



Antibacterial Activity of Ethanolic and Aqueous Leaf Extracts of *Datura* Against *Escherichia coli* and *Staphylococcus aureus* Using the Microtiter Plate Method

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Abstract

Background: The increasing resistance of pathogenic bacteria to conventional antibiotics has renewed interest in medicinal plants as sources of new antimicrobial agents. *Datura* (Solanaceae) is widely used in traditional medicine and is rich in tropane alkaloids and other secondary metabolites.

Objective: This study evaluated the antibacterial activity of ethanolic and aqueous leaf extracts of *Datura*, collected in Misurata, Libya, against a Gram-negative bacterium (*Escherichia coli*) and a Gram-positive bacterium (*Staphylococcus aureus*).

Methods: Leaves were examined microscopically to document their diagnostic features. Extracts were prepared by maceration with 75% ethanol or distilled water for 48 h at 35–45 °C with magnetic stirring. Each extract was tested at 20%, 40% and 60% concentration using the 96-well microtiter plate method; optical density was read at 630 nm after 12 h and 24 h of incubation at 37 °C, and the percentage of growth inhibition was calculated against growth controls.

Results: Microscopy revealed anomocytic stomata, glandular and covering trichomes, lignified vessels and calcium oxalate crystals. Both extracts inhibited bacterial growth to different degrees. The ethanolic extract inhibited *E. coli* by 24.7–43.0% (maximum 42.98% at 60%, 12 h) and *S. aureus* by 7.7–19.5% (maximum 19.51% at 60%, 12 h). The aqueous extract inhibited *E. coli* by 18.1–39.6% (maximum 39.56% at 40%, 12 h) and *S. aureus* by 0–12.6% (maximum 12.63% at 40%, 12 h). Inhibition was generally greater at 12 h than at 24 h and was consistently higher against *E. coli* than against *S. aureus*.

Conclusion: *Datura* leaf extracts show moderate, concentration- and time-dependent growth-inhibitory activity (measured by optical density), with a stronger effect on the Gram-negative test organism; bactericidal activity was not assessed. Further phytochemical characterization and determination of minimum inhibitory concentrations are recommended.

Keywords: *Datura*; antibacterial activity; ethanolic extract; aqueous extract; microtiter plate; *Escherichia coli*; *Staphylococcus aureus*; Libya

1. Introduction

Infectious diseases caused by bacteria remain a major cause of morbidity and mortality worldwide. The widespread and often inappropriate use of antibiotics has accelerated the emergence of multidrug-resistant strains, which compromises the treatment of common infections and increases healthcare costs [1]. Among the organisms of greatest clinical concern are *Staphylococcus aureus*, a Gram-positive coccus responsible for skin, soft-tissue, bloodstream and device-associated infections, and *Escherichia coli*, a Gram-negative bacillus that is the leading cause of urinary tract infections and a frequent agent of gastrointestinal and bloodstream infections. The need for new antimicrobial compounds, including those from natural sources, is therefore well recognized [2].

Plants have long been used as a source of therapeutic agents, and their secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids and phenolic compounds, are known to exert antimicrobial effects through mechanisms such as membrane disruption, enzyme inhibition and interference with nucleic-acid synthesis [3]. Screening of crude plant extracts remains an important first step in identifying promising candidates for further investigation [4]. The genus *Datura* (family Solanaceae) comprises annual herbaceous plants that are distributed in tropical and temperate regions and grow naturally in Libya and other Mediterranean countries. Traditional medicine has used *Datura* for the management of asthma, pain, inflammation and skin disorders. Its leaves contain tropane alkaloids such as hyoscyamine and scopolamine, together with withanolides, flavonoids, tannins and other phenolics [5,6]. Several reports have described antimicrobial activity of *Datura* extracts against a range of bacteria and fungi, although the results vary with the species, plant part, solvent and test method [6,7].

