculations were performed in Microsoft Excel.

#### 2.6. Statistical Analysis

All antioxidant (DPPH) and cytotoxicity (BSLT) assays were conducted in triplicate (n = 3) for each concentration, and mean values were calculated for further analysis. IC<sub>50</sub> values were determined using linear regression, while LC<sub>50</sub> values were calculated using probit regression in IBM SPSS Statistics v.26. Statistical comparisons among plant species were performed using one-way ANOVA followed by Tukey's HSD post-hoc test to assess significant differences (p < 0.05).

#### 2.7. Brine Shrimp Lethality Test (BSLT)

##### 2.7.1. Hatching of *Artemia salina* Nauplii

The cytotoxic activity of the plant extracts was evaluated using the Brine Shrimp Lethality Test (BSLT) following the method of Meyer et al. [16] with minor modifications. *Artemia salina* eggs were in artificial hatched seawater prepared by dissolving 38 g of sea salt in 1 L of distilled water. The hatching chamber was continuously aerated and maintained under constant illumination at 25–28°C for 24–48 hours. After hatching, the phototactic nauplii were collected by directing a light source to one side of the container and transferring the larvae using a micropipette. The nauplii were rinsed three times with fresh seawater to remove egg shells and debris before use in the assay [13].

##### 2.7.2. Toxicity Bioassay

Crude extracts of *Glochidion zeylanicum* var. *zeylanicum*, *Glochidion obscurum*, *Mesua ferrea*, *Vitex pinnata*, and *Salix babylonica* were dissolved in a minimal amount of dimethyl sulfoxide (DMSO), and the volume was adjusted with seawater to obtain a 1000 ppm stock solution. The final DMSO concentration in all test solutions did not exceed 1% (v/v). Series dilutions were prepared to obtain test concentrations of 1000, 500, 100, 10, and 1 ppm.

For each concentration, 10 nauplii were transferred into glass test tubes containing 5 mL of the test solution. All treatments, including controls, were performed in triplicate (n = 3) and incubated under continuous illumination at room temperature (25–28°C) for 24 hours. After incubation, the number of dead nauplii was

**Table 2.** Qualitative phytochemical profile of postpartum medicinal plant extracts.

No.	Plant Species	Alkaloids	Flavonoids	Tannins	Saponins	Terpenoids / Steroids	Phenolic
1	<i>Glochidion zeylanicum</i> var. <i>zeylanicum</i>	++	+	+	-	+	+
2	<i>Glochidion obscurum</i> (Roxb. ex Willd.) Blume	+	+	+	+	++	+
3	<i>Mesua ferrea</i> L.	-	+	+	-	+	+
4	<i>Vitex pinnata</i> L.	+	++	+	-	+	+
5	<i>Salix babylonica</i> L.	-	+	+++	-	++	+

Note: (-) = Not detected reaction | (+) = weak reaction | (++) = Moderately reaction | (+++) = Strong reaction

recorded. Nauplii showing no movement during observation were considered dead.

The percentage mortality was calculated according to the modified Abbott's formula, as shown in Equation (2):

$$\% \text{ Mortality} = \left( \frac{\text{Number of dead nauplii}}{\text{Total number of nauplii}} \right) \times 100 \quad (2)$$

The LC<sub>50</sub> value, defined as the concentration required to kill 50% of the nauplii population, was determined using probit regression analysis based on the dose-response curve. Probit analysis was performed using Microsoft Excel. Extracts with LC<sub>50</sub> values < 100 µg/mL were classified as highly cytotoxic, those between 100–500 µg/mL moderately cytotoxic, and values > 500 µg/mL weakly cytotoxic [17].

### 3. Results and Discussion

#### 3.1. Phytochemical Screening

Phytochemical screening showed that all plant extracts contained phenolic compounds and tannins, while the presence of flavonoids, alkaloids, saponins, and terpenoids varied among species (Table 2). *Vitex pinnata* exhibited the strongest flavonoid expression, whereas *Salix babylonica* showed a predominance of tannins. *Glochidion obscurum* and *Mesua ferrea* demonstrated higher levels of terpenoids/steroids, suggesting enriched biosynthetic pathways associated with antioxidant and cytotoxic functions. The detection of these secondary metabolites aligns with the historical use of these plants in postpartum treatment to reduce inflammation, support wound healing, and restore physiological balance [18]. Phenolics and flavonoids are known for their ability to neutralize free radicals via hydrogen atom transfer (HAT) and single-electron transfer (SET), thereby contributing directly to their antioxidant properties. Meanwhile, terpenoids and alkaloids often contribute to cytotoxic and cell-death regulatory mechanisms, including apoptosis induction, which is relevant to anticancer pharmacology.

