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Original Research Paper

Phytochemical Screening, Antioxidant Activity, and Toxicity of *Imperata Cylindrica* Root Extract Using the Brine Shrimp Lethality Test

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Abstract

Background: Oxidative stress contributes to the pathogenesis of chronic diseases, increasing interest in plant derived antioxidants. Imperata cylindrica root is traditionally used, but its antioxidant potential and preliminary toxicity require evaluation. **Objective:** To characterize the phytochemical profile, antioxidant activity, and preliminary toxicity of methanolic I. cylindrica root extract. **Methods:** Dried roots were extracted by maceration and percolation. Qualitative phytochemical screening was performed. Antioxidant activity was evaluated using the DPPH assay at 100–900 µg/mL, while toxicity was assessed using the Brine Shrimp Lethality Test (BSLT) with Artemia salina at 100–1000 µg/mL for 24 hours. IC_{50} and LC_{50} values were estimated by linear regression. **Results:** Twelve groups of secondary metabolites were detected. DPPH inhibition increased from 30.957% to 72.912%, with an IC_{50} of 437.632 µg/mL, indicating weak antioxidant activity. BSLT mortality increased from 13.462% to 98.684%, with an LC_{50} of 290.084 µg/mL, indicating preliminary toxicity. **Conclusion:** I. cylindrica root extract demonstrated weak DPPH radical-scavenging activity and BSLT lethality. These findings do not establish mammalian or human toxicity and require confirmation through cell-based and in vivo toxicological studies.

Keywords: Imperata cylindrica; antioxidant; DPPH; BSLT; phytochemicals.

Introduction

Free radicals that enter the body are one of the main causes that trigger oxidative stress and play a role in the pathogenesis of various chronic diseases[1]. As neutralizing compounds, antioxidants play a role in protecting the body from the damaging effects of free radicals by donating electrons or hydrogen atoms[2]. These highly reactive compounds can form naturally through body metabolism or due to environmental exposure, such as air pollution, radiation, and hazardous chemicals. An excess of these compounds can cause oxidative stress that damages cellular structures and triggers various degenerative diseases, such as cancer, atherosclerosis, and neurodegenerative disorders[3]. To combat free radicals, the body relies on antioxidants from endogenous production as well as external intake from food and medicinal plants[4].

Natural antioxidant sources from plants have become a focus of research because they are considered safer and have potential for development as preventive therapies. One local plant with great potential that has not been extensively explored scientifically is alang-alang (*Imperata cylindrica*). The root part of this plant has been used traditionally for generations in medical practice to address various health conditions[5]. Previous studies have reported that alang alang root contains

various compounds such as tannins, phenols, saponins, flavonoids, steroids, and terpenoids, which are known to have biological activities[6,7]. However, scientific data evaluating the antioxidant activity and toxicity of *Imperata cylindrica* root extract remain limited, creating a significant research gap in validating its traditional use and ensuring its safety.

The scientific evaluation of crude medicinal plant extracts requires attention to both composition and assay context. Qualitative phytochemical screening can indicate which metabolite classes are detectable, but it does not quantify their concentrations or demonstrate that a particular class is responsible for an observed biological effect. Likewise, antioxidant assays such as DPPH measure a defined chemical reaction under laboratory conditions and may vary with solvent, extract concentration, reaction time, and other analytical parameters. Preliminary lethality assays such as BSLT provide a complementary signal of biological activity, but their value is primarily as a screening step. Combining these approaches is therefore useful for identifying whether an extract warrants further study while avoiding the assumption that antioxidant activity is equivalent to therapeutic efficacy or that brine-shrimp lethality is equivalent to human toxicity. This distinction is especially relevant for *I. cylindrica*, for which traditional use and phytochemical diversity have been described but the biological effects of different plant parts and extraction conditions remain variable[7].

This research is necessary to provide a comprehensive scientific assessment of a widely used traditional herbal remedy. Its novelty lies in the integrated approach, which combines phytochemical screening with both antioxidant (DPPH assay) and preliminary toxicity (Brine Shrimp Lethality Test) evaluations of the methanol extract of *I. cylindrica* root. While some studies have examined specific activities, few have provided this combined profile, which is crucial for understanding both the potential benefits and risks associated with its use. This study's contribution is to offer a more balanced view of the plant's pharmacological profile, moving beyond just efficacy to include essential safety parameters.

The main research question this study aims to answer is: What is the phytochemical profile, antioxidant capacity, and toxicity level of the methanol extract of *Imperata cylindrica* root? The objectives are: 1) To identify the phytochemical constituents present in the methanol extract of *I. cylindrica* root; 2) To determine the in vitro antioxidant activity of the extract using the DPPH radical scavenging assay; and 3) To assess the preliminary toxicity of the extract using the Brine Shrimp Lethality Test (BSLT).

The findings of this study are intended to provide an initial laboratory characterization rather than evidence for clinical use. For academia, the combined evaluation of phytochemical groups, DPPH radical-scavenging activity, and BSLT lethality can help identify questions for subsequent fractionation and mechanistic studies. For public health and medical practice, the results should be interpreted cautiously: DPPH describes chemical radical scavenging under in vitro conditions, whereas BSLT is a preliminary lethality screen and does not determine mammalian or human safety. A favorable antioxidant result would therefore still require toxicological confirmation, while a low BSLT LC_{50} would indicate the need for more specific cell based and in vivo evaluation rather than an immediate conclusion of human toxicity. This benefit safety perspective is particularly important for traditionally used plants, because crude extracts contain multiple constituents whose effects may differ according

to extraction method, dose, and biological system.

Materials and Methods

Study Design

This research uses an in vitro experimental design with the aim of evaluating the phytochemical constituents, antioxidant capacity, and preliminary BSLT lethality of *Imperata cylindrica* root extract. This design is suitable for controlled initial screening of plant derived bioactive compounds before confirmatory cell based, in vivo, or clinical investigations.

Sample

The population in this study was the root of *Imperata cylindrica* (alang-alang). The research sample was selected using a purposive sampling method, with inclusion criteria being fresh, mature roots from healthy plants, and exclusion criteria being roots that showed signs of decay or disease. The number of samples was determined based on the requirements for the extraction and subsequent assays, resulting in approximately 85.57 grams of dried simplicia used for this study.

Data Collection Technique

Data were collected through laboratory procedures including extraction, qualitative phytochemical screening, the DPPH assay, and the Brine Shrimp Lethality Test (BSLT). Fresh alang-alang roots were obtained from the Superindo Intercon area, West Jakarta. The samples were cleaned, cut into small pieces, and dried naturally at room temperature for 3-5 days without direct sunlight exposure. The dried simplicia were ground into a fine powder and extracted with methanol using maceration and percolation, after which the extract was concentrated with a rotary evaporator. Qualitative phytochemical screening used standard reagent based tests for the metabolite groups listed in Table 1[8]. For the DPPH assay, extract concentrations of 100, 300, 500, 700, and 900 µg/mL were evaluated, and percentage inhibition was calculated from the reported absorbance data. The BSLT used *Artemia salina* larvae exposed to extract concentrations of 100, 200, 400, 600, 800, and 1000 µg/mL for 24 hours; mortality percentages were then used to estimate LC_{50}[9].

Data Analysis Technique

The collected data were analyzed descriptively. Extraction yield was calculated as the percentage of thick extract relative to dried simplicia. For DPPH, percentage inhibition was regressed against the untransformed extract concentration, consistent with Figure 1. The fitted equation was $y = 0.0494x + 28.381$ ($R^2 = 0.9787$), and IC_{50} was obtained by setting $y = 50$, yielding 437.632 µg/mL. For BSLT, mortality percentage was regressed against $\log_{10}$ concentration, consistent with Figure 2. The fitted equation was $y = 88.856x - 168.81$ ($R^2 = 0.9851$), and LC_{50} was obtained by setting $y = 50$ and back transforming the concentration, yielding 290.084 µg/mL. Microsoft Excel was used for the linear regression calculations. Antioxidant activity and BSLT lethality were interpreted using published IC_{50} and LC_{50} criteria[10].

Ethical Consideration

This study does not require ethical clearance from an institutional review board because it is an in vitro study that does not directly involve human subjects or vertebrate animals. The BSLT uses invertebrate brine shrimp (*Artemia salina*), which is generally not covered by standard animal ethics regulations. However, all procedures were conducted following standard laboratory safety protocols to ensure researcher safety and proper handling of chemical and biological materials.

Result

The initial sample consisted of fresh *Imperata cylindrica* roots, which were processed into 85.57 grams of dried simplicia. Following extraction with methanol using maceration and percolation, 34.46 grams of thick extract was obtained, resulting in an extraction yield of 40.27%.

Phytochemical Screening

The qualitative phytochemical screening of the methanol extract of *I. cylindrica* root revealed the presence of 12 groups of secondary metabolites. The results are summarized in Table 1.

Table 1. Phytochemical Screening Results of *Imperata cylindrica* Root Extract

Phytochemical Test	Reagent/Method	Result
Alkaloids	Mayer	Positive
Flavonoids	NaOH	Positive
Cardiac Glycosides	Keller-Kiliani	Positive
Glycosides	Modified Borntrager	Positive
Saponins	Foam test	Positive
Coumarins	NaOH	Positive
Phenols	Folin-Ciocalteau	Positive
Quinones	H_2SO_4	Positive
Betacyanin	NaOH	Negative
Steroids	Liebermann-Burchard	Positive
Terpenoids	Liebermann-Burchard	Positive
Tannins	Ferric Chloride	Positive
Anthocyanin	NaOH	Positive

Source: *Primary Data 2025*

Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging assay showed a concentration dependent increase in inhibition, from 30.957% at 100 µg/mL to 72.912% at 900 µg/mL. Linear regression using the untransformed concentration produced y = 0.0494x + 28.381 with R^2 = 0.9787. The high coefficient of determination indicates that the increase in DPPH inhibition was strongly associated with increasing extract concentration within the tested range. The resulting IC_{50} was 437.632 µg/mL. Based on the cited classification, this value indicates weak antioxidant activity[11,12]. The detailed values are presented in Table 2 and Figure 1.

Table 2. DPPH Assay Results of *Imperata cylindrica* Root Extract

Concentration (µg/mL)	Absorbance (reported average)	% Inhibition	IC_{50} (µg/mL)
100	0.339	30.957	437.632
300	0.266	45.825	
500	0.221	54.990	
700	0.193	60.692	
900	0.133	72.912	

Source: *Primary Data 2025*

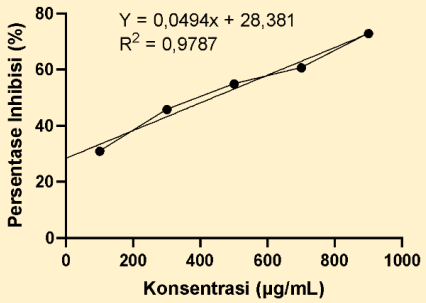

Figure 1. DPPH Radical Scavenging Activity Curve of *Imperata cylindrica* Root Extract

Toxicity (Brine Shrimp Lethality Test)

The BSLT showed increasing Artemia salina mortality with increasing extract concentration, from 13.462% at 100 µg/mL to 98.684% at 1000 µg/mL. Regression of mortality percentage against log_{10} concentration produced y = 88.856x - 168.81 with R^2 = 0.9851. The high coefficient of determination indicates a strong concentration–mortality relationship across the tested extract concentrations. The calculated LC_{50} was 290.084 µg/mL. Under the cited BSLT criterion, an LC_{50} below 1000 µg/mL is classified as toxic in this preliminary lethality assay[9]. This classification is specific to BSLT and should not be interpreted as established mammalian or human toxicity. The results are detailed in Table 3 and Figure 2.

Table 3. BSLT Results of *Imperata cylindrica* Root Extract

Concentration (µg/mL)	Log Concentration	Mortality (%)	LC_{50} (µg/mL)
100	2.00	13.462	290.084
200	2.30	31.915	
400	2.60	56.522	
600	2.78	78.000	
800	2.90	93.333	
1000	3.00	98.684	

Source: *Primary Data 2025*

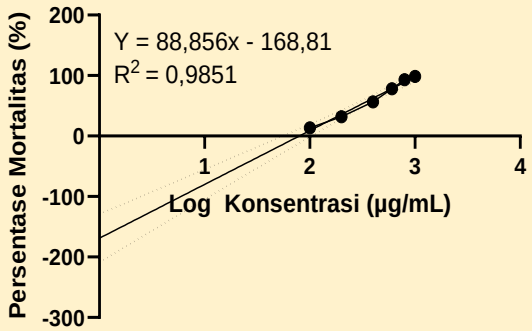

Figure 2. Brine Shrimp Lethality Test (BSLT) Curve of *Imperata cylindrica* Root Extract

