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Phytochemical screening and acute oral toxicity assessment of *Arenga pinnata* leaf aqueous extract in Wistar rats

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ABSTRACT

Background: *Arenga pinnata* leaves have been traditionally used for medicinal purposes; however, their phytochemical composition and toxicity profile remain poorly characterized.

Objective: This study aimed to identify bioactive compounds in *A. pinnata* leaf aqueous extract and evaluate its acute oral toxicity in Wistar rats.

Methods: Qualitative phytochemical screening was performed using standard colorimetric and precipitation methods. Twenty-five male Wistar rats were randomly assigned to five groups (n=5): one control (distilled water) and four treatment groups receiving single oral doses of 200, 400, 800, and 1600 mg/kg body weight of aqueous extract. Animals were monitored for 14 days for mortality, behavioral changes, and clinical toxicity signs.

Results: Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, and triterpenoids. No mortality, adverse clinical signs, or pathological organ changes were observed throughout the study period. All animals exhibited normal behavior and progressive weight gain. The median lethal dose (LD_{50}) exceeded 1600 mg/kg body weight, indicating low acute oral toxicity.

Conclusion: *A. pinnata* leaf aqueous extract contains pharmacologically relevant bioactive compounds and demonstrates a favorable acute safety profile. These findings support traditional use while highlighting the need for sub-chronic and chronic toxicity studies.

Keywords: Acute toxicity testing, Arenga pinnata leaves, herbal medicine safety, phytochemical analysis, sugar palm

Introduction

The use of medicinal plants in traditional healthcare systems continues to gain prominence globally, with *Arenga pinnata* being one such species with a long history of therapeutic applications. Natural medicinal plants are often perceived as safer alternatives to synthetic drugs, contributing to their widespread acceptance [1,2]. However, despite their popularity, most medicinal plants are utilized only in the form of traditional herbal remedies and have not been extensively developed into standardized pharmaceutical products [3]. This limited standardization has raised concerns among researchers regarding the potential risks associated with their use, particularly as studies indicate that many individuals consume medicinal plants without proper consultation with healthcare professionals or herbalists [4]. Such practices pose significant health risks, especially among populations lacking adequate knowledge about appropriate dosages and potential adverse effects. Consequently, rigorous scientific investigation is essential to establish safety parameters and toxicity profiles of medicinal plants, thereby preventing harmful side effects and supporting their evidence-based therapeutic application.

Arenga pinnata, commonly known as sugar palm, has been traditionally used for various medicinal

purposes. While several parts of this plant have been studied, the phytochemical composition and biological activities differ considerably across plant organs. Previous phytochemical investigations have primarily focused on the fruit, roots, and leaf stalks, which are known to contain bioactive compounds including flavonoids, alkaloids, saponins, and tannins, each associated with diverse potential health benefits [5–7]. For instance, ethanol extracts of *A. pinnata* fruit and sap water have been reported to contain alkaloids, flavonoids, and quinones [8–10], while seed extracts contain flavonoids, triterpenoids, saponins, and tannins. Similarly, flour extracts from the stalks have been found to contain saponins, tannins, steroids, triterpenoids, and phenols as secondary metabolites [3,9]. Despite these investigations, the phytochemical profile of *A. pinnata* leaves remains largely unexplored and poorly characterized. Understanding the bioactive constituents present in the leaves is crucial not only to validate their traditional medicinal applications but also to facilitate the development of standardized pharmaceutical products derived from natural sources.

Beyond phytochemical characterization, comprehensive toxicity assessment is fundamental to determining whether a plant material is safe for human consumption. Traditional medicines must undergo both efficacy and safety evaluations through general and specific toxicity testing protocols [11,12]. Among these, acute toxicity testing represents a critical initial step in safety assessment. This test evaluates the adverse effects of a substance following a single administration at various doses, with systematic observations conducted over a 14-day period. Acute toxicity studies serve multiple purposes: they detect the intrinsic toxicity of a substance, identify potential target organs, assess species-specific sensitivity, provide hazard information following acute exposure, and generate preliminary data for establishing safe dose ranges for subsequent studies [13–15]. The median lethal dose (LD_{50}) value, derived from acute toxicity testing, serves as a key parameter for evaluating the safety margin of a substance and is widely recognized in pharmacological and toxicological research.

Despite the potential therapeutic applications of *A. pinnata* leaves, there is currently no available data regarding their acute toxicity profile or safety parameters. This knowledge gap represents a significant limitation in the scientific validation and potential clinical translation of *A. pinnata* leaf-based remedies. Therefore, this study was designed with dual objectives: first, to conduct comprehensive phytochemical screening to identify the bioactive compounds present in *A. pinnata* leaves; and second, to determine the acute toxicity profile and LD_{50} value of the aqueous leaf extract using Wistar rats (*Rattus norvegicus*) as an experimental model. The findings from this investigation will provide foundational safety data essential for the continued development of *A. pinnata* leaves as a potential source of herbal medicine and will contribute to the broader understanding of the pharmacological properties of plants within the Arecaceae family.

Methods

Ethical approval

This study was conducted in accordance with ethical guidelines for animal research. All experimental procedures were approved by the Research Ethics Committee of Medical and Health Research Ethics Review Unit of the Faculty of Medicine, Universitas Riau, with approval number B/063/UN19.5.1.1.8/UEPKK/2024. Animal handling and care followed the principles outlined in the Guide for the Care and Use of Laboratory Animals.

Preparation of experimental animals

Male Wistar rats (*Rattus norvegicus*) served as experimental animals in this study. Animals were selected from a healthy population exhibiting active movement, normal behavior, and no history of prior experimental use. Selected rats were 2–3 months of age with body weights ranging from 150 to 250 grams at the time of selection. Prior to experimentation, animals underwent a 7-day acclimatization period under standard laboratory conditions: temperature maintained at 22 ± 2°C, relative humidity at 50 ± 10%, 12-hour light/dark

cycle (light phase: 06:00–18:00), and ad libitum access to standard pellet feed and tap water [16,17].

Following acclimatization, animals were randomly assigned to five groups using simple random sampling, with each group consisting of five rats (n = 5). One group served as the negative control, while the remaining four groups received different treatment doses. The total sample size (N = 25) was determined using the Resource Equation Method formula [18,19], ensuring adequate statistical power while adhering to the ethical principle of using the minimum number of animals necessary.

Preparation of *Arenga pinnata* extract

Fresh *A. pinnata* leaves were obtained from a plantation in Kampung Baru Village, Bukik Barisan District, Barisan City, Payakumbuh City, Lima Puluh Kota Regency, West Sumatra, Indonesia. Leaf extract was prepared through the following procedure: Fresh leaves were thoroughly washed under running tap water to remove dirt and debris. Clean leaves were cut into small pieces (approximately 2 × 2 cm) and dried in an oven at 50°C for 30 minutes to reduce moisture content while preserving bioactive compounds. Dried leaves were ground using an electric grinder to obtain a fine powder, which was then stored in sterile, airtight containers at room temperature.

For aqueous extraction, the leaf powder was mixed with distilled water at a ratio of 1:10 (w/v) and macerated at room temperature (25°C) for 24 hours with periodic stirring every 6 hours to facilitate compound extraction. The mixture was then filtered through Whatman No. 1 filter paper to remove particulate matter. The resulting filtrate was concentrated using a rotary evaporator at 50°C under reduced pressure until a viscous extract was obtained. The concentrated extract was subsequently stored at 4°C in sterile, amber-colored bottles until further use [20]. The extraction yield was calculated as follows:

Extraction yield (%) = (Weight of dried extract / Weight of dried powder) × 100

Phytochemical screening of *A. pinnata* leaf extract

Qualitative phytochemical screening was performed on the aqueous extract to identify the presence of major classes of bioactive compounds using standard protocols [3,21–23].

Alkaloids: Three milliliters of extract was acidified with 2 mL of 2% hydrochloric acid and divided into two test tubes. Mayer's reagent (2 drops) was added to the first tube, and Dragendorff's reagent (2 drops) to the second tube. Formation of a white precipitate with Mayer's reagent or an orange-red precipitate with Dragendorff's reagent indicated the presence of alkaloids [3].

Flavonoids: Three milliliters of extract was mixed with 0.5 g magnesium powder and 3 drops of concentrated hydrochloric acid. The appearance of red, yellow, or orange coloration within 3 minutes indicated the presence of flavonoids [3].