The choice of extraction solvent strongly influences the profile of compounds recovered. Ethanol extracts a broad range of moderately polar and non-polar constituents, whereas water mainly recovers highly polar compounds such as glycosides, some phenolics and tannins [8,9]. Comparing the two solvents therefore provides information about which fractions contribute most to activity, and water is also the solvent most relevant to traditional preparations. Microscopic examination of plant material is a recognized method of authentication in pharmacognosy because the shape and distribution of stomata, trichomes, vascular elements and crystals provide a characteristic fingerprint of the drug [10]. Documenting these features in the plant material also allows other researchers to verify the identity of the material used. The broth microdilution method, performed in 96-well microtiter plates, is a standard approach for evaluating antimicrobial activity. It requires small volumes, allows multiple concentrations and replicates to be tested simultaneously, and permits objective spectrophotometric quantification of growth [11,12].

The present study aimed to (i) describe the diagnostic microscopic features of *Datura* leaves collected in Misurata, Libya; (ii) prepare ethanolic and aqueous leaf extracts; and (iii) evaluate and compare their effect, at three concentrations and two incubation times, on the growth of *E. coli* and *S. aureus* by the microtiter plate method.

2. Materials and Methods

2.1. Plant material collection and preparation

Datura plants were collected on 28 December 2024 from the Al-Kharruba area and the grounds of a college campus in the city of Misurata, Libya. Leaves were separated, cleaned of soil and adhering impurities, and dried in the shade at room temperature in a well-ventilated room for two weeks to preserve the integrity of the plant material to the greatest extent possible. The dried leaves were ground to a powder in an electric grinder and stored in airtight containers until use.

2.2. Microscopic examination

Fresh green leaf material was cut into small pieces and boiled in 10% sodium hydroxide (NaOH) solution for 20 minutes, then filtered through filter paper. A drop of the cleared material was placed on a glass slide, a drop of glycerin:water solution (1:3) was added, and a cover slip was applied. Slides were examined first with the 10× objective and then with the 40× objective of a microscope fitted with a camera. Images were captured and compared with reference specimens and published descriptions of the diagnostic elements of *Datura* leaves.

2.3. Preparation of extracts

Extraction was carried out by maceration at 35–45 °C on a hot plate in the medicinal plants laboratory of the Faculty of Medical Technology, Misurata. Two portions of 25 g of powdered leaves were weighed on a sensitive balance. The first portion was placed in a 250 mL conical flask and 250 mL of 75% ethanol was added; the second was placed in a second 250 mL conical flask and 250 mL of distilled water was added. Both flasks were placed on a hot plate and stirred continuously with magnetic stirrer bars for 48 h.

After extraction, each mixture was filtered through gauze and then filter paper. The filtrates were placed in an oven at 40 °C to eliminate most of the solvent, and the concentrated residues were transferred to glass Petri dishes and dried. The dried extracts were redissolved in the corresponding solvent to give a stock solution of each extract. The test concentrations of 20%, 40% and 60% are expressed as percentages of this stock solution; the extract concentration in mg/mL is therefore not reported. In the microtiter plates, 100 µL of each concentration was added to 100 µL of bacterial suspension, so the final concentration in each well was half of the stated value. All solutions were filtered through a 0.45 µm membrane filter to remove large particles and to sterilize them, and were stored at 4 °C until used [13].

2.4. Pathogenic bacterial isolates

The pathogenic bacteria used were *Staphylococcus aureus* (Gram-positive cocci) and *Escherichia coli* (Gram-negative bacilli). Identified isolates were obtained from a diagnostic laboratory in Misurata. Nutrient broth (OXOID, UK) was prepared as a liquid medium and nutrient agar (OXOID, UK) as a solid medium for culture and maintenance. Cultures were incubated for 24 h at 37 °C. The identity of the isolates was confirmed by Gram staining and by the catalase test, and the isolates were then stored at 4 °C for use in the study [14].

