

EVALUATION OF ANTIOXIDANT ACTIVITY AND ACUTE TOXICITY OF KALANCHOE PINNATA IN VIETNAM

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Received: 16/6/2026; Reviewed: 20/6/2026; Revised: 26/6/2026; Accepted: 29/6/2026

DOI: <https://doi.org/10.58902/tcnckhpt.v5i2.388>

Abstract: *Kalanchoe pinnata* is a widely distributed medicinal plant long utilized in traditional medicine in Vietnam. This study comprehensively evaluates the total antioxidant capacity (TAC) of the hydroalcoholic extract from fresh leaves of *K. pinnata* collected in Vietnam using integrated quantified analytical models, alongside acute toxicity screening of its solvent fractions. Antioxidant activities were evaluated via Folin-Ciocalteu assay (FCR), ferric reducing antioxidant power (FRAP), cupric ion reducing antioxidant capacity (CUPRAC), and the phosphomolybdenum (PM) assay. Methodological reliability for all quantitative spectrophotometric responses was strictly monitored through systematic standard calibration, baseline zeroing, and triplicate analysis. Acute toxicity was assessed using the Zebrafish (*Danio rerio*) acute toxicity bioassay according to OECD 203 guidelines. The results indicated that *K. pinnata* extract possesses concentration-dependent reducing activity, which are highly concentration-dependent and linked to endogenous polyphenol content. Notably, in the bioactivity screening, the Ethyl Acetate fraction exhibited the highest acute toxicity against Zebrafish with an LC50 value of 34.2 µg/mL, suggesting a strong concentration of bioactive secondary metabolites. These findings suggest that Vietnamese *K. pinnata* leaves may serve as a promising source of antioxidant constituents and bioactive fractions for further bioassay-guided phytochemical investigation.

Keywords: Zebrafish; Antioxidant; Acute toxicity; *Kalanchoe pinnata*.

1. Introduction

Kalanchoe pinnata (Crassulaceae family), locally known as "Cay Thuoc Bong", is a perennial herb that thrives in tropical climates. In Vietnam, owing to its remarkable viability and high adaptability, *K. pinnata* is considered a valuable indigenous medicinal plant, growing wild and widely cultivated from lowland plains to mountainous regions (Nkollo, et al. 2025). According to long-standing experience in traditional medicine, crushed fresh leaves of *K. pinnata* are commonly applied topically to treat burns and heal infected wounds, or decocted for oral administration to support the treatment of gastric ulcers (Singh, et al. 2022).

Despite its wide spectrum of applications, the efficacy of this medicinal material depends heavily on climatic factors, soil conditions, and ecological pressures of specific geographical regions, leading to variations in the content and

activity of its bioactive compounds (Qaderi, et al. 2023). While international studies have thoroughly documented the chemical profiles of *K. pinnata* populations in Africa and South America, there remains a critical knowledge gap regarding the specific chemical and biological properties of *K. pinnata* acclimatized to the distinct monsoon tropical climate and soil chemistry of Northern Vietnam (Stefanowicz-Hajduk et al., 2023; Singh et al., 2019; Biswas, 2011). To optimize the value of this species, conducting quantified evaluations to systematize physicochemical data and accurately determine the biosafety profiles of domestic *K. pinnata* populations is warranted an urgent requirement (Yang, et al. 2018).

Therefore, this study was conducted to map the physicochemical activities and determine the acute toxicity of *K. pinnata* populations adapted to the specific natural conditions of Vietnam

(Nkollo, et al. 2025). The obtained results not only establish a scientific rationale for traditional remedies but also serve as a provides a preliminary basis for the targeted downstream fractionation of highly active pharmaceutical compounds to develop safe and high-value healthcare products in the future.

2. Research overview

Phytochemical studies globally have demonstrated that species belonging to the *Kalanchoe* genus possess an exceptionally rich and highly bioactive secondary metabolic profile. Literature reports suggest that this chemical diversity is characterized by the presence of various compound classes including flavonoids (such as quercetin glycosides, kaempferol glycosides, and luteolin), phenolic acids (caffeic acid, p-coumaric acid, and ferulic acid), and highly polar bufadienolides (such as bryophyllin A, bryophyllin C, and bersaldegenin derivative scaffolds). These specialized molecules have been documented in international literature to possess diverse pharmacological profiles, including potential anti-proliferative, anti-inflammatory, and specific free-radical scavenging activities (Nascimento, et al. 2023; Pattewar and Research 2012).

