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Exploring antioxidant and cytotoxic activity of ultrasound-assisted extraction of Javanese ginseng (*Talinum paniculatum*) roots: Potential halal bioactive source

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ABSTRACT
The roots of Javanese ginseng (*Talinum paniculatum* (Jacq.) Gaertn.) are widely used in traditional medicine, yet their scientific evaluation remains limited. This study compared ethanol and butanol extracts of *T. paniculatum* roots obtained through ultrasound-assisted extraction (UAE) in terms of phytochemical composition, antioxidant activity, and cytotoxicity. Phytochemical tests confirmed the presence of phenolics, flavonoids, terpenoids, and other compounds, with the ethanolic extract showing a more diverse profile. Total phenolic content (TPC) was relatively low for both extracts, with ethanol slightly higher (0.64 ± 0.02 mg GAE/g) than butanol (0.57 ± 0.01 mg GAE/g). In contrast, antioxidant activity measured by the DPPH assay was stronger in the butanol extract (IC_{50} = 39.45 mg/L) than in ethanol (IC_{50} = 94.07 mg/L). Cytotoxicity screening using the brine shrimp lethality test revealed a notable difference, as the butanol extract exhibited a very low LC_{50} value of 2.36 mg/L, indicating strong activity compared to 20.99 mg/L for the ethanolic extract. These findings suggest that *T. paniculatum* roots, particularly the butanol fraction, may offer bioactive compounds with promising antioxidant and anticancer potential. These findings also support the potential development of *T. paniculatum* as a halal-compliant source of bioactive compounds for nutraceutical and functional food applications.

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INTRODUCTION

Degenerative diseases remain a major global health concern, and the side effects associated with synthetic drugs have prompted a shift toward natural alternatives. Medicinal plants are widely recognized for their rich content of bioactive compounds with antioxidant, anti-inflammatory, and anticancer properties. Antioxidants help neutralize free radicals, reducing the risk of chronic conditions such as Alzheimer's and Parkinson's disease (Mittal et al., 2023). Recent reviews also highlight their neuroprotective potential, though challenges such as bioavailability and standardization remain (Nahar et al., 2025).

In the context of halal science, there is growing interest in plant-based bioactive compounds as safe and sustainable alternatives to synthetic or animal-derived ingredients that may pose halal compliance concerns. The concept of *halal* is intrinsically tied to *tayyib*, which stresses not only religious permissibility but also safety, quality, efficacy, and health benefits. Plant-derived formulations have been increasingly investigated as functional ingredients and cosmetics that satisfy critical halal

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points while maintaining high biological safety and activity (Amalina et al., 2024; Silviandari et al., 2024). Therefore, exploring medicinal plants such as *Talinum paniculatum* as sources of bioactive compounds directly supports the expanding market demand for halal-certified nutraceuticals and functional food ingredients.

Javanese ginseng (*Talinum paniculatum* (Jacq.) Gaertn.), although widely used in Indonesian traditional medicine, has not received the same scientific attention as other ginseng species such as *Panax ginseng*. Previous studies have reported the presence of phenolic compounds, flavonoids, and various phytochemicals in *T. paniculatum*, which are associated with antioxidant, anti-inflammatory, and other pharmacological activities (Aini & Susilo, 2023). Its roots are particularly rich in saponins (Ahmad & Anggita, 2019) and are commonly consumed as stew or soup in several countries, including Indonesia and other parts of Asia (Achieng et al., 2024). Despite these findings, research on the roots and their bioactive properties remains limited, and the plant is still underutilized compared to other medicinal herbs.

Oxidative stress is known to contribute to various chronic diseases, making antioxidant activity a critical parameter when evaluating natural products. At the same time, safety assessment is essential before any bioactive plant extract can be developed into nutraceutical or pharmaceutical products (Pilipović et al., 2023). While some research has identified bioactive compounds in *T. paniculatum*, few studies have systematically examined both its antioxidant potential and cytotoxicity. This gap highlights the need for further investigation to confirm its efficacy and safety.

To address this gap, the present study aims to characterize the phytochemical profile of *T. paniculatum* root extracts obtained using two different solvents, ethanol and butanol, through ultrasonication-assisted extraction. Moreover, the study evaluates the antioxidant activity of these extracts and assesses their cytotoxicity using an in vivo bioassay. The results are expected to provide scientific evidence for the potential use of Javanese ginseng as a natural source of bioactive compounds, supporting its application in pharmaceutical and nutraceutical development, particularly within the framework of halal-compliant natural product innovation.

RESEARCH METHOD

Materials

The plant material used in this study consisted of roots of *Talinum paniculatum* obtained from cultivated plants in Bogor, West Java, Indonesia. Ethanol (70%, analytical grade) and n-butanol (analytical grade) were used as extraction solvents, while methanol p.a. and distilled water were used for solution preparation and analysis. Phytochemical screening was carried out using standard reagents, including Wagner's, Dragendorff's, and Mayer's reagents, as well as ferric chloride ($FeCl_3$) and other reagents for detecting flavonoids, saponins, tannins, terpenoids, and steroidal glycosides. Total phenolic content (TPC) analysis employed Folin–Ciocalteu reagent, sodium bicarbonate (Na_2CO_3), and gallic acid as a standard. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), with butylated hydroxytoluene (BHT) as a positive control. Cytotoxicity testing utilized brine shrimp (*Artemia salina*) eggs, saline water, and ethanol (96%) for sample preparation, with potassium dichromate used as a positive control. All chemicals and reagents used were of analytical grade.

Methods

Plant materials

The roots of *T. paniculatum* used in this study were obtained from plants cultivated in Bogor City, West Java Province, Indonesia (6°32'29.3"S, 106°49'22.0" E). The plant was determined by Herbarium Bogoriense, BRIN, and confirmed as *Talinum paniculatum* (Jacq.) Gaertn., family *Talinaceae*, through letter No. B-1169/IV/DI.05.07/4/2022 dated 25 April 2022. The roots collected were washed, dried, and ground into powder prior to extraction.

Extraction Procedure

The ultrasound-assisted extraction method was employed for the preparation of *T. paniculatum* root extract. The process involved comparing two solvents, ethanol and butanol, for their efficiency in root extraction. Javanese ginseng roots (JGR) were washed, dried, and then chopped into small pieces.

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The sample was ground into a fine powder. Fifteen grams of root powder were weighed, and 70% solvent was added (1:10, w/v%). The mixture of sample and solvent was sonicated using an ultrasonic device (VCX 750, Sonics and Materials Inc., USA) for 35 minutes at 65% amplitude. After sonication, the mixture was filtered and evaporated using a rotary evaporator (Rotary Evaporator Hei-VAP Value, Heidolph, Germany) at 50 °C until it thickened. Finally, the sample was dried in an oven (LabTech, Daihan LabTech, South Korea) at 40 °C for 24 hours. The selection of ethanol and butanol as extraction solvents was also considered from a halal processing perspective. Ethanol is commonly used as an extraction solvent and is generally acceptable in halal production when not present in the final product. Butanol, as a non-intoxicating solvent, may serve as an alternative extraction medium with potentially fewer concerns regarding halal compliance, depending on its source and purity. Residual ethanol, n-butanol, and other volatile compounds were not quantified. Therefore, the dried extracts were not evaluated against a residual-solvent specification.

Phytochemical screening

The phytochemical composition of JGR was assessed to identify the presence of various bioactive compounds. The qualitative tests included alkaloid detection using Wagner's, Dragendorff's, and Meyer's reagents. In addition, the extract was analyzed for phenolics, flavonoids, terpenoids, saponins, tannins, steroidal glycosides, and anthraquinones (Maheshwaran et al., 2024).

Determination of Total Phenolic Content (TPC)

Initially, a 0.4 mL aliquot of the extract (1 mg/mL) was transferred into a 10 mL volumetric flask, followed by the addition of 6 mL of distilled water and 1 mL of Folin–Ciocalteu reagent. The mixture was homogenized and incubated in the dark at room temperature for 3 minutes. Subsequently, 2.5 mL of 10% (w/v) sodium bicarbonate solution was added, and the volume was adjusted to the mark with distilled water. The solution was further incubated in the dark at room temperature for 1 hour. After incubation, the absorbance was measured at 650 nm using a UV–Vis spectrophotometer (Analytik Jena, Germany). A calibration curve was prepared using gallic acid standards at concentrations ranging from 2 to 8 mg/L. All measurements were conducted in triplicate. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of plant extract (mg GAE/g) (Irawan, Elya, et al., 2021; Irawan, Utami, et al., 2021; Moncayo et al., 2021).

In vitro antioxidant assay

The antioxidant activity of the JGR extract was evaluated using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay. To conduct the DPPH assay, a total of 5 mg of JGR extract was dissolved in methanol p.a. in a 5 mL volumetric flask to prepare a solution with a concentration of 1000 mg/L. This stock solution was subsequently diluted to concentrations ranging from 8 to 128 mg/L. Then, 1 mL of a 39 mg/L DPPH solution was added to the extract fractions and incubated at room temperature for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at 515 nm. Butylated hydroxytoluene (BHT) was used as a positive control (Irawan, Utami, et al., 2021). The percent inhibition was calculated using the formula below:

$$Inhibition\ (\%) = \left(\frac{A_{Blank} - A_{Sample}}{A_{Blank}}\right) \times 100\%$$

The terms A_{Blank} and A_{Sample} refer to the absorbance of the DPPH solution (negative control) and the extract combined with DPPH at 515 nm, respectively. The percentage of inhibition was plotted on the Y-axis against concentration on the X-axis, following the equation $y = ax + b$. The IC_{50} value was calculated using the formula:

$$IC_{50} = \frac{50 - b}{a}$$

Cytotoxicity assay

The cytotoxic potential of the JGR extract was assessed using the brine shrimp lethality test (BSLT). Artemia salina eggs (30 mg) were incubated in saline water under constant aeration and

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illumination provided by a 25 W lamp at room temperature. After 24 hours, the eggs hatched into nauplii (larvae). The JGR extract was serially diluted in saline water and 96% ethanol to obtain concentrations ranging from 10 to 1000 µg/mL. For the assay, 10 actively swimming nauplii were transferred into test tubes containing 5 mL of each extract concentration. A negative control (saline with ethanol) and a positive control (e.g., potassium dichromate) were included. After 24 hours of exposure, the number of dead nauplii was counted, and the percentage mortality was calculated. All treatments were conducted in triplicate; percent mortality was calculated based on Abbott's formula, and LC_{50} values were determined using probit analysis (Ansari et al., 2025; Bogavac et al., 2019).

$$Mortality\ (\%) = \left(\frac{Observed\ mortality\ (\%) - Control\ mortality\ (\%)}{100 - Control\ mortality\ (\%)} \right) \times 100\%$$

Data Analysis

TPC is reported as mean ± SD from three analytical measurements. Extraction yield, qualitative phytochemical results, DPPH IC50, and brine shrimp LC50 are descriptive results from the available experiment. Because the DPPH and brine shrimp assays were not independently replicated, no t-test, p-value, confidence interval, or claim of statistically significant difference was calculated.

RESULT AND DISCUSSION

The extraction process produced different yields depending on the solvent used. Ethanol produced 4.80 g of dried extract (16.78%), whereas n-butanol produced 4.07 g (14.18%) (Table 1). The higher ethanol yield is consistent with its ability, particularly as an aqueous mixture, to recover a broad range of polar and moderately polar constituents. Recent solvent-comparison studies also demonstrate that yield, phenolic recovery, and DPPH activity respond differently to solvent polarity (El Mannoubi, 2023; Palaiogiannis et al., 2023). A recent comparative study of Centella asiatica similarly showed that solvent selection in ultrasound-assisted extraction altered phenolic and flavonoid recovery, DPPH activity, and triterpenoid composition, confirming that extraction yield and biological response are governed by the compounds each solvent preferentially recovers (Khursheed et al., 2024).

Table 1. Weight and yield of extract produced by UAE.

Sample	Weight (g)	Yield (%)
Ethanolic extract	4.80	16.78
Butanol extract	4.07	14.18

Table 2. Qualitative phytochemical screening of *T. paniculatum* root extracts

Secondary Metabolite	Test Results	
	Ethanolic Extract	Butanol Extract
Alkaloid	+	+
Flavonoid	+	-
Phenolic	+	+
Terpenoid	+	+
Saponin	+	-
Tanin	+	-
Steroidal glycoside	+	+
Anthraquinone	-	-

Qualitative screening confirmed the presence of alkaloids, phenolics, and terpenoids in both extracts, while flavonoids, saponins, tannins, and steroidal glycosides were detected exclusively in the ethanol extract (Table 2). This finding indicates that ethanol's polarity favors the extraction of polyphenolic compounds and glycosides, which are often associated with antioxidant properties. The absence of these components in the butanol fraction implies a more concentrated presence of lipophilic compounds such as terpenoids, which may explain differences in biological activities observed later.

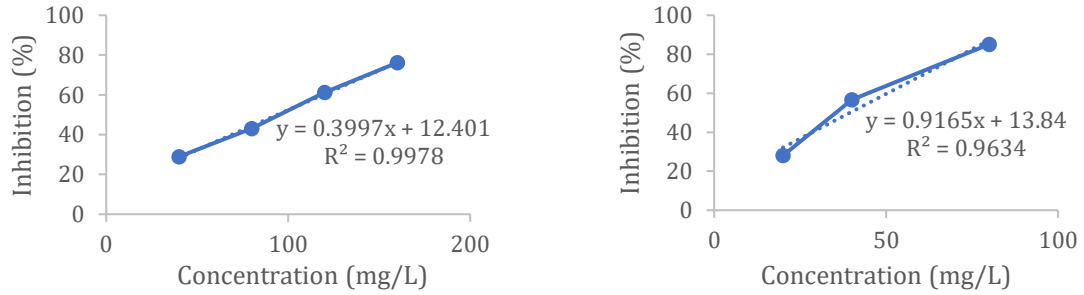
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The data highlight the importance of solvent selection in determining the chemical profile and biological activity of *T. paniculatum* root extracts. Ethanol provided the highest yield and extracted a broader array of phytochemicals, including flavonoids and saponins, whereas butanol yielded less material but showed greater bioactivity in antioxidant and cytotoxic assays. This outcome underscores a critical point: extraction yield does not necessarily predict bioactivity, a phenomenon also reported in previous phytochemical studies (Mehta et al., 2022). From a halal perspective, the choice of extraction solvent is a critical factor in ensuring compliance in the production of bioactive ingredients. The use of plant-derived raw materials combined with controlled extraction processes supports the development of halal-certified products, provided that solvent residues are minimized and raw materials are sourced appropriately.

Table 3. Total phenolic content, DPPH radical-scavenging, and cytotoxic activity of JGR extract.

Sample	TPC (mg GAE/g extract)	DPPH IC_{50} (µg/mL)	LC_{50} (mg/L)
Ethanolic extract	0.64 ± 0.02	94.07	20.99
Butanol extract	0.57 ± 0.01	39.45	2.36

Both extracts had low total phenolic contents (< 1 mg GAE/g extract) as shown in Table 3. These values were substantially lower than those recently reported for other *T. paniculatum* materials, possibly because of differences in the plant part, cultivation conditions, extraction procedure, and basis used to express the results (Agatha et al., 2026). However, antioxidant results tell a different story. Despite its lower TPC, the butanol extract exhibited stronger free radical scavenging ability than the ethanol extract. This finding implies that non-phenolic compounds, possibly terpenoids and certain alkaloids, play a significant role in antioxidant defense. Similar patterns have been documented in other medicinal plants, where phenolic content and antioxidant activity do not always correlate (Srividya et al., 2012).



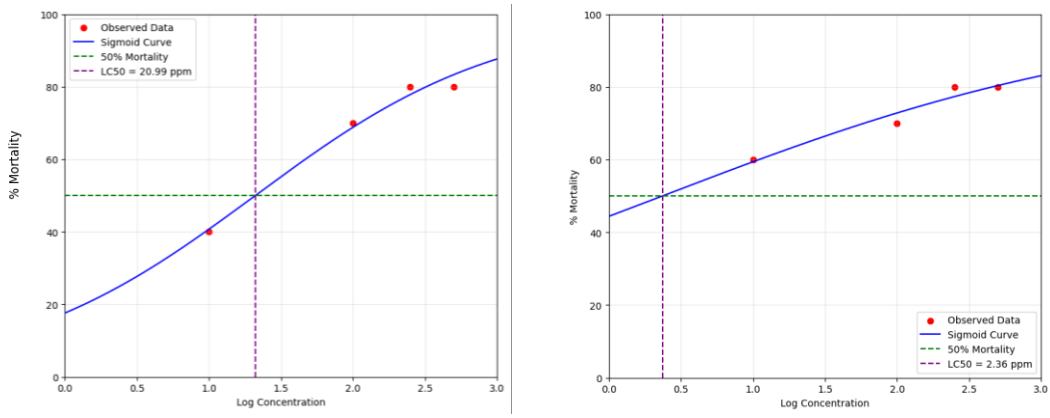
A B

Figure 1. Concentration-dependent DPPH radical-scavenging activity of the *Talinum paniculatum* root ethanol extract (A) and butanol extract (B).

As illustrated in Figure 1, both the ethanolic and n-butanol extracts of *Talinum paniculatum* roots exhibited a clear concentration-dependent DPPH radical-scavenging activity, where percentage inhibition increased progressively with higher extract concentrations. Across the tested range, the n-butanol extract demonstrated stronger radical scavenging efficiency, reaching a lower IC_{50} value of 39.45 mg/L compared to 94.07 mg/L for the ethanolic extract (Table 3). This concentration-dependent behavior reflects typical radical neutralization kinetics, where increasing bioactive concentrations provide greater electron- or hydrogen-donating capacity to stabilize DPPH radicals. Similar concentration-dependent DPPH inhibition profiles and IC_{50} evaluations have been observed in bioactive botanical studies, where extract concentration directly governs radical scavenging potency (Fadli & Lasanudin, 2024; Permadi et al., 2026). Despite displaying a slightly lower total phenolic content (0.57 ± 0.01 mg GAE) than the ethanolic fraction (0.64 ± 0.02 mg GAE/g), the superior radical scavenging potential of the n-butanol extract suggests significant contributions from non-phenolic metabolites such as terpenoids and alkaloids. This absence of a direct correlation between total phenolic content and

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antioxidant efficacy is consistent with findings in other plant-derived bioactive formulations (Albana et al., 2025), as well as general solvent extraction studies where high phenolic yields do not necessarily produce the highest antioxidant response (Tymczewska et al., 2023). Because DPPH responses are also influenced by molecular structure, solvent, reaction time, and temperature, the IC50 values reported here should be interpreted under the defined assay conditions rather than directly compared across studies that use different protocols (Yamauchi et al., 2024).



A B

Figure 2. Probit analysis for brine shrimp lethality test with JGR ethanol extract (A) and butanol extract (B).

Cytotoxicity screening using the brine shrimp lethality test revealed a marked difference between the two extracts (Figure 2). The butanol extract exhibited strong cytotoxicity with an LC_{50} value of 2.36 mg/L, classifying it as highly active according to established criteria (< 1000 µg/mL). In contrast, the ethanol extract was less potent, with an LC_{50} of 20.99 mg/L (Table 3). This strong activity in the butanol fraction could indicate an enrichment of compounds such as steroidal glycosides and terpenoids, which are often associated with cytotoxic and anticancer properties (Ramosa et al., 2010). The cytotoxicity findings are particularly noteworthy. The butanol extract demonstrated an LC_{50} of 2.36 mg/L, which is considered highly toxic and indicative of potential anticancer activity. Previous research has shown that non-polar extracts often contain steroidal glycosides and triterpenes, compounds known for their cytotoxic properties (Ramosa et al., 2010). These metabolites may account for the strong lethality observed in this study. The ethanol extract, while less potent, still displayed moderate activity, suggesting that both extracts contain compounds with biological relevance.

The pronounced toxicity of the butanol extract (LC_{50} = 2.36 mg/L, equivalent to 2.36 µg/mL) indicates the presence of highly bioactive constituents. This LC_{50} value is substantially lower than the commonly used 1000 µg/mL threshold for identifying extracts with significant toxicity in preliminary brine shrimp lethality screening. However, the strong lethality observed in this assay should be interpreted cautiously. The brine shrimp lethality test (BSLT) is primarily a preliminary general-toxicity and bioactivity screening method and does not demonstrate selective cytotoxicity toward cancer cells or establish an anticancer mechanism. Therefore, although the butanol fraction represents a promising source of bioactive compounds for further investigation, its activity should not yet be interpreted as evidence of anticancer efficacy. This interpretation is consistent with a recent evaluation of the Artemia salina test, which supports its use for preliminary screening and bioactivity-guided isolation while emphasizing that positive lethality alone cannot identify a specific pharmacological mechanism or therapeutic selectivity (Olmedo et al., 2024).

Previous phytochemical studies have reported the presence of triterpenoids, steroidal saponins, and alkaloids in *T. paniculatum*, classes of secondary metabolites that have been associated with diverse biological activities, including apoptosis induction, cell-cycle modulation, and inhibition of angiogenesis in experimental cancer models (Ramosa et al., 2010). The relatively polar nature of the butanol fraction may favor the extraction of certain saponins, glycosylated compounds, and other

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moderately polar metabolites, which could contribute to the strong toxicity observed in the present study. Nevertheless, the contribution of individual compounds cannot be established without further chemical characterization and bioactivity-guided fractionation. Recent evidence further describes triterpenoid saponins as structurally diverse plant metabolites with broad biological activities and substantial pharmaceutical potential, supporting their consideration as candidate contributors to the activity of the butanol fraction (Kundu et al., 2025).

Several classes of plant-derived metabolites, including terpenoids and steroidal saponins, have been reported to influence pathways associated with cell survival and programmed cell death. Terpenoids, for example, may affect mitochondrial function and oxidative balance, potentially leading to reactive oxygen species generation and apoptosis under certain experimental conditions. Steroidal saponins may interact with membrane sterols and alter membrane integrity, thereby influencing intracellular signaling pathways. Although such mechanisms provide a plausible rationale for further investigation, the present BSLT data alone cannot confirm that these mechanisms are responsible for the observed toxicity. The relationship between the metabolites present in the butanol extract and its biological activity therefore requires direct experimental verification. Current reviews indicate that steroidal saponins can modulate apoptosis, proliferation, angiogenesis, and related cancer hallmarks, although bioavailability and limited preclinical evidence remain important constraints (Bouabdallah et al., 2023). Plant-derived terpenoids likewise act on interconnected metabolic and signaling pathways in tumor models, but these mechanistic observations require compound-specific validation and cannot be inferred directly from a crude-extract BSLT result (Pandey et al., 2025).

The strong lethality observed in the brine shrimp model supports the use of the butanol fraction as a candidate for further bioactivity screening, consistent with the role of BSLT as an initial and relatively simple assay for identifying biologically active natural-product extracts. However, the LC_{50} estimate should be interpreted with particular caution because the assay was not independently replicated, and the use of ten nauplii per concentration does not substitute for biological replication. Confirmation through independently repeated BSLT experiments is therefore necessary to establish the reproducibility and reliability of the observed LC_{50} value.

Subsequent investigations should include bioassay-guided fractionation and chemical characterization to identify the compounds responsible for the observed toxicity. More importantly, the extract and its active fractions should be evaluated using both normal and human cancer cell lines. Determination of selectivity indices would be essential to distinguish general cytotoxicity from preferential toxicity toward cancer cells. Mechanistic studies examining relevant endpoints, such as apoptosis, cell-cycle arrest, oxidative stress, mitochondrial dysfunction, and molecular signaling pathways, would also be required before any anticancer potential can be established. Further in vivo toxicity, pharmacokinetic, and efficacy studies would subsequently be necessary to evaluate the safety and therapeutic relevance of potential lead compounds.

In addition to its potential pharmacological relevance, *T. paniculatum* may represent a promising plant-derived source of bioactive compounds for pharmaceutical and nutraceutical development, including products intended for halal-conscious consumers. Plant-derived ingredients may provide advantages in terms of avoiding certain concerns associated with bioactive ingredients derived from non-halal animal sources, particularly regarding ingredient origin, traceability, and potential contamination (Norazmi & Lim, 2015). However, the halal status of a final product cannot be inferred solely from its plant origin. Halal compliance depends on the entire production chain, including the origin and processing of raw materials, extraction solvents, processing aids, formulation ingredients, manufacturing practices, and prevention of contamination with non-halal materials.

The use of ultrasound-assisted extraction may offer additional advantages by improving extraction efficiency and potentially reducing processing time and solvent requirements. These characteristics may support cleaner and more resource-efficient production. Nevertheless, describing the extraction method as inherently halal-compatible requires verification that all materials and processing conditions comply with applicable halal requirements. Consequently, the development of *T. paniculatum*-derived bioactive products should integrate chemical characterization, safety and efficacy evaluation, and halal assurance throughout the production chain.

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Overall, the present findings identify the butanol fraction of *T. paniculatum* roots as a highly bioactive extract warranting further investigation. Its strong activity in the BSLT provides a useful starting point for the isolation and characterization of bioactive constituents, but additional experiments are required to determine whether the observed toxicity is selective, reproducible, and relevant to cancer or other therapeutic targets. A systematic progression from repeated preliminary screening to cell-based assays, mechanistic studies, animal experiments, and comprehensive safety evaluation will be necessary before therapeutic or anticancer claims can be substantiated.

CONCLUSION

It was demonstrated through this study that ultrasound-assisted extraction successfully extracted bioactive compounds from *Talinum paniculatum* roots using ethanol and butanol solvents. The butanol extract showed stronger antioxidant activity (IC_{50} = 39.45 mg/L) and higher cytotoxic activity (LC_{50} = 2.36 mg/L) than the ethanolic extract, despite having lower extraction yield and total phenolic content. Phytochemical screening confirmed the presence of alkaloids, phenolics, and terpenoids in both extracts, while flavonoids and saponins were mainly detected in the ethanolic extract. These findings indicate that *T. paniculatum* roots, particularly the butanol fraction, have potential as a source of natural antioxidant and cytotoxic compounds for halal-compliant nutraceutical and phytopharmaceutical applications.

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