2.5. Antibacterial assay by the microtiter plate method

The inhibitory effect of the extracts on the pathogenic bacteria was evaluated in sterile 96-well flat-bottom microtiter plates (Biotek, Germany) [15]. For each test, 100 µL of nutrient broth

containing 10^6 bacterial cells per mL was dispensed into the wells. One hundred microliters of the aqueous extract at concentrations of 20%, 40% and 60% was then added to separate wells. The same procedure was applied to the ethanolic extract at 20%, 40% and 60%, with 100 μ L added to separate wells containing bacteria-inoculated broth. Plates were incubated at 37 °C, and absorbance was measured at 630 nm after 12 h and 24 h using a microplate (ELISA) reader (Biotek, Germany).

Two controls were included. The **negative control** consisted of the plant extract with sterile nutrient broth and no bacteria, which allowed correction for the intrinsic color and turbidity of the extracts. The **positive (growth) control** consisted of nutrient broth inoculated with the test bacterium without extract.

2.6. Calculation of percentage inhibition

The percentage of growth inhibition was calculated as:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the blank-corrected absorbance of the growth control and A_{sample} is the blank-corrected absorbance of the well containing bacteria and extract. Blank correction was made by subtracting, at each concentration and time point, the absorbance of the corresponding negative-control well (extract plus sterile broth, without bacteria), so that the intrinsic color and turbidity of the extract did not contribute to the optical-density reading at 630 nm.

2.7. Statistical analysis

Results are presented as mean $\pm$ standard deviation. Normality and homogeneity of variance were checked before analysis, and differences among extract concentrations, incubation times and solvents were tested by [two-way ANOVA followed by Tukey's post hoc test; $p < 0.05$ considered significant] using Minitab version 16.

2.8. Safety precautions

Datura contains toxic tropane alkaloids. Plant material and extracts were handled with gloves, a laboratory coat and eye protection; grinding and solvent evaporation were carried out in a ventilated area; extract containers were labelled as toxic; and waste was discarded as hazardous laboratory waste .