#### 3.2. Antioxidant Activity (DPPH Radical Scavenging Assay)

The antioxidant activity of the six postpartum medicinal plant species was assessed using the DPPH radical

scavenging assay at six concentration levels (100–3.125 µg/mL). All extracts demonstrated dose-dependent radical scavenging activity, indicating that their bioactive constituents can donate hydrogen atoms or electrons to neutralize free radicals (Table 3). Among the tested species, *Glochidion obscurum* exhibited the strongest antioxidant activity, with inhibition values ranging from 92.58% (100 µg/mL) to 51.92% (3.125 µg/mL) and an IC<sub>50</sub> of 2.38 µg/mL, placing it in the very strong antioxidant category. In contrast, *Salix babylonica* showed the weakest scavenging response, with inhibition values ranging from 92.22% to 0.758%, and the highest IC<sub>50</sub> (262.11 µg/mL). The remaining species, *Glochidion zeylanicum* var. *zeylanicum*, *Mesua ferrea*, and *Vitex pinnata*, exhibited moderate to strong activity, with IC<sub>50</sub> values of 188.05, 16.83, and 176.71 µg/mL, respectively. Ascorbic acid, used as a positive control, showed consistently high inhibition levels at all concentrations (IC<sub>50</sub> = 16.43 µg/mL).

The markedly superior radical scavenging capacity of *G. obscurum* is consistent with phytochemical reports describing a high abundance of ellagitannins, gallotannins, and catechin derivatives within the genus. These phenolic constituents contain multiple hydroxyl groups that serve as effective hydrogen and electron donors. At the same time, their conjugated ring systems allow for extensive resonance stabilization, enabling rapid and thermodynamically favorable DPPH neutralization [19, 20]. Likewise, the strong antioxidant performance of *M. ferrea* can be attributed to prenylated xanthenes such as mesuaferrin A, mesuaferrin B, and mesuol, which have been reported to activate the Nrf2/ARE pathway, enhance antioxidant enzyme expression (CAT, SOD, GPx), and suppress pro-inflammatory mediators [21–23]. This mechanistic profile helps explain the traditional use of *M. ferrea* in postpartum recovery, particularly for reducing inflammation and accelerating wound healing [24].

*Vitex pinnata* also demonstrated notable antioxidant potency, likely due to its richness in vitexicarpin, apigenin, luteolin derivatives, and other flavones known for strong single-electron transfer (SET) activity and inhibition of oxidative-stress-mediated signaling [25, 26].

**Table 3.** Antioxidant activity of postpartum plant extracts using the DPPH method.

Plant Species	Inhibition (%)						IC <sub>50</sub> (µg/mL)
	100 (µg/mL)	50 (µg/mL)	25 (µg/mL)	12,5 (µg/mL)	6,25 (µg/mL)	3.125 (µg/mL)	
<i>Glochidion zeylanicum</i> var. <i>zeylanicum</i>	81.08 <sup>b</sup>	74.41 <sup>b</sup>	54.75 <sup>c</sup>	42.50 <sup>c</sup>	23.83 <sup>b</sup>	15.91 <sup>b</sup>	188.05
<i>Glochidion obscurum</i> (Roxb ex Willd) Blume	92.58 <sup>a</sup>	89.51 <sup>a</sup>	82.09 <sup>a</sup>	73.29 <sup>a</sup>	60.93 <sup>a</sup>	51.92 <sup>a</sup>	2.38
<i>Mesua ferrea</i> L.	92.51 <sup>a</sup>	81.81 <sup>a</sup>	61.96 <sup>b</sup>	49.08 <sup>b</sup>	20.36 <sup>b</sup>	5.756 <sup>b</sup>	16.83
<i>Vitex pinnata</i> L.	91.68 <sup>b</sup>	75.80 <sup>b</sup>	60.27 <sup>b</sup>	40.20 <sup>b</sup>	22.14 <sup>b</sup>	9.925 <sup>b</sup>	176.71
<i>Salix babylonica</i> L.	92.22 <sup>c</sup>	64.36 <sup>c</sup>	43.88 <sup>d</sup>	25.87 <sup>d</sup>	10.99 <sup>c</sup>	0.758 <sup>c</sup>	262.11
Ascorbic Acid	95.60 <sup>a</sup>	79.43 <sup>a</sup>	64.46 <sup>a</sup>	47.77 <sup>a</sup>	18.73 <sup>c</sup>	5.756 <sup>c</sup>	16.43

Note: Different superscript letters denote statistically significant differences ( $p < 0.05$ ) among species at each concentration level.

These compounds may contribute to uterine tissue recovery after childbirth by preventing lipid peroxidation and supporting connective tissue stabilization [27]. In contrast, the moderate antioxidant activity of *S. babylonica* suggests that its traditional postpartum application may derive more from its salicylate-based analgesic and antipyretic properties than from direct free-radical scavenging, aligning with the known COX-2 inhibitory mechanisms of the willow family [28, 29].

Overall, the antioxidant profiles observed in this study strongly support the ethnomedicinal use of these species during postpartum recovery. The presence of high-potency phenolic and flavonoid metabolites provides a biochemical rationale for their traditional roles in reducing inflammation, improving circulation, enhancing tissue repair, and restoring physiological balance following childbirth. Moreover, the exceptionally strong antioxidant responses exhibited by *G. obscurum*, *M. ferrea*, and *V. pinnata* indicate broader pharmacological potential for managing oxidative-stress-related disorders, including chronic inflammation, metabolic dysregulation, and cancer.

### 3.3. Cytotoxicity (Brine Shrimp Lethality Test)

The Brine Shrimp Lethality Test (BSLT) revealed a clear concentration-dependent increase in nauplii mortality across all plant extracts ( $p < 0.05$ ). As shown in Table 4, each species demonstrated its highest lethality at 1000 and 500 ppm, with markedly lower mortality at the lowest concentrations (50–100 ppm). These findings are consistent with the established sensitivity of BSLT as a rapid screening tool for general bioactivity and toxicity in medicinal plants [14].

*Glochidion zeylanicum* var. *zeylanicum* exhibited mortality ranging from 33<sup>a</sup> at 250 ppm to 97<sup>a</sup> at 1000 ppm, indicating significant variation across concentrations. *Glochidion obscurum* showed a similar dose–response trend, with mortality increasing from 27% at 50 ppm to 77% at 1000 ppm. *Mesua ferrea* and *Vitex pinnata* exhibited very high lethality at upper concentrations (97<sup>a</sup>–

100<sup>a</sup> at 500–1000 ppm), while lethality declined considerably at lower concentrations. *Salix babylonica* showed moderate toxicity, ranging from 47<sup>c</sup> at 50–100 ppm to 97<sup>a</sup> at 1000 ppm. Tukey's HSD analysis confirmed that these differences were statistically significant within each species, as represented by the superscript letters.

Probit regression further distinguished the toxicity levels of the extracts. *V. pinnata* (LC<sub>50</sub> = 3.87 ppm), *M. ferrea* (LC<sub>50</sub> = 4.48 ppm), and *S. babylonica* (LC<sub>50</sub> = 5.32 ppm) were categorized as highly cytotoxic (LC<sub>50</sub> < 30 ppm) [11], while *G. zeylanicum* showed moderate-to-high toxicity (LC<sub>50</sub> = 13.61 ppm). *G. obscurum*, however, displayed moderate cytotoxicity (LC<sub>50</sub> = 54.90 ppm), consistent with a milder toxic profile compared to the other species.

The variations in cytotoxicity are consistent with the known phytochemical compositions of these plant species. *M. ferrea* contains prenylated xanthenes (mesuaferriin, mesuol) that have been widely documented for their strong redox activity and influence on cellular stress responses [21, 23, 24, 30, 31]. In *V. pinnata*, flavonoids such as casticin, luteolin, and vitexicarpin are known to modulate oxidative pathways and contribute to biological activity in [25–27]. The toxicity profile of *S. babylonica* aligns with its salicin-derived glycosides and condensed tannins, which exhibit antimicrobial and pro-oxidant effects [26, 27]. Thus, the high cytotoxicity seen in these three species is consistent with their phytochemical richness.

Interestingly, *G. obscurum*, although previously shown to possess strong antioxidant activity, exhibited only moderate cytotoxicity in the BSLT. This contrast highlights the functional distinction between antioxidant and cytotoxic phytochemicals: antioxidants primarily mitigate oxidative stress by scavenging ROS [7, 10], whereas cytotoxic compounds induce stress-mediated pathways in sensitive cells [32–35]. The lower toxicity of *G. obscurum* and *G. zeylanicum* at low-to-medium concentrations therefore supports their traditional use in postpartum recovery, where safety and restorative functions are prioritized [18].

**Table 4.** LC<sub>50</sub> values of plant extracts against *Artemia salina*.

No	Plant Species	Percent Death at 24 h (%)					LC <sub>50</sub> (ppm)
		50 (ppm)	100 (ppm)	250 (ppm)	500 (ppm)	1000 (ppm)	
1	<i>Glochidion zeylanicum</i> var. <i>zeylanicum</i>	40 <sup>bc</sup>	40 <sup>bc</sup>	33 <sup>c</sup>	80 <sup>ab</sup>	97 <sup>a</sup>	13.61
2	<i>Glochidion obscurum</i> (Roxb. ex Willd.) Blume	27 <sup>c</sup>	37 <sup>b</sup>	40 <sup>b</sup>	67 <sup>a</sup>	77 <sup>a</sup>	54.90
3	<i>Mesua ferrea</i> L.	37 <sup>c</sup>	53 <sup>c</sup>	80 <sup>b</sup>	97 <sup>a</sup>	100 <sup>a</sup>	4.48
4	<i>Vitex pinnata</i> L.	37 <sup>c</sup>	63 <sup>b</sup>	73 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	3.87
5	<i>Salix babylonica</i> L.	47 <sup>c</sup>	47 <sup>c</sup>	60 <sup>c</sup>	83 <sup>b</sup>	97 <sup>a</sup>	5.32

Different superscript letters within a row denote significant differences among concentrations ( $p < 0.05$ ).

These toxicity patterns have important implications for postpartum applications. Ethnobotanical records indicate that women commonly utilize these plants in decoctions, massage oils, herbal baths, and steam therapy during the recovery period [4]. Although traditional preparations are considerably more diluted than the laboratory concentrations tested here, the high toxicity observed in *M. ferrea*, *V. pinnata*, and *S. babylonica* indicates the need for cautious use, especially when oral consumption is involved [24, 36]. Potential risks include gastrointestinal irritation, hepatotoxicity, and possible transfer of phytochemicals to breastfeeding infants [5, 9]. Therefore, further toxicological assessments in mammalian cell lines or animal models are needed to determine safety thresholds.

Conversely, the moderate cytotoxicity and strong antioxidant characteristics of *G. obscurum* and *G. zeylanicum* suggest a more favorable safety profile for postpartum recovery. Their traditional use to reduce inflammation, promote tissue regeneration, and restore vitality aligns with their laboratory-assessed milder toxicity and documented phytochemistry [16, 18].

Overall, the BSLT results reveal substantial variability in toxicity across postpartum medicinal plants used in Aceh. Highly toxic species such as *M. ferrea*, *V. pinnata*, and *S. babylonica* contain potent bioactive constituents that merit further phytochemical isolation and controlled pharmacological evaluation. Meanwhile, *G. obscurum* and *G. zeylanicum* appear more appropriate for postpartum support due to their lower cytotoxicity and strong antioxidant capacity. Integrating ethnomedicinal knowledge with laboratory-based toxicity screening is therefore essential to ensuring both the therapeutic value and safety of postpartum herbal practices.

#### 4. Conclusions

This study provides an integrated phytochemical, antioxidant, and cytotoxic evaluation of five postpartum medicinal plants traditionally used in Aceh, Indonesia. All extracts contained secondary metabolites associated with biological activity; however, their safety profiles varied substantially. *Glochidion obscurum* and *Glochidion zeylanicum* showed strong antioxidant activity and only

moderate cytotoxicity, suggesting they may be comparatively safer for postpartum applications when used at traditional low concentrations. In contrast, *Mesua ferrea*, *Vitex pinnata*, and *Salix babylonica* demonstrated high cytotoxicity (LC<sub>50</sub> < 10 µg/mL), indicating that these species pose potential toxicity risks and should be used with extreme caution, particularly in orally consumed preparations. Therefore, the findings do not uniformly validate the traditional postpartum use of all plants studied. Instead, they highlight that some species may offer benefits through antioxidant activity, whereas others may require dosage adjustment, restricted use, or reconsideration in postpartum contexts due to their strong cytotoxicity. These results underscore the importance of safety assessment alongside ethnopharmacological knowledge, particularly for vulnerable groups such as postpartum women. Further research, especially dose–response studies, bioassay-guided fractionation, mechanistic toxicology, and animal-based safety evaluations, is essential before any of these plants can be recommended for standardized or clinical postpartum use. This study emphasizes the need to integrate traditional knowledge with evidence-based safety considerations to ensure that culturally important medicinal practices remain both effective and safe.

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