However, the concentration and accumulation of these secondary metabolites within plant tissues are not static; rather, they are tightly regulated by geo-ecological factors, including soil characteristics, temperature ranges, solar radiation, and biotic pressures of each native region (Qaderi, et al. 2023). The majority of previously published works were predominantly conducted on plant specimens collected from other continents with vastly different climates (such as Africa, South America, or South Asia). Meanwhile, the bioactivity profiles and chemical architectures of *K. pinnata* populations long adapted to Vietnam remain a major knowledge gap requiring systematic investigation (Yang, et

al. 2018).

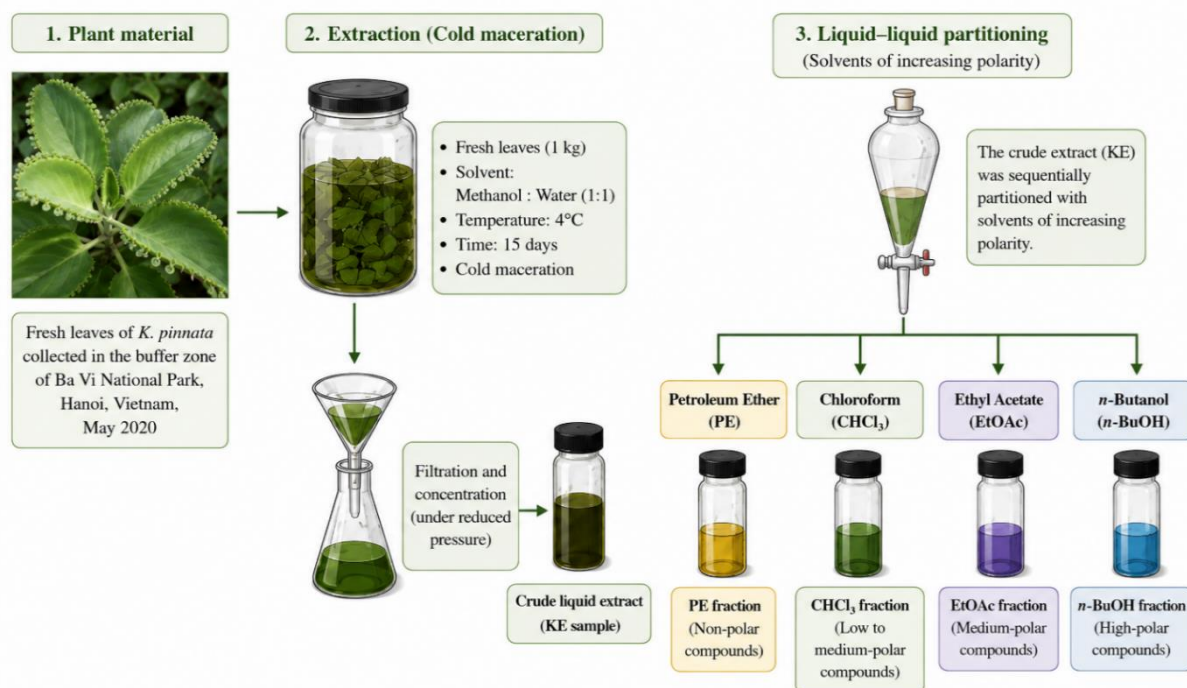
In particular, the comprehensive evaluation of the antioxidant capacity of this medicinal species in Vietnam has not yet been deeply explored through modern Single Electron Transfer (SET) mechanisms, represented by the CUPRAC (Cupric Reducing Antioxidant Capacity) method (Apak, et al. 2004). This technique offers high reliability and stability due to its optimal reaction capability at near-physiological pH and its accurate measurement of both lipophilic and hydrophilic antioxidants, thereby overcoming the limitations of traditional assays. Linking this SET activity mapping with acute bio-toxicity screening on experimental aquatic animal models such as zebrafish (*Danio rerio*) under standardized international validation frameworks will provide a rapid, visual dual-screening dataset (Modarresi Chahardehi, et al. 2020). This provides the core kinetic and biological rationale, serving as a "guiding compass" for subsequent bio-guided isolation of highly active pure compounds intended for drug development.

3. Research methods

3.1. Materials and Extraction

The fresh leaves of *K. pinnata* were collected in the buffer zone of Ba Vi National Park, Hanoi, Vietnam, in May 2020, and taxonomically identified by Dr. Nguyen Anh Duc (Faculty of Biology, VNU University of Science). A voucher specimen (Code: KP 01) has been deposited in the botanical collection of Ba Vi National Park, Hanoi, Vietnam. Fresh leaves (1 kg) were macerated in a Methanol: Water (1:1) solvent mixture at 4°C for 15 days to maximize the preservation of thermolabile phenolic compounds. The crude liquid extract (KE sample) was subjected to liquid-liquid partitioning with solvents of increasing polarity: Petroleum Ether (PE), Chloroform (CHCl₃), Ethyl Acetate (EtOAc), and n-Butanol (n-BuOH).

Figure 1. Schematic representation of the extraction and solvent partitioning procedure for *Kalanchoe pinnata* leaf extract (KE) and its fractions



3.2. Antioxidant Activity

To ensure rigorous Quality Assurance/Quality Control (QA/QC), all physicochemical assays were conducted in triplicate ($n = 3$) under temperature-controlled laboratory conditions ($25 \pm 1^\circ\text{C}$). All spectrophotometric measurements were performed using a calibrated Shimadzu UV-1800 spectrophotometer, and blank solutions containing only solvents and reagents were utilized to zero the instrument before each measurement series.

FCR (Folin-Ciocalteu Reagent) Assay: This assay was utilized as a primary screening tool for electron-donating capacity. Based on the reduction of the Folin-Ciocalteu reagent by phenolic compounds in an alkaline medium, generating a blue complex with maximum absorbance at a wavelength of 765 nm. A standard calibration curve of Gallic Acid (10 -100 $\mu\text{g/mL}$) was established to express the total phenolic content quantitatively as milligrams of Gallic Acid Equivalent per gram of dried extract (mg GAE/g).

FRAP (Ferric Reducing Antioxidant Power) Model: Measures the Single Electron Transfer (SET) capability via the reduction of a ferric complex Fe^{3+} to a ferrous complex Fe^{2+} , producing an intense Prussian blue color measured

at 700 nm. Results were normalized and expressed as mg Trolox Equivalent per gram of dried extract (mg TE/g) via a Trolox standard curve (20 - 200 $\mu\text{g/mL}$).

CUPRAC (Cupric Ion Reducing Antioxidant Capacity) Assay: Utilizes a Cu(II)-Neocuproine complex in a neutral physiological buffer medium ($\text{pH} = 7.0$) to measure the cupric ion reducing capacity at 450 nm. Activity was quantitatively converted to mg Trolox Equivalent per gram of dried extract (mg TE/g).

Phosphomolybdenum (PM) Method: Evaluates the Total Antioxidant Capacity (TAC) based on the reduction of Molybdenum Mo(VI) to Mo(V) in an acidic environment at 95°C for 90 minutes, with spectrophotometric measurement at 695 nm. Results were quantified as mg Trolox Equivalent per gram of dried extract (mg TE/g).

3.3. Toxicity Evaluation

Acute toxicity was evaluated based on the mortality rate of the Zebrafish (*Danio rerio*) model after 96 hours of exposure according to the standardized OECD 203 guideline. Wild-type adult zebrafish (aged 3 - 4 months, average weight 350 ± 50 mg, body length 3.0 ± 0.5 cm) were acclimated for 14 days in dechlorinated water under a continuous 14h light/10h dark

photoperiod, temperature maintained at $25 \pm 1^\circ\text{C}$, pH 7.2 ± 0.2 . Tests were performed in 5L glass tanks containing 10 fish per group ($n = 10$) exposed to a wide concentration range of each solvent fraction (10, 50, 100, 200, 400, and 500 $\mu\text{g/mL}$). A negative control (system water containing 0.1% DMSO) and a positive control (Vincristine Sulfate) were run simultaneously. Dead fish, identified by the absence of visible gill movement and lack of response to mechanical touching, were recorded and removed at 24, 48, 72, and 96 hours. The median lethal concentration (LC_{50}) along with its 95% confidence intervals (95% CI) was calculated and analysis via PRISM 8.

3.4. Data Analysis and Statistical Processing

Statistical analyses were performed using PRISM 8 software. All quantitative experimental data are expressed as Mean \pm Standard Deviation (SD) ($n = 3$ for chemical assays and $n = 10$ for animal bioassays). Statistical significance was determined using Student's *t*-test or ANOVA combined with Bonferroni's post-hoc test ($p < 0.05$).

4. Research results

Table 1. Total phenolic reducing capacity (FCR assay) of the KE sample and the GA standard

Sample Volume (μL)	Absorbance of KE (A_{765})	Absorbance of GA (A_{765})	Calculated TPC value (mg GAE/g extract)
100	0.418 ± 0.03	0.155 ± 0.01	41.15 ± 1.82
200	0.742 ± 0.05	0.308 ± 0.02	43.24 ± 2.04
300	1.156 ± 0.09	0.452 ± 0.03	45.18 ± 1.95
400	1.395 ± 0.11	0.585 ± 0.02	45.42 ± 2.26
500	1.764 ± 0.14	0.728 ± 0.04	45.22 ± 2.15

4.2. Ferric Reducing Capacity via FRAP Model

The FRAP model is widely applied to evaluate antioxidant capacity via the Single Electron Transfer (SET) mechanism. This assay directly measures the ability of bioactive components in the KE extract to reduce the ferric complex into an intense blue-colored ferrous complex at a wavelength of 700 nm. The ferric reducing power of the extract at the highest investigated volume was converted to 38.65 ± 1.48 mg TE/g extract. From a volume concentration of 400 μL onwards,

4.1. Evaluation of Total Phenolic Content (TPC) via FCR Assay

In this study, the reducing capacity tightly coupled with the total phenolic content of the hydroalcoholic extract from Vietnamese *K. pinnata* fresh leaves (KE sample) was quantified based on the reduction of the Folin-Ciocalteu reagent (FCR), directly referenced against the Gallic Acid (GA) standard. The calculated total phenolic content of the KE sample reached 45.22 ± 2.15 mg GAE/g dried extract. The results indicated that the Absorbance (A) of the KE sample consistently maintained a prominently higher level compared to the Gallic Acid standard across all investigated volume ranges ($p < 0.01$). At the maximum volume of 500 μL , the A value of KE reached 1.764, which was 2.42-fold higher than that of GA (0.728), at the equivalent concentration volume, demonstrating strong reducing characteristics. It is critical to note that the FCR assay measures total reducing capacity, meaning non-phenolic reducing agents natively present in the fresh leaf matrix - such as ascorbic acid, reducing sugars, and free amino acids - may also contribute to this final absorbance signal.

the A value of the KE sample increased sharply and formally surpassed Ascorbic Acid, peaking at 0.642 at the 500 μL dose (compared to 0.438 for AA), confirming the robust electron-donating characteristics of the extract. This initial comparison, based on the absolute optical absorbance at corresponding tested volumes, indicates a robust concentration-dependent electron-donating activity within the extract matrix.

Table 2. Ferric reducing antioxidant power (FRAP) of KE extract compared to Ascorbic Acid (AA)

Sample Volume (μL)	Absorbance of KE (A_{700})	Absorbance of AA (A_{700})	Quantified Activity (mg TE/g extract)
100	0.392 ± 0.05	0.308 ± 0.01	24.12 ± 1.05
200	0.415 ± 0.04	0.345 ± 0.02	28.34 ± 1.12
300	0.428 ± 0.06	0.392 ± 0.01	32.56 ± 1.35
400	0.495 ± 0.09	0.424 ± 0.02	35.48 ± 1.22
500	0.642 ± 0.12	0.438 ± 0.01	38.65 ± 1.48

4.3. Cupric Ion Reducing Capacity via CUPRAC Physicochemical Assay

The CUPRAC assay was conducted in a physiological pH environment ($\text{pH} = 7.0$), allowing a more accurate reflection of biological interactions that may occur within living organisms. The final calculated CUPRAC activity for the KE extract was 58.42 ± 3.12 mg TE/g extract. The CUPRAC assay recorded the most

statistically significant difference. At all measurement points, the absorbance of the *K. pinnata* extract was significantly higher than that of Ascorbic Acid ($p < 0.001$), peaking at 2.654 at the 500 μL dose. These raw optical density trends highlight the substantial metal-reducing capacity of the crude extract under the assigned volumetric conditions.

Table 3. Cupric ion reducing capacity (CUPRAC) of KE extract compared to Ascorbic Acid (AA)

Sample Volume (μL)	Absorbance of KE (A_{450})	Absorbance of AA (A_{450})	Quantified Activity (mg TE/g extract)
100	0.945 ± 0.04	0.458 ± 0.01	40.15 ± 1.95
200	1.468 ± 0.05	0.518 ± 0.02	48.64 ± 2.15
300	1.982 ± 0.06	0.574 ± 0.01	52.34 ± 2.84
400	2.245 ± 0.03	0.628 ± 0.02	55.12 ± 2.45
500	2.654 ± 0.04	0.684 ± 0.03	58.42 ± 3.12

4.4. Quantification of Total Antioxidant Capacity (TAC) by Phosphomolybdenum Method

The Phosphomolybdenum (PM) method evaluates total antioxidant activity based on the reduction of Mo(VI) to Mo(V) in an acidic pH environment driven by prolonged thermal

activation (95°C for 90 minutes). Data from table 4 reaffirmed that the total antioxidant capacity (TAC) of the KE sample reached a converted standard value of 72.18 ± 4.25 mg TE/g extract, exhibiting higher absorbance values compared to the ascorbic acid control at equivalent volume doses ($p < 0.01$).

Table 4. Total antioxidant capacity via Phosphomolybdenum (PM) assay

Sample Volume (μL)	Absorbance of KE (A_{695})	Absorbance of AA (A_{695})	Quantified Activity (mg TE/g extract)
100	0.345 ± 0.02	0.135 ± 0.01	45.12 ± 2.18
200	0.722 ± 0.04	0.318 ± 0.03	52.64 ± 3.11

Sample Volume (μL)	Absorbance of KE (A_{695})	Absorbance of AA (A_{695})	Quantified Activity (mg TE/g extract)
300	1.094 ± 0.03	0.515 ± 0.02	61.35 ± 2.94
400	1.412 ± 0.04	0.842 ± 0.03	68.42 ± 3.52
500	1.715 ± 0.06	1.108 ± 0.02	72.18 ± 4.25

4.5. Acute Toxicity Screening on Zebrafish (*Danio rerio*) Model

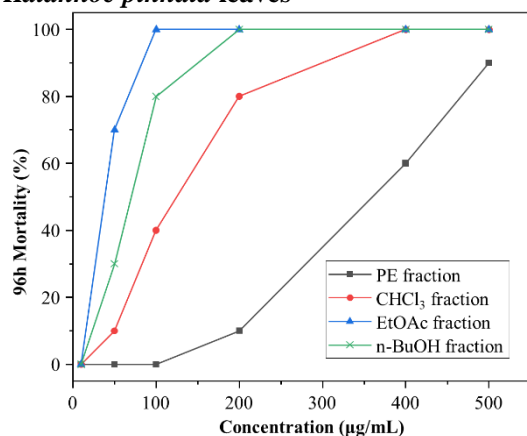
The negative control group (0.1% DMSO) maintained a 0% mortality rate throughout the 96-hour experiment, validating the baseline health of the test system. The acute toxicity of the four obtained fractions was evaluated based on the mortality rate of the model organism Zebrafish (*Danio rerio*) after 96 hours of continuous

exposure to determine the median lethal concentration (LC_{50}). The Ethyl Acetate (EtOAc) fraction possessed the lowest LC_{50} value ($34.2 \pm 2.1 \mu\text{g/mL}$), demonstrating the strongest acute potency, which was 3.45-fold higher than the Chloroform fraction and up to 10.47-fold higher than the Petroleum Ether fraction, verifying that highly potent bioactive components are concentrated within this phase.

Table 5. LC_{50} values of Vietnamese *Kalanchoe pinnata* leaf fractions on the Zebrafish model

No.	Solvent Fraction	96h Mortality at 100 $\mu\text{g/mL}$ (%)	LC_{50} ($\mu\text{g/mL}$)	Toxicity Potency Assessment
1	Petroleum Ether (PE) fraction	0%	358.4 ± 12.6	Low toxicity / Insignificant
2	Chloroform (CHCl_3) fraction	40%	118.2 ± 8.5	Moderate toxicity
3	Ethyl Acetate (EtOAc) fraction	100%	34.2 ± 2.1	High toxicity (Strongest)
4	n-Butanol (n-BuOH) fraction	80%	75.6 ± 4.8	High toxicity
5	Positive Control (Vincristine Sulfate)	100% (at 5 $\mu\text{g/mL}$)	0.84 ± 0.03	Extreme toxicity

Figure 2. 96h Mortality of Zebrafish model exposed to different fractions of Vietnamese *Kalanchoe pinnata* leaves



5. Discussion

The simultaneous integration of four physicochemical antioxidant assays (FCR, FRAP, CUPRAC, and PM) alongside a biological systemic monitoring model provides a systematic evaluation of *Kalanchoe pinnata* adapted to the specific ecological conditions of Vietnam. The baseline data generated in this study maps the concentration-dependent electron-donating profile of the leaf extract, showing that the low-temperature cold maceration process effectively preserved the matrix's functional properties, as evidenced by a steady increase in absorbance across all models (tables 1 - 4). Within the multi-layered mechanistic framework, the extract exhibited

prominent single-electron transfer (SET) kinetics across diverse experimental conditions (Huang et al. 2005). Particularly, the high spectrophotometric response observed in the CUPRAC model at physiological pH 7.0 (table 3), which peaked at an absorbance of 2.654 at the 500 μ L dose, suggests that the extract's active constituents remain stable and structurally optimized to exert reducing capabilities under near-physiological environments. This strong anti-oxidative response is well aligned with the acute toxicity profiles derived from the zebrafish (*Danio rerio*) bioassay under the OECD 203 framework (table 5 and figure 2), wherein the intermediate-polarity ethyl acetate fraction was identified as the highly active phase, yielding the lowest LC₅₀ value of 34.2 ± 2.1 μ g/mL. (Apak, et al. 2004).

Despite these promising insights, we recognize that this screening-level study has certain technical limitations that warrant precise clarification. First, while the FCR assay indicates a high concentration of reducing matrices - quantified at 45.22 ± 2.15 mg GAE/g dried extract at the maximum evaluated volume (table 1) - this colorimetric method inherently captures total reducing capacity rather than exclusive phenolic footprints. Because the Folin-Ciocalteu reagent is vulnerable to cross-reactivity with other endogenous electron-donating components natively present in fresh leaves - such as organic acids, reducing sugars, and free amino acids - the reported values represent an upper bound of the sample's reducing traits (Pérez et al., 2023). Second, a direct mass-for-mass or mol-for-mol potency advantage over pure ascorbic acid cannot be conclusively claimed based solely on the raw optical density trends recorded at equivalent tested volumes, such as the extract formally surpassing ascorbic acid from 400 μ L onwards in the FRAP model (table 2). Because the crude extract is a complex, multi-component mixture, these relative differences were not normalized against individual active compound weights or exact molar concentrations (Prior et al., 2005). Lastly, since this initial phase of our research relied primarily on spectrophotometric screening, direct high-performance liquid

chromatography (HPLC-DAD) or liquid chromatography-mass spectrometry (LC-MS/MS) profiling was not conducted. Consequently, attributing the observed biological and toxicological profiles to specific targeted compound classes - such as flavonoids, triterpenoids, or bufadienolides - remains a deductive hypothesis derived from established literature rather than a direct experimental conclusion of this specific dataset (Nascimento, Casanova et al. 2023).

To strengthen the scientific rigor of these findings and overcome the limitations, our future research roadmap will prioritize two critical trajectories. Characterization will be expanded by employing advanced hyphenated separation platforms (HPLC-MS/MS and NMR) to definitively isolate, identify, and quantify the specific secondary metabolites residing within the bioactive Ethyl Acetate fraction (Cieřla & Moaddel, 2016; Seger et al., 2013). Furthermore, to bridge the gap between systemic acute toxicity in lower vertebrates and therapeutic translation, downstream investigations will transition into targeted in vitro cellular cytotoxicity assays using mammalian and human cell lines to evaluate selective anti-proliferative and cellular antioxidant signaling pathways. This stepwise validation strategy is consistent with the broader literature showing that *K. pinnata* contains multiple bioactive classes with both antioxidant and cytotoxic potential, supporting a bioassay-guided fractionation workflow (Modarresi Chahardehi, Arsad et al. 2020).

Nevertheless, the immediate significance of this study remains highly valuable as it meaningfully contributes to the global and regional research landscape of this medicinal species. By mapping the specific bioactivity profile across five comprehensive datasets (tables 1–5) and establishing a verified toxicological safety baseline for the *K. pinnata* population acclimatized to Northern Vietnam, these findings complement existing geographical data on the species' phytochemistry and bioactivity. By verifying that the intermediate-polarity solvent phase effectively isolates the highly active

constituents, this work serves as an essential biological guide for downstream fractionation and isolation (Sarker & Nahar, 2012). It provides the mandatory experimental justification and standardizes the screening logistics required to initiate targeted, bioassay-guided discovery of high-value pharmaceutical derivatives from this abundant domestic medicinal resource (Modarresi Chahardehi, Arsad et al. 2020).