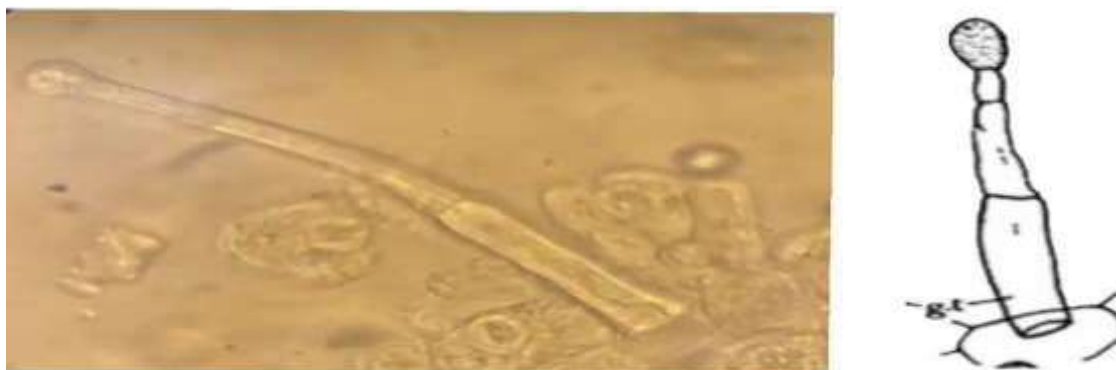
3. Results

3.1. Microscopic features of the leaves

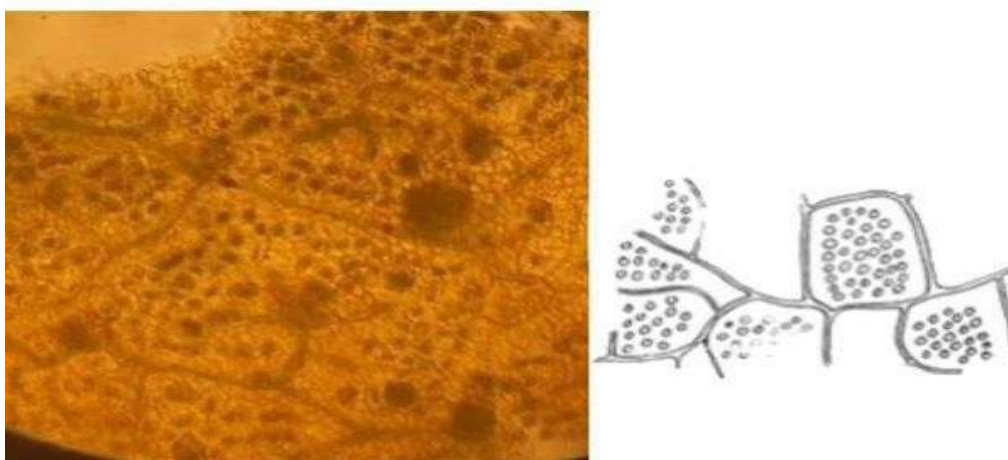
Microscopic examination of the cleared leaf material at 10 $\times$ and 40 $\times$ showed features that agreed with the reference specimens (Figures 1–5). The principal diagnostic elements were:



Epidermal cells and stomata (Figure 1): polygonal epidermal cells with irregular walls, and stomata distributed on the leaf surface.



Trichomes (Figure 2): scattered unicellular and multicellular covering hairs and glandular hairs, the latter bearing a multicellular stalk and a rounded secretory head.



Vascular tissue and crystals (Figures 3–4): lignified xylem vessels with annular and spiral thickening, accompanied by calcium oxalate crystals.



Calcium oxalate (Figure 5): star-shaped (stellate) calcium oxalate crystals.

These features, in particular the glandular trichomes and stellate calcium oxalate crystals, are characteristic of *Datura* leaves and served as microscopic markers of the plant material used.

3.2. Effect of the ethanolic extract on *E. coli*

The three concentrations of the ethanolic extract were effective against *E. coli*, although to different degrees (Table 1, Figure 6). Inhibition was higher at 12 h than at 24 h at all concentrations. The highest value, 42.98%, was recorded at 60% extract after 12 h, whereas the lowest, 24.67%, was recorded at 20% after 24 h. At 24 h, inhibition rose with concentration (24.67% < 31.63% < 31.98%). At 12 h, the 20% and 40% extracts gave similar values (38.92% and 38.06%), and the 60% extract was the most active.

Table 1. Percentage inhibition of *E. coli* growth by the ethanolic leaf extract of *Datura*.

Extract concentration	12 h	24 h
20%	38.9166	24.6726
40%	38.0613	31.6334
60%	42.9793	31.9779

[Figure 6: bar chart of Table 1.]

3.3. Effect of the ethanolic extract on *S. aureus*

The ethanolic extract produced lower and less regular inhibition of *S. aureus* (Table 2, Figure 7). The highest inhibition, 19.51%, occurred at 60% after 12 h, and the lowest, 7.75%, at 40% after 24 h. The 40% extract was the least active at both times (10.47% at 12 h; 7.75% at 24 h), while the 60% extract was the most active at 12 h. Unlike the pattern in *E. coli*, inhibition of *S. aureus* by the 20% extract was greater at 24 h (16.57%) than at 12 h (11.70%).

Table 2. Percentage inhibition of *S. aureus* growth by the ethanolic leaf extract of *Datura*.

Extract concentration	12 h	24 h
20%	11.7043	16.5710
40%	10.4723	7.74749
60%	19.5072	14.4189

[Figure 7: bar chart of Table 2.]

3.4. Effect of the aqueous extract on *E. coli*

The aqueous extract showed clear activity against *E. coli* at all concentrations (Table 3, Figure 8). Values ranged from 18.13% to 39.56%. The highest inhibition (39.56%) was recorded at 40% after 12 h, and the lowest (18.13%) at 20% after 24 h. At 12 h the 40% extract was the most active, followed by 20% and 60%; at 24 h inhibition increased progressively with concentration (18.13%, 21.36% and 22.12%).