Tannins: Three milliliters of extract was treated with 3 drops of 1% ferric chloride ($FeCl_3$) solution. Development of a blackish-green or blue-black coloration indicated the presence of tannins [3,22,23].

Saponins: Three milliliters of extract was mixed with 5 mL of distilled water in a test tube and shaken vigorously for 30 seconds. Formation of stable foam (height > 1 cm) persisting for at least 10 minutes indicated the presence of saponins [3,21].

Triterpenoids and steroids: Two milliliters of extract was evaporated to dryness, and the residue was dissolved in 2 mL of chloroform. Subsequently, 1 mL of anhydrous acetic acid and 2 drops of concentrated sulfuric acid were added carefully along the side of the tube (Liebermann-Burchard test). Formation of a red or purple color indicated triterpenoids, whereas blue or green coloration suggested the presence of steroids [3].

All tests were performed in triplicate to ensure reproducibility, and results were recorded immediately upon color development.

Determination of LD_{50} value in water extract

The acute oral toxicity study was conducted following the Organization for Economic Cooperation and Development (OECD) guideline 423 with modifications [24,25]. After acclimatization, animals were fasted overnight (12–16 hours) with free access to water before extract administration.

On day 8 (first day of treatment), rats were randomly assigned to five experimental groups (n = 5 per group): group C (Control): received 1 mL distilled water orally; group T1: received aqueous extract at 200 mg/kg BW orally; group T2: Received aqueous extract at 400 mg/kg BW orally; group T3: received aqueous extract at 800 mg/kg BW orally; group T4: received aqueous extract at 1600 mg/kg BW orally.

The dose levels were selected based on preliminary range-finding studies and traditional usage patterns. Extract doses were calculated individually for each animal based on body weight measured immediately before administration. All administrations were performed as a single oral dose via gavage using a stainless steel feeding tube.

Following extract administration, animals were observed intensively for the first 4 hours for immediate toxic signs, including changes in skin, fur, eyes, mucous membranes, respiratory patterns, circulatory signs, autonomic effects (salivation, lacrimation, piloerection, pupil size, unusual respiratory patterns), central nervous system effects (tremors, convulsions, sedation, excitation, abnormal locomotion), and behavioral changes. Subsequently, animals were monitored daily for 14 consecutive days for morbidity, mortality, changes in body weight, food and water consumption, and any signs of delayed toxicity or behavioral abnormalities.

Body weight was recorded on days 0, 3, 7, and 14 post-administration. At the end of the observation period (day 14), all surviving animals were humanely euthanized using a combination of ketamine (80 mg/kg BW) and xylazine (10 mg/kg BW). The test animals were sacrificed by cervical dislocation in accordance with ethical guidelines for the use of laboratory animals and gross necropsy was performed to examine major organs for any macroscopic pathological changes.

The median lethal dose (LD_{50}) was estimated based on mortality data recorded within the 14-day observation period. Since no mortality occurred at the highest tested dose, the LD_{50} value was estimated to be greater than 1600 mg/kg BW [9,24,25].

Data analysis

Survival data were analyzed and presented using Kaplan-Meier survival curves. Body weight changes were expressed as mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test for multiple comparisons. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism 10.

Results

Extraction yield

The aqueous extraction of *A. pinnata* leaves yielded a dark green, viscous extract with a characteristic odor. From 500 g of dried leaf powder, 55 g of crude extract was obtained, corresponding to an extraction yield of 11% (w/w).

Phytochemical composition of *A. pinnata* leaf extract

Qualitative phytochemical screening of the aqueous extract revealed the presence of multiple bioactive compound classes (Table 1). Alkaloids were detected through positive reactions with both Mayer's reagent (formation of white precipitate) and Dragendorff's reagent (formation of orange-red precipitate). Flavonoids were confirmed by the appearance of reddish-brown coloration following the addition of magnesium powder and concentrated hydrochloric acid. The presence of tannins was indicated by the development of blackish-green coloration upon treatment with ferric chloride solution. Triterpenoids were detected through the Liebermann-Burchard test, evidenced by reddish-brown coloration. However, the test for saponins was negative, as indicated by the absence of stable

Table 1. Phytochemical composition of aqueous extract of *Arenga pinnata* leaves

Phytochemical class	Test/reagent	Observation	Result
Alkaloids	Mayer's reagent	White precipitate	+
	Dragendorff's reagent	Orange-red precipitate	+
Flavonoids	Mg powder + HCl	Reddish-brown coloration	+
Tannins	$FeCl_3$ 1%	Blackish-green coloration	+
Saponins	Foam test	No stable foam	–
Triterpenoids	Liebermann-Burchard test	Reddish-brown coloration	+
Steroids	Liebermann-Burchard test	No blue/green coloration	–

(+) = Present; (–) = Absent

Table 2. Mortality data of male Wistar rats following single oral administration of *Arenga pinnata* leaf aqueous extract

Group	Dose (mg/kg BW)	Number of animals	Deaths (Day 1–14)	Survival rate (%)
Control	0	5	0	100
T1	200	5	0	100
T2	400	5	0	100
T3	800	5	0	100
T4	1600	5	0	100

Table 3. Body weight changes (g) in Wistar rats following oral administration of *A. pinnata* leaf extract (mean ± SD, n=5)

Group	Dose (mg/kg BW)	Day 0	Day 3	Day 7	Day 14	Weight gain (%)
Control	0	182 ± 11	185 ± 12	192 ± 13	200 ± 14	9.9
T1	200	185 ± 12	188 ± 13	195 ± 14	203 ± 15	9.7
T2	400	187 ± 13	190 ± 14	197 ± 15	204 ± 16	9.1
T3	800	190 ± 14	192 ± 15	198 ± 16	205 ± 17	7.9
T4	1600	192 ± 15	193 ± 16	199 ± 17	204 ± 18	6.3

foam formation after vigorous shaking. Similarly, steroids were not detected in the aqueous extract, as no blue or green coloration was observed in the Liebermann-Burchard test.

The detection of alkaloids, flavonoids, tannins, and triterpenoids in the aqueous extract is consistent with the phytochemical profiles reported for other parts of *A. pinnata* and related species within the Arecaceae family. However, the absence of saponins in the leaf extract differs from previous findings in *A. pinnata* seeds and stalks, suggesting tissue-specific variation in secondary metabolite accumulation.

Clinical observations and mortality

Throughout the 14-day observation period, no mortality was recorded in any treatment group, including those receiving the highest dose of 1600 mg/kg BW (Table 2). All animals in both control and treatment groups survived until the end of the study, resulting in a 100% survival rate across all experimental groups (Figure 1).

During the intensive observation period (first 4 hours post-administration), no abnormal behavioral signs or acute toxic symptoms were observed in any treatment group. Animals exhibited normal locomotor activity, respiratory patterns, and grooming behavior comparable to control animals. No signs of salivation, lacrimation, piloerection, tremors, convulsions, sedation, or diarrhea were noted. Throughout the subsequent 14-day observation period, all animals maintained normal feeding and drinking patterns, with no visible signs of distress, illness, or abnormal behavior.

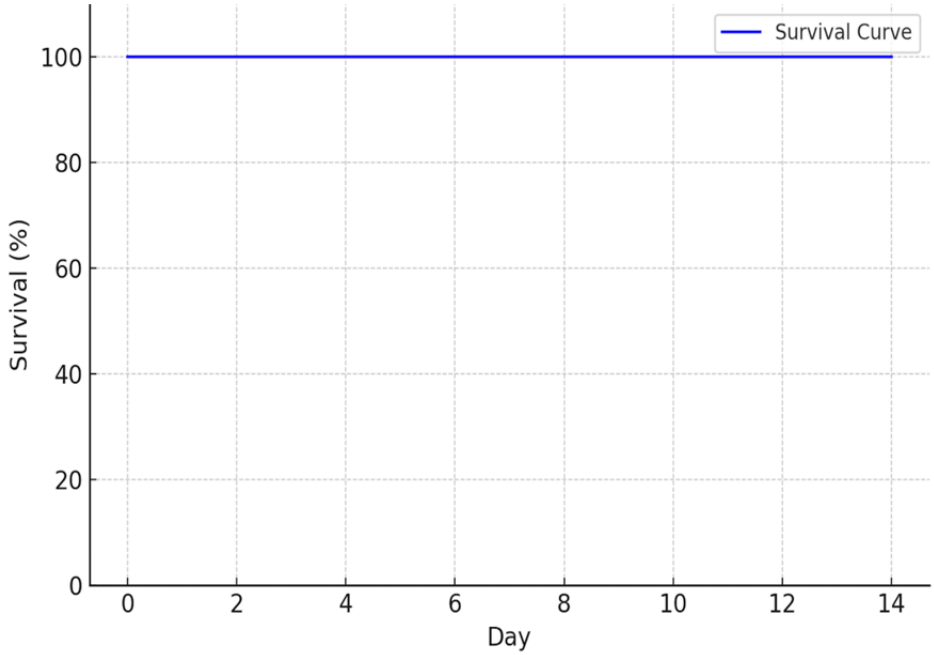

Figure 1. Kaplan-Meier survival curve of male Wistar rats over 14 days following single oral administration of *Arenga pinnata* leaf aqueous extract at doses of 0 (control), 200, 400, 800, and 1600 mg/kg body weight. All groups showed 100% survival throughout the observation period (n = 5 per group).

Body weight changes

Body weight measurements recorded on days 0, 3, 7, and 14 post-administration showed progressive weight gain in all groups, consistent with normal growth patterns in healthy rats (Table 3). No significant differences in body weight changes were observed between treatment groups and the control group at any time point ($p > 0.05$), indicating that the aqueous extract did not adversely affect normal growth and development.

Values are expressed as mean ± standard deviation. No significant differences were observed among groups (one-way ANOVA, $p > 0.05$).

Gross pathological examination

Macroscopic examination of vital organs (liver, kidneys, heart, lungs, spleen, and stomach) at necropsy revealed no visible abnormalities or pathological lesions in any treatment group. Organ appearance, color, and texture were comparable to those of control animals, suggesting the absence of gross organ toxicity at all tested doses.

LD_{50} determination

Based on the absence of mortality across all dose levels tested (200–1600 mg/kg BW), the median lethal dose (LD_{50}) of the aqueous extract of *A. pinnata* leaves was determined to be greater than 1600 mg/kg BW in male Wistar rats following single oral administration (Figure 1). The 100% survival rate observed across all treatment groups, combined with the absence of clinical toxicity signs and normal body weight progression, indicates that the aqueous extract of *A. pinnata* leaves demonstrates a favorable acute safety profile at doses up to 1600 mg/kg BW when administered orally to Wistar rats.

Discussion

This study provides the first comprehensive report on the phytochemical composition and acute oral toxicity profile of *A. pinnata* leaf aqueous extract in Wistar rats. The findings contribute important baseline data for the safety assessment and potential therapeutic development of this traditionally used medicinal plant.

Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and triterpenoids in the aqueous extract of *A. pinnata* leaves. The detection of alkaloids was confirmed through positive reactions with both Mayer's reagent (white precipitate formation) and Dragendorff's reagent (orange-red precipitate formation). Alkaloids are nitrogen-containing organic compounds widely recognized for their diverse pharmacological properties, including analgesic, antibacterial, anti-inflammatory, and anticancer activities [26,27]. The presence of these compounds in *A. pinnata* leaves suggests potential therapeutic applications consistent with traditional medicinal uses.

Flavonoids, identified by the characteristic reddish-brown coloration in the presence of magnesium powder and concentrated hydrochloric acid, are polyphenolic compounds renowned for their potent antioxidant properties. These compounds play crucial roles in neutralizing reactive oxygen species (ROS) and protecting cells against oxidative stress-related damage, which is implicated in numerous chronic diseases including cardiovascular disorders, neurodegenerative conditions, and metabolic syndromes [26,27]. The antioxidant capacity of flavonoids supports the hypothesis that *A. pinnata* leaves may contribute to cellular protection and disease prevention mechanisms, particularly in conditions characterized by oxidative stress such as diabetes mellitus and aging-related pathologies.

The identification of tannins, evidenced by blackish-green coloration upon treatment with ferric chloride solution, indicates the presence of polyphenolic compounds with known astringent properties. Tannins have been associated with antimicrobial, anti-inflammatory, and wound-healing activities in various medicinal plants [28,29]. Their presence in *A. pinnata* leaves may contribute to the traditional use of this plant in treating inflammatory conditions and infections.

Triterpenoids were detected through the Liebermann-Burchard test, as indicated by reddish-brown coloration. These compounds are known for their anti-inflammatory, hepatoprotective, and immunomodulatory properties [28,29]. The presence of triterpenoids in *A. pinnata* leaves aligns with previous reports of these compounds in other parts of the plant, suggesting a consistent biosynthetic capacity across different plant organs.

Notably, the aqueous extract tested negative for saponins, as evidenced by the absence of stable foam formation. This finding contrasts with previous studies reporting saponin content in *A. pinnata* seeds and stalks [3,9], indicating tissue-specific variation in secondary metabolite accumulation. Saponins are typically associated with immunomodulatory, hypocholesterolemic, and expectorant activities [28,29]. Their absence in leaf extracts may reflect differences in biosynthetic pathways, developmental stage, or environmental factors influencing secondary metabolite production. Alternatively, the aqueous extraction method employed in this study may have been insufficient to extract saponins effectively, as these compounds are sometimes better extracted using alcohol-based solvents. Similarly, steroids were not detected in the aqueous extract, which may also reflect solvent-specific extraction limitations or genuine absence in leaf tissue.

The phytochemical profile identified in this study is partially consistent with previous investigations of *A. pinnata*. Li et al. [9] reported that ethanol extracts of *A. pinnata* fruit contain alkaloids, flavonoids, and quinones, while seed extracts contain flavonoids, triterpenoids, saponins, and tannins. The presence of alkaloids, flavonoids, tannins, and triterpenoids in the leaf extract suggests that leaves share common biosynthetic pathways with fruits and seeds, although the absence of saponins distinguishes the leaf phytochemical profile. These findings underscore the importance of investigating different plant organs separately, as phytochemical composition can vary significantly depending on tissue type, developmental stage, and environmental conditions.

The bioactive compounds identified in this study may be responsible for the immunomodulatory effects previously reported for *A. pinnata* leaf extract. Yuliastri et al. [30] demonstrated that aqueous extract of *A. pinnata* leaves significantly enhanced macrophage phagocytosis in mice, suggesting active modulation of innate immune responses. The

increased phagocytic index observed in their study implies that the extract components—particularly flavonoids, alkaloids, and triterpenoids—may play a role in boosting host defense mechanisms. Flavonoids are known to modulate immune cell function through antioxidant pathways, while alkaloids and triterpenoids can directly influence immune cell activation and cytokine production. These immunomodulatory properties, combined with the low toxicity profile established in the present study, support the potential development of *A. pinnata* leaf extracts as safe immunostimulatory agents. However, further mechanistic studies are needed to elucidate the specific compounds and pathways responsible for these effects.

The acute toxicity evaluation revealed no mortality or observable toxic symptoms in Wistar rats at any of the tested doses (200, 400, 800, and 1600 mg/kg BW) throughout the 14-day observation period. All animals exhibited normal behavioral patterns, locomotor activity, feeding and drinking habits, and progressive body weight gain comparable to control animals. Macroscopic examination of vital organs at necropsy revealed no visible abnormalities or pathological lesions in any treatment group. These findings collectively indicate that the aqueous extract of *A. pinnata* leaves demonstrates low acute oral toxicity, with an LD_{50} value exceeding 1600 mg/kg BW.

According to the GHS classification system [31], the extract falls within Category 4 (LD_{50} between 300 and 2000 mg/kg BW) or potentially Category 5 (LD_{50} between 2000 and 5000 mg/kg BW), both indicating relatively low acute oral toxicity. This safety profile is consistent with previous toxicity studies of plants within the Arecaceae family. For example, Syahmi et al. [32] reported that aqueous leaf extracts of oil palm (*Elaeis guineensis*), another member of the Arecaceae family, demonstrated an LD_{50} greater than 5000 mg/kg BW in rats. Similarly, Lima et al. [23] reviewed the safety profile of coconut (*Cocos nucifera*) and noted that various extracts from this closely related species exhibited minimal acute toxicity. The phylogenetic relationship among Arecaceae species may contribute to similar toxicity profiles, possibly reflecting comparable secondary metabolite compositions and mechanisms of action.

The absence of acute toxicity at doses up to 1600 mg/kg BW suggests that *A. pinnata* leaf extract possesses a wide safety margin for oral administration. This is particularly significant given that traditional medicinal preparations typically employ much lower doses than those tested in this study. For context, if a 70 kg human were to consume an equivalent dose (based on body surface area conversion), the corresponding human equivalent dose for the highest tested dose (1600 mg/kg in rats) would be approximately 258 mg/kg, or roughly 18 grams for a 70 kg individual—a dose far exceeding typical traditional use levels. This substantial safety margin provides reassurance regarding the acute safety of traditionally prepared *A. pinnata* leaf remedies.

The favorable safety profile observed in this study aligns with emerging evidence regarding the therapeutic potential of *Arenga* species. Previous studies have demonstrated various pharmacological activities of *A. pinnata* extracts, including anti-nociceptive and anti-inflammatory effects [9], antioxidant activity [10], and lipid-lowering properties [25]. The low acute toxicity observed in the present study complements these pharmacological findings, suggesting that *A. pinnata* leaves can be developed as a relatively safe source of bioactive compounds for therapeutic applications.

However, it is noteworthy that different extraction solvents may yield different phytochemical profiles and, consequently, different toxicity profiles. Lubis et al. [24] and Rambe et al. [25] investigated ethanol extracts of *A. pinnata* fruit and reported beneficial effects on liver function and lipid profiles in rats, although detailed toxicity parameters were not comprehensively evaluated in those studies. The use of aqueous extraction in the present study is more reflective of traditional preparation methods, which typically involve boiling or steeping plant materials in water, thus providing more directly applicable safety data for traditional use contexts.

While this study provides valuable preliminary safety data, several limitations should be acknowledged. First, the acute toxicity model employed assesses only single-dose exposure effects over a 14-day period and does not provide information regarding potential cumulative toxicity, organ-specific damage, or biochemical alterations that may develop with repeated or prolonged exposure. Sub-chronic (28–90 days) and chronic (>90 days) toxicity studies are necessary to establish comprehensive safety profiles for long-term use [11,12,15,33].

This study utilized only male Wistar rats, which limits the generalizability of findings. Sex-specific differences in drug metabolism, hormonal influences, and toxicokinetics may result in different toxicity profiles between males and females. Future studies should include both sexes to ensure comprehensive safety assessment across populations. While no gross pathological abnormalities were observed, histopathological examination of tissues and assessment of biochemical parameters (liver enzymes, renal function markers, hematological parameters) were not performed in this study. Such analyses are essential for detecting subtle organ damage or metabolic disturbances that may not be evident through macroscopic examination alone [13,14].

Despite these limitations, the findings of this study have important implications for both traditional medicine practices and modern pharmaceutical development. The demonstrated presence of multiple bioactive compounds with established pharmacological properties, combined with the favorable acute safety profile, provides scientific validation for the traditional use of *A. pinnata* leaves in herbal medicine. These data can inform traditional practitioners about safe dose ranges and support the standardization of herbal preparations.

Furthermore, the low acute toxicity and presence of antioxidant and anti-inflammatory phytochemicals suggest potential applications in managing conditions associated with oxidative stress and inflammation, such as diabetes mellitus, cardiovascular disease, and age-related degenerative disorders. The immunomodulatory activity previously reported for this extract [30], combined with its safety profile, positions *A. pinnata* leaves as a promising candidate for development as a natural immunostimulatory supplement.

Conclusion

This study demonstrates that the aqueous extract of *A. pinnata* leaves contains multiple bioactive compounds—alkaloids, flavonoids, tannins, and triterpenoids—with established pharmacological potential. The LD_{50} exceeding 1600 mg/kg BW and the absence of acute toxicity signs indicate a favorable preliminary safety profile for oral administration. These findings provide a scientific foundation for the traditional use of *A. pinnata* leaves and support further investigation into their therapeutic applications, while highlighting the need for comprehensive long-term safety studies before widespread clinical translation.

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Author contributions

UU: Conceptualization, Methodology, Writing – Original Draft. HHMZ, UU: Data Curation, Formal Analysis, Visualization. UU, SPB, RR: Data Collection. UU, HHMZ, AA: Investigation, Resources, Validation. UU, HHMZ, AA: Supervision, Writing – Review & Editing.

Declaration of interest

The authors declare that none of them has any conflict of interest with any private, public or academic party related to the information contained in this manuscript.

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