Overall, the methanol extract of *Imperata cylindrica* root contained multiple secondary-metabolite groups, including alkaloids, flavonoids, saponins, and phenols. Its DPPH radical-scavenging activity was weak (IC_{50} = 437.632 µg/mL), while its BSLT LC_{50} was 290.084 µg/mL, indicating preliminary lethality within the BSLT classification. These outcomes describe the measured in vitro assays only and do not establish systemic toxicity or clinical safety.

Discussion

This study identified a diverse range of secondary metabolites in the methanol extract of *Imperata cylindrica* root, including alkaloids, flavonoids, cardiac glycosides, saponins, phenols, steroids, and terpenoids. These findings are consistent with Maharini et al[13] and Jung and Shin[7], who also reported bioactive constituents in *Imperata* extracts. Such compounds have been associated with antioxidant, anti-inflammatory, and other biological activities[14]. Flavonoids and phenolic compounds can contribute to radical scavenging activity through electron or hydrogen donation mechanisms[2,15]. However, the Folin Ciocalteu entry in Table 1 is a qualitative positive reaction only; it does not constitute a quantitative total phenolic-content assay. Therefore, no total phenolic content value is claimed in this study.

The extraction yield of 40.27% indicates that a substantial fraction of the dried root material was recovered in the methanol extract, but extraction yield should not be interpreted as a measure of antioxidant potency or of any specific secondary metabolite. Methanol can solubilize a broad range of polar and moderately polar constituents, so a crude methanol extract may contain compounds with different and even opposing biological effects. The qualitative detection of phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, glycosides, coumarins, quinones, and anthocyanins therefore provides a compositional profile rather than quantitative evidence of abundance. This is important when interpreting the weak DPPH result: a positive reagent reaction for phenols or flavonoids does not imply that these constituents are present at a concentration sufficient to produce strong radical scavenging. It also does not exclude interactions among constituents in a crude matrix. Future quantitative work should therefore measure total phenolic and flavonoid content with calibration curves and report the result in an appropriate equivalent unit, while chromatographic methods are needed to identify individual compounds[2,15].

The DPPH assay yielded an IC_{50} of 437.632 µg/mL, which was classified as weak. Munadi et al[16] reported substantially stronger activity for a methanol extract/fractions of *Imperata cylindrica* leaves, including an IC_{50} of 14.786 µg/mL for the methanol extract.

Differences in plant part, solvent system, extraction conditions, geographic origin, and the concentration of antioxidant constituents can substantially influence measured bioactivity[17]. Dhianawaty and Ruslin[18] reported an IC_{50} of 0.32 mg/mL (320 µg/mL) for a methanol root extract, which is closer to the present value but still indicates inter-study variability. Because phytochemical screening in the present study was qualitative, the weak DPPH activity cannot be attributed to a measured concentration of any single phenolic or flavonoid constituent.

Direct comparison of IC_{50} values between studies should also be made cautiously because DPPH estimates are protocol-dependent. The apparent IC_{50} can be influenced by the concentration of the DPPH reagent, the solvent system, wavelength, reaction or incubation time, blank correction, reference antioxidant, and the range of extract concentrations used for regression[10,15]. In the present dataset, the concentration-response relationship was strong at the descriptive level (R^2 = 0.9787), and the reported IC_{50} is mathematically consistent with the fitted equation. Nevertheless, the original laboratory record available for this manuscript contained only reported average absorbance values and did not retain replicate level readings or control details. Consequently, the precision of the IC_{50} estimate cannot be expressed as a confidence interval or standard deviation, and the result should be interpreted as a point estimate from the available experiment rather than as a fully replicated comparative potency measure. Confirmatory experiments should prespecify technical replicates, report mean ± SD, include a recognized positive control such as ascorbic acid or Trolox, and document all spectrophotometric conditions so that the assay can be reproduced and compared reliably across laboratories.

The BSLT produced an LC_{50} of 290.084 µg/mL, which meets the commonly used criterion for toxicity in this preliminary lethality screen[9,19]. BSLT is useful for rapid screening of biologically active or potentially cytotoxic plant extracts, but it is not a substitute for mammalian toxicology and cannot establish safety or harm in humans. Some plant constituents, including saponins and alkaloids, may contribute to biological activity and can also produce toxic effects at particular concentrations[20]. Accordingly, the present result should be interpreted as a signal for further toxicological evaluation rather than evidence that the crude extract is unsafe for human consumption.

The concentration-response pattern in the BSLT was monotonic, and the regression of mortality on $\log_{10}$ concentration showed a high descriptive fit (R^2 = 0.9851). This supports the internal mathematical calculation of the reported LC_{50} but does not by itself establish biological precision, because an R^2 value summarizes the fit of the plotted percentages rather than variation among independent replicate experiments. Classical BSLT methodology uses brine-shrimp lethality as a convenient general screen for biologically active natural products[19], while comparative work has shown that brine shrimp assays are screening tools rather than direct surrogates for toxicity in more complex organisms[19]. For that reason, the LC_{50} of 290.084 µg/mL is appropriately described as toxic in the BSLT or as preliminary lethality/cytotoxicity. It should not be converted into a predicted human dose, a clinical contraindication, or a statement that traditional preparations are unsafe. A stronger toxicological inference would require standardized cell based testing, characterization of exposure to identified constituents, and in vivo studies with appropriate species, dose selection, clinical observation, hematologic and

biochemical measurements, and histopathology.

The principal implication of these findings is methodological and hypothesis generating. The weak DPPH activity indicates that the crude methanol root extract was not a strong radical scavenger under the conditions tested, while the BSLT result identifies a concentration range that warrants confirmatory investigation. Bioactivity guided fractionation could determine whether antioxidant and lethality signals arise from the same constituents or from different fractions. Safety conclusions should await more specific cell based assays and appropriately designed in vivo studies, including dose response evaluation and characterization of target-organ effects. Thus, the present study supports further investigation of *I. cylindrica* rather than direct therapeutic or public health recommendations.

The choice of a methanol crude extract also limits the extent to which these findings can be transferred to customary preparations of alang-alang. Traditional use may involve water based decoctions, mixed herbal formulations, different plant to solvent ratios, or substantially different exposure durations, whereas methanol is primarily an analytical extraction solvent that can recover constituents not present at the same concentration in traditional preparations. Therefore, neither the DPPH IC_{50} nor the BSLT LC_{50} should be assumed to represent the activity or safety profile of a household preparation. At the same time, methanol extraction remains useful in early natural product research because it provides broad recovery of phytochemical constituents and can reveal compounds that merit subsequent isolation[2,17]. A useful translational strategy would compare the present methanol extract with preparations that more closely reflect traditional use, while maintaining standardized plant identification, drying conditions, particle size, solvent ratio, extraction time, and final concentration. Such comparisons could determine whether the weak antioxidant activity and preliminary BSLT lethality are properties of the root itself, consequences of the methanol extraction process, or features of particular chemical fractions. Reporting these preparation variables is therefore essential for distinguishing laboratory screening results from conclusions about real world herbal exposure.

A logical next phase is therefore a staged experimental program. First, the crude extract should be reproduced under a fully documented extraction protocol and tested with replicate DPPH and complementary antioxidant assays such as ABTS or FRAP. Second, total phenolic and total flavonoid contents should be quantified rather than inferred from qualitative screening, followed by chromatographic profiling to identify dominant constituents. Third, bioactivity guided fractionation can determine whether the fractions with the greatest radical-scavenging activity are the same fractions that produce BSLT lethality. Fourth, selected fractions or isolated compounds should be evaluated in mammalian cell systems to distinguish general cytotoxicity from more specific biological actions. Only after these steps should in vivo toxicological work be used to investigate exposure-response relationships and systemic effects. This sequence would allow the potential antioxidant signal and the preliminary lethality signal to be separated analytically and would provide a more defensible basis for any later discussion of therapeutic relevance or safety.