Table 3. Percentage inhibition of *E. coli* growth by the aqueous leaf extract of *Datura*.

Extract concentration	12 h	24 h
20%	35.4241	18.1254
40%	39.5581	21.3646
60%	34.5688	22.1227

[Figure 8: bar chart of Table 3.]

3.5. Effect of the aqueous extract on *S. aureus*

The aqueous extract had the weakest effect on *S. aureus* (Table 4, Figure 9). The highest inhibition (12.63%) was obtained at 40% after 12 h, and 9.90% was recorded for the same concentration after 24 h. The 20% extract produced 9.96% inhibition at 12 h and no inhibition at 24 h. The 60% extract showed no inhibition at 12 h and only 3.37% at 24 h.

Table 4. Percentage inhibition of *S. aureus* growth by the aqueous leaf extract of *Datura*.

Extract concentration	12 h	24 h
20%	9.95893	0
40%	12.6283	9.89957
60%	0	3.37159

[Figure 9: bar chart of Table 4.]

3.6. Summary comparison

Table 5 summarizes the range and maximum inhibition for each extract–organism combination. Across both solvents and all concentrations, *E. coli* was inhibited to a greater extent (18.1–43.0%) than *S. aureus* (0–19.5%). The ethanolic extract gave the single highest value (42.98% against *E. coli*), although the aqueous extract was only slightly less effective against that organism (maximum 39.56%). Against *S. aureus*, the ethanolic extract was more consistent, with all values above 7%, whereas the aqueous extract failed to inhibit growth in two of six treatment conditions.

Table 5. Summary of the antibacterial effect of *Datura* leaf extracts (inhibition, %).

Extract	Organism	Range	Maximum (concentration, time)
Ethanolic	<i>E. coli</i>	24.67–42.98	42.98 (60%, 12 h)
Ethanolic	<i>S. aureus</i>	7.75–19.51	19.51 (60%, 12 h)
Aqueous	<i>E. coli</i>	18.13–39.56	39.56 (40%, 12 h)
Aqueous	<i>S. aureus</i>	0–12.63	12.63 (40%, 12 h)

Note on the data. Values in Tables 1–5 are mean percentage inhibition from two independent runs. Standard deviations and the results of statistical tests [to be inserted from the raw data]

are needed before differences among concentrations, incubation times or solvents can be described as significant; the comparisons above are therefore descriptive.

4. Discussion

4.1. Microscopic characterization

The microscopic examination documented the combination of features that is usually used to recognize *Datura* leaves in powdered or fragmented form: stomata and polygonal epidermal cells, glandular and covering trichomes, lignified vessels and calcium oxalate crystals [10]. Their presence in the material used here, and their agreement with reference specimens, supports the identity and integrity of the plant material before extraction. Because pharmacological results depend on the plant material tested, this authentication step adds value to the antibacterial data and allows the work to be reproduced.

4.2. Antibacterial activity of the extracts

Both the ethanolic and aqueous extracts reduced the growth of the test bacteria, but the magnitude of the effect depended on the organism, the solvent, the concentration and the incubation time. The most evident finding is the consistently stronger effect on *E. coli* than on *S. aureus*: the ethanolic extract inhibited *E. coli* by up to 42.98%, compared with a maximum of 19.51% for *S. aureus*, and the aqueous extract reached 39.56% against *E. coli* but only 12.63% against *S. aureus*.

This result is somewhat unexpected, since Gram-negative bacteria are generally regarded as less susceptible to plant extracts because their outer membrane, with its lipopolysaccharide layer, restricts the penetration of many hydrophobic compounds [16]. Several explanations may be considered, and the present data cannot distinguish among them. First, susceptibility to plant extracts is strain-dependent, and the clinical isolates used here may differ from reference strains in their intrinsic resistance. Second, the active constituents of *Datura* leaves, including tropane alkaloids and polar phenolic compounds, may be able to cross the outer membrane through porins or to act on targets that are more readily reached in *E. coli* than in the *S. aureus* isolate; comparable or higher activity against Gram-negative organisms has been reported for *Datura* extracts by other authors [7]. Third, *S. aureus* is a fast-growing organism that forms dense cultures in nutrient broth, and the inoculum effect may have reduced the apparent inhibition. Finally, optical-density readings are influenced by the turbidity and pigmentation of crude extracts and by cell aggregation, which differs between cocci and bacilli. The inclusion of an extract-plus-broth blank in this study reduced, but may not have eliminated, this interference.

4.3. Effect of solvent

The ethanolic extract gave the highest single inhibition value (42.98% against *E. coli*) and was more consistently active against *S. aureus*, whereas the aqueous extract produced no measurable inhibition in two of six *S. aureus* conditions. This is in line with the ability of ethanol to extract a wider range of compounds, including less polar alkaloids and phenolics, than water [8,9]. However, the aqueous extract was almost as effective against *E. coli* (maximum 39.56%), suggesting that the compounds responsible for this effect are sufficiently polar to be extracted by water. This is of practical interest because water is inexpensive, non-toxic and corresponds

to the way in which the plant is traditionally prepared. It should be noted that the extracts were not standardized by yield or by marker compounds, so differences between solvents may reflect differences in the amount of extractable material as well as in its composition.

4.4. Effect of concentration and time

The relationship between concentration and inhibition was not strictly linear. For the ethanolic extract against *E. coli* at 24 h, inhibition increased with concentration (24.67%, 31.63%, 31.98%), consistent with a dose-dependent effect, with a plateau between 40% and 60%. At other time–concentration combinations, the 40% extract was equal to or more active than the 60% extract (aqueous extract against *E. coli* and *S. aureus* at 12 h), while the opposite pattern was seen for the ethanolic extract against *S. aureus*, in which the 40% extract was the least active. Non-monotonic responses of this kind are often seen with crude extracts and may arise from limited solubility or aggregation of active constituents at high concentration, from interactions among extract components, or from increased background absorbance at high extract concentrations. They also underline that measurement variability should be reported, and the limits of a two-run design should be recognized.

Inhibition was generally lower at 24 h than at 12 h, particularly for *E. coli* (for example, 42.98% to 31.98% for the 60% ethanolic extract and 35.42% to 18.13% for the 20% aqueous extract). Because the assay measures turbidity, a reduction in optical density cannot distinguish inhibition of growth from killing, and the extracts are therefore described here only as growth-inhibitory. The lower values at 24 h are compatible with partial regrowth of surviving cells, but other explanations, such as degradation or binding of active compounds in the medium, cannot be excluded. Demonstrating a bactericidal effect would require subculturing of the wells to determine the minimum bactericidal concentration (MBC), viable colony counts or time-kill assays [11].

4.5. Limitations

Several limitations should be acknowledged. (i) The plant species and voucher information must be stated explicitly, because identification at genus level alone is insufficient for a published study. (ii) The extract concentrations were expressed as percentages of the prepared solutions; expressing them in mg/mL, together with extract yield, is necessary for comparison with other studies. (iii) Only two independent runs were performed, which limits statistical inference; the number of replicates, means with standard deviations and an appropriate statistical test are required. (iv) Growth was measured only as optical density at 630 nm. Although extract-plus-broth blanks were subtracted, the absence of interference from extract color and turbidity was not independently verified, for example by comparison with colony counts or a viability indicator. (v) Optical density cannot establish a bactericidal effect, so no killing activity is claimed; MBC determination or viable counts are needed. (vi) Minimum inhibitory concentrations were not determined and no reference antibiotic was included. (vii) The extracts were not analysed phytochemically, so the compounds responsible for the activity are unknown. (viii) Only one Gram-positive and one Gram-negative species were tested. These findings should therefore be regarded as preliminary screening data [4].

5. Conclusions

The microscopic features of *Datura* leaves collected in Misurata, