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# Comparative Evaluation of Acute Toxicity and Dose-Dependent Mortality of n-Hexane, Petroleum Ether, Ethanol, and Aqueous Leaf Fractions of *Sida acuta*

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## ABSTRACT

*Sida acuta* is widely used in traditional medicine for managing various ailments; however, the safety profile of its solvent fractions requires scientific evaluation to support its pharmacological and therapeutic relevance. This study investigated the comparative acute toxicity and dose-dependent mortality response of n-hexane, petroleum ether, ethanol and aqueous fractions of *Sida acuta* extracted in this order and sequence of fractionation starting from the highly non-polar to the polar fractions using Lorke's method. A total of 52 male rats were used across the four solvent-fraction treatment groups. The fractions were administered orally by gavage using an intubation cannula, and the animals were observed continuously during the first few hours after administration and periodically for 24 h for behavioural changes and mortality. Mortality data were used to determine the median lethal dose ($LD_{50}$), while dose-dependent mortality response was assessed from deaths recorded across increasing dose levels. The $LD_{50}$ values were 2154 mg/kg body weight for the n-hexane and ethanol fractions, 3808 mg/kg body weight for the petroleum ether fraction, and > 5000 mg/kg body weight for aqueous fraction. These findings show fraction-specific acute toxicity, with the aqueous fraction exhibiting the highest safety margin.

***Keywords****: Sida acuta*; acute toxicity; dose-dependent mortality response; $LD_{50}$; Lorke's method; solvent fractions

## Introduction

*Sida acuta* Burm. F. is a medicinal plant belonging to the family Malvaceae and is

widely used in traditional medicine for the management of several ailments. Members of the genus *Sida* have been reported to possess important ethnomedicinal, phytochemical, and pharmacological properties, which support their continued use in traditional healthcare systems. S*ida acuta*, in particular, has been traditionally used in the treatment of conditions such as ulcers, intestinal worms, diabetes, malaria and other fevers (Donkor *et al*., 2023). Its broad ethnomedicinal application has made it an important plant of interest in experimental pharmacology and natural product research (Jiménez-Ferrer *et al*., 2025). The therapeutic relevance of *S. acuta* has been associated with the presence of bioactive secondary metabolites, which may contribute to its reported medicinal effects and justify further scientific investigation into its pharmacological and toxicological properties (Karou *et al*., 2007; Jiménez-Ferrer *et al*., 2025). The ethanolic leaf extracts of *Ficus exasperata* and *Sida acuta* possess antidiabetic activity that may be linked to their phytochemical composition and bioactive properties (Ukogo *et al*., 2026). However, the traditional use of medicinal plants does not automatically guarantee their safety. Although plant-derived remedies are often perceived as natural and safe, they may contain bioactive constituents capable of producing adverse effects, especially when consumed at high doses or when prepared using methods that concentrate potentially toxic compounds. The evidence of improved biochemical parameters when administered singly or in combination with *Sida acuta* and *Ficus exasperata* support the safety and ethnomedicinal relevance of both plants (Ukogo *et al*., 2026). The safety of herbal preparations may also be influenced by factors such as plant part used, extraction method, dosage, duration of exposure, contamination, and possible herb-drug interactions. Therefore, experimental toxicological evaluation is necessary to establish the safety profile of medicinal plants before their therapeutic use can be fully supported scientifically. This is particularly important because the same phytochemicals responsible for beneficial biological activity may also produce harmful effects under certain conditions. Toxicity studies therefore provide essential baseline information on possible adverse effects, safe dose ranges, and the relative risk associated with plant extracts or fractions (Mugale *et al*., 2024).

The need for toxicity assessment becomes even more important when plant materials are separated into different solvent fractions. Solvent fractionation is a useful phytochemical approach because different solvents extract different classes of compounds based on their polarity and chemical affinity. Non-polar solvents tend to extract lipophilic constituents such as oils, fats, waxes, steroids, terpenoids, and other non-polar compounds, while polar solvents such as ethanol and water extract more polar constituents, including phenolics, flavonoids, glycosides, tannins, and other hydrophilic compounds. Consequently, solvent choice can influence not only the phytochemical composition of an extract but also its biological and toxicological effects. A fraction obtained with one solvent may therefore show a wider safety margin, while another fraction from the same plant may display greater toxicity due to the concentration of different bioactive or toxic constituents (Lee *et al*., 2024). Despite extensive reports on the pharmacological properties of *Sida acuta*, comparative toxicological data on its different solvent fractions remain scarce. A side-by-side evaluation of the n-hexane, petroleum ether, ethanol, and aqueous fractions is therefore necessary because these solvents are

expected to recover different groups of secondary metabolites. Although n-hexane and petroleum ether are both non-polar solvents with overlapping extraction properties, their inclusion in a sequential extraction process may still yield operationally distinct lipophilic fractions. N-hexane is a more defined hydrocarbon solvent, while petroleum ether is a mixture of low-boiling aliphatic hydrocarbons; therefore, both solvents may not extract exactly the same range or proportion of non-polar constituents. Comparing these fractions helps to determine whether differences in solvent extraction influence toxicity, mortality response, and $LD_{50}$ estimates.

Acute toxicity testing provides an important first-line assessment of the safety of medicinal plant preparations. It helps to identify immediate harmful effects following single-dose exposure and provides an estimate of the median lethal dose ($LD_{50}$), which is useful in classifying the relative toxicity of test substances. Mortality data obtained from acute toxicity studies can also be used to assess dose-dependent mortality patterns, showing how lethality changes with increasing dose levels. This is particularly useful when comparing different fractions of the same plant, as it helps to identify fractions with higher toxic potential and those with wider safety margins. The Lorke method remains a recognized and commonly used approach for preliminary acute toxicity screening and $LD_{50}$ estimation in experimental animals (Chinedu *et al*., 2013). Therefore, this study was designed to evaluate and compare the acute toxicity and dose-dependent mortality response of the n-hexane, petroleum ether, ethanol, and aqueous fractions of *Sida acuta* in Wistar rats. The study aimed to determine the $LD_{50}$ values of the different fractions, observe the signs of acute toxicity associated with each fraction, and compare their relative safety profiles in order to provide baseline toxicological data for further pharmacological investigation of *Sida acuta*.

## Materials and Methods

### Plant Collection and Authentication

The plants were authenticated by Dr. (Mrs.) Bibian Aziagba of the Department of Botany, Nnamdi Azikiwe University, Awka. Voucher specimens were deposited with reference numbers N.A.U.H. No: $75^{A}$ for *Sida acuta*. The fresh leaf samples of *Sida acuta* were collected, thoroughly rinsed, and air-dried at room temperature. After drying, the leaves were pulverized into fine powder using a Buchymix electric blender (Turbocrush BM2016JS).

### Extraction Procedure

The dried leaves of *Sida acuta* were ground into fine powder and subjected to successive solvent extraction using n-hexane, petroleum ether, ethanol and distilled water in this order of polarity: n-hexane, petroleum ether, ethanol and water. The leaf samples were fractionated by the method of Wu *et al.* (2005) which involved successive extraction by increasing polarity. N-hexane, being a more defined hydrocarbon solvent, was used first to remove readily extractable highly non-polar constituents, while petroleum ether, a mixture of low-boiling aliphatic hydrocarbons, was used subsequently to recover residual non-polar constituents that may not have been completely extracted by n-hexane. The use of both solvents was intended to improve lipophilic fractionation and allow comparison of the acute toxicity profiles of separate non-polar fractions rather than treating all non-polar constituents as a single fraction.

For each extraction, 1000 g of the powdered plant material was soaked in 5 L of the respective solvent for 24 h with occasional

stirring with a glass stirring rod. The extract was filtered first through muslin cloth and subsequently through Whatman No. 1 filter paper. The resulting filtrate was concentrated using a water bath, while the marc was air-dried and re-extracted with the next solvent in the sequence.

The n-hexane and petroleum ether fractions were concentrated to dryness in a water bath at 40 °C, while the ethanol and aqueous fractions were concentrated at 50 °C and 65 °C, respectively. Although n-hexane and petroleum ether are both non-polar solvents with overlapping extraction properties, both were included in the extraction sequence to obtain operationally distinct lipophilic fractions for comparative toxicity assessment

**Ethical Approval**

All experimental procedures involving animals were conducted in accordance with standard guidelines for the care and use of laboratory animals. Ethical approval for the study was obtained from the Nnamdi Azikwe University Animal Research Ethics Committee (AREC) of Nnamdi Azikiwe University with approval number **NAU/AREC/2023/00027.**

**Experimental Animals and Housing**

A total of fifty-two (52) healthy male Wistar rats weighing between 100 and 120 g were purchased from Chris Animal Farms and Research Laboratory, Mgbakwu, Awka North Local Government Area Anambra State, Nigeria. The area is situated within Awka North of Anambra State, in south-eastern Nigeria, with approximate geographical coordinates of Latitude 6° 16' 20" N (6.27234), and Longitude 7° 3' 21" E (7.05605). The location lies within the tropical climatic zone of South-eastern Nigeria, characterized by distinct wet and dry seasons, with relatively high humidity and moderate to warm ambient temperatures.

Only male rats were used for the study; male and female animals were not mixed. The use of animals of the same sex was intended to reduce possible sex-related variation in toxicity response. The animals were housed in clean, well-ventilated cages under standard laboratory conditions and allowed to acclimatize to the experimental environment at Chris Animal Farms and Research Laboratory, Mgbakwu, Awka, for one week before commencement of the study. During the acclimatization and experimental periods, the rats were fed with Vital Growers' Mash pellets purchased from Vital Feed Distributor, Awka, and were allowed access to clean drinking water ad libitum throughout the study. Before administration of the test fractions, the animals were weighed, grouped, and properly labelled for identification.

**Animal Purchase, Grouping and Acute Toxicity**

The study was carried out using male Wistar rats obtained from Chris Animal Farms and Research Laboratory, Mgbaukwu, Awka North Local Government Area, Anambra State, Nigeria.

The animals were grouped according to the dose levels used in the two phases of Lorke's acute toxicity method. Dose selection was based on the standard Lorke (1983) acute toxicity protocol, which employs graded low, intermediate, and high dose levels to establish the lethal range and estimate the median lethal dose ($LD_{50}$). Each solvent fraction of *Sida acuta* was tested separately using the selected dose levels of 10, 100, 1000, 1600, 2900 and 5000mg/kg. For each solvent fraction, thirteen (13) male rats were assigned, giving a total of 52 rats for the four solvent fractions: n-hexane,

petroleum ether, ethanol, and aqueous fractions. Out of the thirteen rats assigned to each fraction, twelve (12) rats were used for the graded-dose acute toxicity assessment, while one (1) rat served as a reserve/backup animal. Thus, a total of forty-eight (48) rats were used for the actual $LD_{50}$ determination, while four (4) rats served as backup animals, one for each fraction. The backup animals were maintained under the same housing and feeding conditions and were not included in the $LD_{50}$ calculation. For each fraction, the Lorke acute toxicity assessment was carried out in two phases. In phase one, nine (9) rats were divided into three groups of three rats each. The groups received 10, 100, and 1000 mg/kg body weight of the respective fraction, respectively. In phase two, three (3) rats were individually administered 1600, 2900, and 5000 mg/kg body weight of the respective fraction, respectively. So for each fraction, three rats were used per dose group in phase one, while one rat was used per dose level in phase two.

**Route of Administration, Observation Period and Monitored Toxicity Signs**

The leaf fractions were administered orally to the rats by gavage using an intubation cannula. Following administration, the animals were closely monitored for signs of toxicity and mortality. Observations were carried out continuously during the first few hours after dosing and subsequently at periodic intervals for 24 h. The animals were observed for mortality as well as behavioural, physical, autonomic, and neurological changes following administration of the fractions. The monitored toxicity signs included weakness, reduced activity, tremors, jerking movements, aggression, changes in general behaviour, and visible skin or fur changes.

**Determination of $LD_{50}$**

The median lethal dose ($LD_{50}$) for each fraction was estimated based on the outcome of the two phases of Lorke's method. The $LD_{50}$ was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose for each fraction where mortality occurred. For the aqueous fraction, where no mortality was recorded, the $LD_{50}$ could not be determined within the tested dose range.

**Data Analysis**

Data obtained from the acute toxicity study were analyzed descriptively. Mortality and observed toxicity signs were recorded for each fraction and dose level during the observation period. The number of deaths at each dose was expressed as frequency and percentage mortality. Behavioural, physical, autonomic, and neurological signs of toxicity were also described based on the observations made after administration of the fractions. The median lethal dose ($LD_{50}$) for each fraction was determined using Lorke's method. The $LD_{50}$ was calculated as the geometric mean of the highest dose that produced no mortality and the lowest dose that produced mortality, using the formula:

$$LD_{50} = \sqrt{(HNLD \times LLD)}$$

where HNLD represents the highest non-lethal dose, and LLD represents the least lethal dose. For fractions in which no mortality was recorded within the tested dose range, the $LD_{50}$ was considered not reached within the administered dose levels. The dose-dependent mortality response was assessed by comparing the pattern of mortality across increasing dose levels for each solvent fraction. Since the study was based on Lorke's acute toxicity method, which uses mortality outcome and observable toxicity signs to estimate $LD_{50}$, no inferential statistical test was applied. Results were therefore presented as percentage mortality, observed toxicity signs,

and $LD_{50}$ values expressed in mg/kg body weight.

## Results

The acute toxicity study of the n-hexane, petroleum ether, ethanol, and aqueous fractions of *Sida acuta* revealed differences in mortality pattern, behavioral response, and median lethal dose ($LD_{50}$) among the fractions. Mortality was observed in the n-hexane, petroleum ether, and ethanol fractions at higher doses, while the aqueous fraction produced no mortality within the tested dose range. Animals exposed to doses that did not cause death appeared normal, whereas those exposed to toxic doses showed weakness before mortality. Animals that survived the administered doses generally maintained normal behavior and physical appearance during the observation period. Doses associated with toxicity and mortality were characterized mainly by weakness, tremors, and jerking movements prior to death.

### Acute Toxicity ($LD_{50}$) of n- Hexane Fraction of *Sida acuta* Leaf Extracts.

The acute toxicity profile of the hexane fraction of *Sida acuta* leaf extract is presented in Table 1. In the first phase, no mortality was observed at 10, 100, and 1000 mg/kg body weight, and the animals appeared normal at all dose levels. In the second phase, the animal treated with 1600 mg/kg body weight also appeared normal, with no mortality recorded. However, at 2900 mg/kg body weight, the animal appeared weak and mortality was recorded, while at 5000 mg/kg body weight, the animal appeared very weak and mortality was also recorded. Using the highest non-lethal dose of 1600 mg/kg body weight and the least lethal dose of 2900 mg/kg body weight, the $LD_{50}$ was calculated as 2154 mg/kg body weight.

**Table 1: Acute Toxicity ($LD_{50}$) of n-Hexane Fraction of *Sida acuta* Leaf Extracts**

| Dose | Observation | Mortality |
|---|---|---|
| **First Phase** | | |
| **10** | The experimental Wistar rats showed normal behavior | **0/3** |
| **100** | The experimental Wistar rats showed normal behavior | **0/3** |
| **1000** | The experimental Wistar rats showed normal behavior | **0/3** |
| **Second Phase** | | |
| **1600** | The experimental Wistar rat showed normal behavior | **0/1** |
| **2900** | The experimental Wistar rat was weak | **1/1** |
| **5000** | The experimental Wistar rat was very weak | **1/1** |

$LD = \sqrt{1600 \times 2900} = \sqrt{4640000} = 2154.0659 \approx 2154 mg/kg\ b.w.$

**Acute Toxicity (LD $_{50}$) of Petroleum-ether Fraction of *Sida acuta* Leaf Extracts**

The acute toxicity profile of the pet-ether fraction of *Sida acuta* leaf extract is presented in Table 2. In the first phase, no mortality was observed at 10, 100, and 1000 mg/kg body weight, and the animals appeared normal at all dose levels. In the second phase, the animal treated with 1600 mg/kg body weight and 2900 mg/kg body weight also appeared normal. However at 5000 mg/kg body weight, the animal appeared very weak and mortality was also recorded. Using the highest non-lethal dose of 2900 mg/kg body weight and the least lethal dose of 5000 mg/kg body weight, the $LD_{50}$ was calculated as 3,808 mg/kg body weight.

**Table 2: Acute Toxicity (LD $_{50}$) of Petroleum-Ether Fraction of *Sida acuta* Leaf Extracts**

| Dose | Observation | Mortality |
|---|---|---|
| **First Phase** | | |
| **10** | The experimental Wistar rats showed normal behavior | **0/3** |
| **100** | The experimental Wistar rats showed normal behavior | **0/3** |
| **1000** | The experimental Wistar rats showed normal behavior | **0/3** |
| **Second Phase** | | |
| **1600** | The experimental Wistar rat showed normal behavior | **0/1** |
| **2900** | The experimental Wistar rat showed normal behavior | **0/1** |
| **5000** | The experimental Wistar rat was very weak | **1/1** |

$LD = \sqrt{2900 \times 5000} = \sqrt{14500000} = 3807.88655 \approx 3808 mg/kg$ b.w.

**Acute Toxicity (LD $_{50}$) of Ethanol Fraction of *Sida acuta* Leaf Extracts**

The acute toxicity profile of the ethanol fraction of *Sida acuta* leaf extract is presented in Table 3. In the first phase, no mortality was observed at 10, 100, and 1000 mg/kg body weight, and the animals appeared normal at all dose levels. In the second phase, the animal treated with 1600 mg/kg body weight also appeared normal, with no mortality recorded. However, at 2900 mg/kg body weight, the animal appeared weak and mortality was recorded, while at 5000 mg/kg body weight, the animal appeared very weak and mortality was also recorded. Using the highest non-lethal dose of 1600 mg/kg body weight and the least lethal dose of 2900 mg/kg body weight, the $LD_{50}$ was calculated as 2154 mg/kg body weight

**Table 3: Acute Toxicity (LD $_{50}$) of Ethanol Fraction of *Sida acuta* Leaf Extracts**

| Dose | Observation | Mortality |
|---|---|---|
| **First Phase** | | |
| **10** | The experimental Wistar rats showed normal behavior | **0/3** |
| **100** | The experimental Wistar rats showed normal behavior | **0/3** |
| **1000** | The experimental Wistar rats showed normal behavior | **0/3** |
| **Second phase** | | |
| **1600** | The experimental Wistar rat showed normal behavior | **0/1** |
| **2900** | The experimental Wistar was weak | **1/1** |
| **5000** | The experimental Wistar rat was weak and jerking | **1/1** |

LD= $\sqrt{1600 \times 2900} = \sqrt{4640000} = 2154.0659 \approx 2154$mg/kg b.w.

**Acute Toxicity (LD $_{50}$) of Aqueous Fraction of *Sida acuta* Leaf Extracts**

Table 4 shows the acute toxicity result of the aqueous fraction of *Sida acuta* leaf extract. The extract produced no visible signs of toxicity or mortality in both phases of the study. All animals treated with 10, 100, 1000, 1600, 2900, and 5000 mg/kg body weight appeared normal, with mortality records of 0/3 in the first phase and 0/1 in the second phase. Therefore, the median lethal dose ($LD_{50}$) of the aqueous fraction of *Sida acuta* leaf extract was estimated to be greater than 5000 mg/kg body weight.

**Table 4: Acute Toxicity ($LD_{50}$) Of Aqueous Fraction of *Sida acuta* Leaf Extracts**

| Dose | Observation | Mortality |
|---|---|---|
| **First Phase** | | |
| **10** | The experimental Wistar rats showed normal behavior | **0/3** |
| **100** | The experimental Wistar rats showed normal behavior | **0/3** |
| **1000** | The experimental Wistar rats showed normal behavior | **0/3** |
| **Second Phase** | | |
| **1600** | The experimental Wistar rat showed normal behavior | **0/1** |
| **2900** | The experimental Wistar rat showed normal behavior | **0/1** |
| **5000** | The experimental Wistar rat showed normal behavior | **0/1** |

No mortality was observed up to 5000 mg/kg body weight; therefore, $LD_{50}$ was considered to be greater than 5000 mg/kg body weight.

**Dose Dependent Mortality of the four fraction extracts**

The dose dependent mortality of n-hexane, petroleum ether, ethanol and aqueous fractions of *Sida acuta* are presented below.

**Dose Dependent Mortality Response of *Sida acuta* Leaf Fractions**

The dose-dependent mortality response plot of the four fractions of *Sida acuta* leaf extract is presented in Figure 1. Mortality was not recorded at the lower dose levels of 10, 100, 1000, and 1600 mg/kg body weight in all the tested fractions. However, differences in mortality became evident at the higher dose levels, especially between 2900 and 5000 mg/kg body weight.

The combined dose-dependent mortality response curve showed that the n-hexane

and ethanol fractions produced identical mortality patterns. Both fractions showed no mortality up to 1600 mg/kg body weight, but mortality occurred at 2900 mg/kg body weight and persisted at 5000 mg/kg body weight. Based on Lorke's method, the $LD_{50}$ values of both the n-hexane and ethanol fractions were calculated as 2154 mg/kg body weight, indicating that these two fractions had the highest acute toxicity among the tested fractions.

The petroleum ether fraction showed a comparatively lower acute toxicity. No mortality was observed up to 2900 mg/kg body weight, while mortality occurred at 5000 mg/kg body weight. The $LD_{50}$ of the petroleum ether fraction was therefore calculated as 3808 mg/kg body weight, showing that it had a wider acute safety margin than the n-hexane and ethanol fractions.

The aqueous fraction produced no mortality across all tested dose levels. Its mortality curve remained at 0% mortality throughout the tested dose range. Therefore, the $LD_{50}$ of the aqueous fraction was not determined because no lethal dose was reached within the administered dose

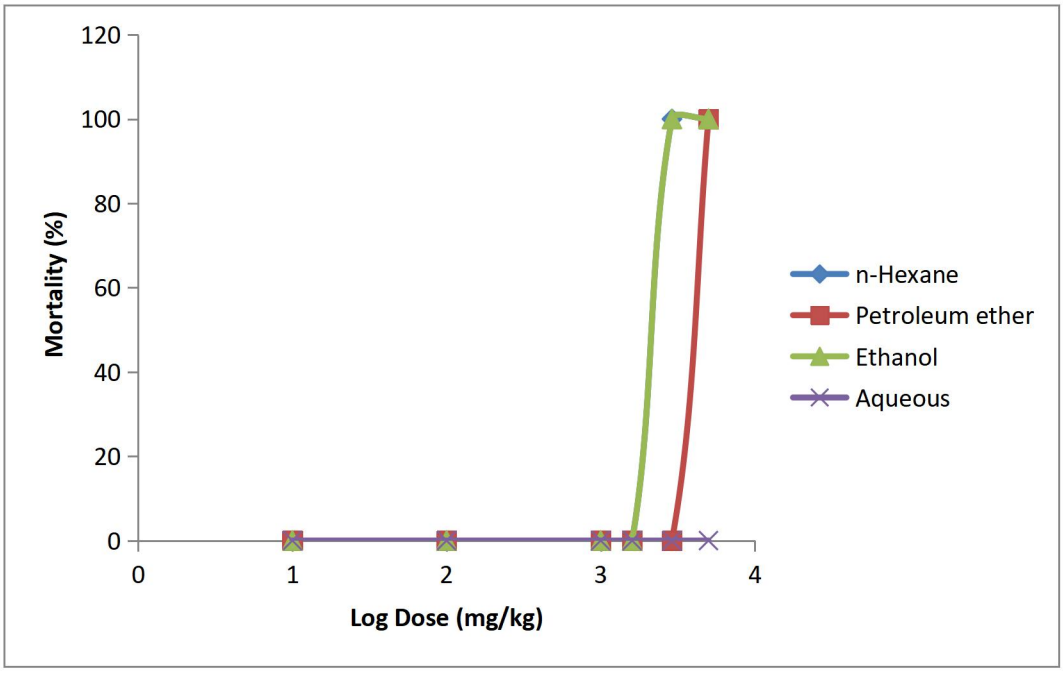



Fig. 1: Combined dose-dependent mortality response plot of n-hexane, petroleum ether, ethanol, and aqueous leaf fractions of *Sida acuta*.

**Discussion**

$LD_{50}$ is an important index of acute lethality and is defined as the median dose predicted to kill 50% of a test population (Morris-Schaffer and McCoy, 2021). In this study, the solvent fractions of *Sida acuta* showed fraction-dependent differences in acute toxicity. The n-hexane and ethanol fractions had the lowest $LD_{50}$ values, each at 2154 mg/kg body weight, indicating greater acute toxicity than the petroleum ether fraction, which had an $LD_{50}$ of 3808 mg/kg body

weight. The aqueous fraction produced no mortality within the tested dose range, showing that its $LD_{50}$ was not reached. These findings indicate that the safety profile of *Sida acuta* cannot be generalized across all preparations and support the need for toxicity evaluation of specific plant fractions rather than treating all extracts as equivalent (Jiménez-Ferrer *et al*., 2025).

The differences observed among the fractions may be linked to solvent-driven variation in phytochemical composition. Solvent selection influences both the qualitative and quantitative recovery of phytochemicals from medicinal plants (Lee *et al*., 2024). N-hexane may concentrate lipophilic constituents such as fixed oils, waxes, sterols, terpenoids, chlorophyll-rich materials, and other hydrophobic compounds. Thus, each fraction may differ toxicologically because each solvent enriches a different pool of constituents.

The greater toxicity of the n-hexane fraction may reflect the concentration of lipophilic compounds with strong membrane interaction or higher membrane permeability. Some terpenoid or steroid-like compounds in non-polar fractions may be pharmacologically active but toxic at higher doses. Similarly, the ethanol fraction may have shown increased toxicity because it can concentrate several bioactive classes simultaneously. Previous work on the ethanolic leaf extract of *Sida acuta* identified phenols, flavonoids, tannins, terpenoids, steroids, alkaloids, and glycosides, confirming the presence of biologically active constituents (Chatepa *et al*., 2024). Fractionation can alter the concentration and interaction of such constituents, thereby modifying toxicity even when fractions originate from the same plant material (Enechi *et al*., 2021).

The lower toxicity of the petroleum ether fraction compared with the n-hexane and ethanol fractions shows that non-polar fractions do not necessarily have identical toxicological behavior. Although petroleum ether and n-hexane have overlapping non-polar extraction properties, they may not recover the same compounds in the same concentrations. Small differences in solvent characteristics can affect metabolite recovery (Tsaturyan *et al*., 2025). Therefore, toxicity is not determined by polarity alone, but by the specific chemical profile produced by the solvent system, including the presence and concentration of toxic constituents and the possible absence of protective compounds (Lee *et al*., 2024).

The absence of mortality in the aqueous fraction suggests that the water-soluble constituents of *Sida acuta* were safer in terms of acute lethal effect within the tested dose range. This is relevant because aqueous preparations are common in traditional practice as decoctions or infusions. However, absence of acute mortality should not be interpreted as absolute safety, since acute toxicity only assesses immediate harmful effects following single-dose exposure. A fraction may be non-lethal acutely but still produce subacute, chronic, reproductive, hepatic, renal, or hematological toxicity after repeated exposure (Sall *et al*., 2025).

The observed behavioral pattern also supports the mortality findings in this current study. Animals that received non-lethal doses generally maintained normal behavior, whereas weakness occurred at doses associated with mortality. Such weakness may indicate systemic toxicity, central nervous system depression, reduced locomotor activity, metabolic distress, or early organ dysfunction. In acute toxicity studies, behavioral changes are useful early indicators of toxic insult before death occurs; therefore, the weakness observed before mortality suggests that death was preceded

by physiological compromise rather than occurring abruptly (Canadian Centre for Occupational Health and Safety, 2025).

The $LD_{50}$ values provide a basis for comparing the short-term poisoning potential of the fractions. Although the n-hexane and ethanol fractions were more toxic than the petroleum ether fraction, the $LD_{50}$ values of all lethal fractions were above 2000 mg/kg body weight, suggesting that they were not highly toxic under the experimental conditions. The aqueous fraction showed the widest acute safety margin because no $LD_{50}$ was reached. These distinctions are useful for dose selection, formulation development, and future pharmacological studies (Canadian Centre for Occupational Health and Safety, 2025).

The ethanol fraction result differs from a previous report in which crude ethanol leaf extract of *Sida acuta* produced no lethality at 5000 mg/kg body weight and no marked behavioral changes (Enechi *et al*., 2021). This difference is plausible because the previous study used a crude ethanol extract, whereas the present study evaluated an ethanol fraction obtained after successive solvent extraction. Fractionation can enrich specific bioactive molecules and remove constituents that may dilute or buffer toxicity in a crude extract. Therefore, the lower $LD_{50}$ observed in this study does not contradict previous work but emphasizes the need for fraction-specific safety assessment.

Solvent fractionation altered the acute toxicological profile of *Sida acuta*. The n-hexane and ethanol fractions were the most toxic, the petroleum ether fraction showed intermediate toxicity, and the aqueous fraction was the least toxic. These findings suggest that aqueous preparations may have a better acute safety margin, while n-hexane and ethanol fractions require greater caution in dose selection. This indicates that the aqueous fraction had the widest acute safety margin among the four tested fractions. These findings are consistent with the combined graph in Figure 1, where the n-hexane and ethanol curves overlapped, the petroleum ether curve showed delayed mortality, and the aqueous curve remained flat at 0% mortality. The safety of *Sida acuta* should therefore not be generalized across all extracts or fractions, since toxicity depends strongly on the chemical nature of the preparation tested (Jiménez-Ferrer *et al*., 2025).

## Conclusion

This study showed solvent-dependent differences in the acute toxicity of *Sida acuta* fractions. The findings provide preliminary toxicological evidence supporting the relative safety of aqueous preparations, as no mortality occurred within the tested dose range. The n-hexane and ethanol fractions were more toxic, each with an $LD_{50}$ of 2,154 mg/kg body weight, while petroleum ether showed lower toxicity with an $LD_{50}$ of 3,808 mg/kg body weight. These results highlight the need for careful dose consideration when using non-aqueous fractions and support further sub-acute, sub-chronic, chronic toxicity, and bioactivity-guided pharmacological studies.

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## Conflict of Interest

No conflict of interest

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**Authors Contribution**
Adanma Eunice Ukogo carried out the experiments, analyzed the data, and drafted the manuscript. Author Chinwe Nonyelum Ezekwesili provided guidance on the study design and revised the manuscript. Author Josephine Ozioma Ezekwesili-ofili revised the manuscript and provided additional scholarly input. All authors read and approved the final manuscript

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