6. Conclusion

This study successfully establishes the baseline antioxidant profiles and acute toxicological thresholds of *Kalanchoe pinnata* acclimatized to the distinct ecological conditions of Vietnam. Through an integrated panel of multi-mechanism spectrophotometric models, the hydroalcoholic leaf extract demonstrated robust, concentration-dependent radical-scavenging and metal-reducing capabilities, characterized by a maximum total phenolic reducing value of $45,22 \pm 2,15$ mg

GAE/g (table 1) and a total antioxidant capacity of $72,18 \pm 4,25$ mg TE/g (table 4). Furthermore, the systemic biological screening in the Zebrafish (*Danio rerio*) model effectively identified the intermediate-polarity Ethyl Acetate fraction as the primary reservoir for the plant's highly potent bioactive constituents, exhibiting a prominent acute toxicity threshold with an LC_{50} value of $34,2 \pm 2,1$ μ g/mL (table 5). While further chromatographic quantification and targeted in vitro cell-line assays are mandatory to map the definitive phytochemical architectures and selective mechanisms, these screening benchmarks meaningfully enrich the regional research landscape of this medicinal species. Consequently, this work provides the essential experimental rationale and a strategic toxicological compass to guide future bioassay-directed discovery of high-value pharmaceutical derivatives from this abundant domestic resource.

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ĐÁNH GIÁ HOẠT TÍNH CHỐNG OXY HÓA VÀ ĐỘC TÍNH CẤP TÍNH CỦA CÂY KALANCHOE PINNATA TẠI VIỆT NAM

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Ngày nhận bài: 16/6/2026; Ngày phản biện: 20/6/2026; Ngày tác giả sửa: 26/6/2026;

Ngày duyệt đăng: 29/6/2026

DOI: <https://doi.org/10.58902/tcnckhpt.v5i2.388>

Tóm tắt: *Kalanchoe pinnata* là một loài cây thuốc phân bố rộng rãi và đã được sử dụng từ lâu trong y học cổ truyền tại Việt Nam. Nghiên cứu này đánh giá toàn diện tổng năng lực chống oxy hóa (Total Antioxidant Capacity – TAC) của dịch chiết hydroalcol từ lá tươi *K. pinnata* thu hái tại Việt Nam bằng các mô hình phân tích tiên tiến tích hợp, đồng thời khảo sát độc tính cấp tính của các phân đoạn dung môi thu được. Hoạt tính chống oxy hóa được đánh giá thông qua các phương pháp Folin-Ciocalteu (FCR), khả năng khử sắt (FRAP), khả năng khử ion đồng (CUPRAC) và phép thử phosphomolybdenum (PM). Độ tin cậy của phương pháp các phản ứng định lượng quang phổ được kiểm soát chặt chẽ thông qua đường chuẩn tiêu chuẩn, hiệu chuẩn nền và phân tích lặp lại ba lần. Độc tính cấp tính được xác định bằng thử nghiệm độc tính cấp trên cá ngựa vằn (*Danio rerio*) theo hướng dẫn OECD 203. Kết quả cho thấy dịch chiết *K. pinnata* có hoạt tính khử phụ thuộc vào nồng độ, với mức độ chống oxy hóa tăng theo nồng độ và có mối liên hệ chặt chẽ với hàm lượng polyphenol nội sinh. Đáng chú ý, trong quá trình sàng lọc hoạt tính sinh học, phân đoạn ethyl acetat thể hiện độc tính cao nhất đối với cá ngựa vằn với giá trị LC_{50} là 34,2 $\mu\text{g/mL}$, cho thấy sự tập trung cao của các chất chuyển hóa thứ cấp có hoạt tính sinh học. Những phát hiện này cho thấy lá *K. pinnata* tại Việt Nam có thể là nguồn tiềm năng chứa các hợp chất chống oxy hóa và các phân đoạn giàu hoạt tính sinh học, phù hợp cho các nghiên cứu hóa thực vật chuyên sâu định hướng theo hoạt tính sinh học trong tương lai.

Từ khóa: Cá ngựa vằn; Chống oxy hóa; Độc tính cấp tính; *Kalanchoe pinnata*.