The antioxidant and BSLT findings should also be considered as separate endpoints rather than combined into a single judgment of benefit or risk. A crude extract can show weak radical scavenging activity while still producing lethality in a simple biological screen because different constituents may dominate the two responses. Conversely, a fraction with stronger

antioxidant activity is not necessarily less toxic. The present data therefore do not support a benefit risk ratio in the clinical sense; they support only the observation that, under the conditions tested, the crude methanol root extract required a relatively high concentration to inhibit 50% of DPPH radicals and a lower concentration to produce 50% brine-shrimp lethality. This distinction is important because antioxidant capacity assays quantify chemical reactivity, while BSLT measures organismal survival in an invertebrate model. The biological meaning, exposure conditions, and relevant concentration ranges are different for the two assays[10,19]. A more informative interpretation will require fraction specific testing, identification of major compounds, and assessment of whether the concentration that produces a desired biochemical effect can be separated from concentrations associated with cytotoxic or toxic effects. Until those data are available, the results should be described as an initial laboratory profile rather than evidence for or against therapeutic use.

The regression results also illustrate the importance of reporting the analytical pathway used to derive IC_{50} and LC_{50} values. In this study, DPPH inhibition was modeled against the original concentration scale, whereas BSLT mortality was modeled against $\log_{10}$ concentration. These approaches are consistent with the displayed figures and reproduce the reported point estimates when y is set to 50. However, a high R^2 does not provide information about between replicate variability, measurement error, or uncertainty around the estimated 50% effect concentration. For future work, raw replicate data should be retained and presented so that mean responses, standard deviations, confidence intervals, and the stability of the regression can be evaluated. Controls should be reported alongside treated groups, and any correction for background or control mortality should be specified. The chosen concentration range should bracket the 50% response whenever possible so that the estimate depends on interpolation rather than extrapolation. These reporting practices would strengthen both reproducibility and the biological interpretation of the assay and would allow meaningful comparison with published antioxidant and BSLT studies[10,19].

The strength of this research is the integrated assessment of phytochemical groups, DPPH radical-scavenging activity, and BSLT lethality within the same crude extract. Nevertheless, important limitations must be considered. The DPPH assay is a chemical-based test and may not reflect antioxidant behavior in a living biological system[21]. Likewise, BSLT is a preliminary lethality/cytotoxicity screen and does not directly predict toxicity in mammals[22]. In addition, the available source laboratory record reported averaged absorbance and mortality percentages but did not preserve the technical replicate counts, DPPH reagent concentration, incubation time, measurement wavelength, detailed blank/solvent and positive control composition, the number of *Artemia salina* larvae per concentration, or replicate-level mortality counts. Consequently, standard deviations and mortality n/N values could not be reconstructed retrospectively. This incomplete procedural documentation limits reproducibility and should be corrected in confirmatory experiments.

Future research should address these limitations through a fully prespecified laboratory protocol with documented negative and positive controls, replicate level measurements, and transparent reporting of variability. Bioactivity guided fractionation is needed to isolate antioxidant and BSLT active constituents. In vivo toxicity studies are important for establishing a more informative safety profile of *Imperata cylindrica* preparations[23]. Complementary antioxidant

assays such as ABTS and FRAP could provide a broader assessment of antioxidant capacity[24]. Chromatographic and spectrometric approaches, including HPLC, GC-MS, or LC-MS, should also be used to identify and quantify specific phytochemicals[25].

Several relevant studies reinforce the importance of transparency in reporting extraction and testing procedures. Septiani et al[26] employed the DPPH method to evaluate the antioxidant activity of plant derived products, demonstrating that formulations can significantly alter measured antioxidant capacity. Zai et al[27] detailed maceration based preparation and antimicrobial testing of botanical extracts across varying concentrations, while Sitanggang et al[28] integrated extraction, phytochemical screening, the use of various concentration levels and controls, and replicated bioactivity testing. These studies provide a valuable context for interpreting the current findings and underscore the necessity of comprehensive methodological documentation in plant extract experiments.

Conclusion

The methanol extract of *Imperata cylindrica* root contained multiple secondary metabolite groups and showed weak DPPH radical scavenging activity (IC_{50} = 437.632 µg/mL). In the Brine Shrimp Lethality Test, the LC_{50} was 290.084 µg/mL, which is classified as toxic within this preliminary BSLT assay. These measured outcomes do not establish mammalian or human toxicity. Further chemical characterization, cell based testing, and in vivo toxicological studies are required before conclusions regarding clinical safety, human consumption, or therapeutic development can be made.

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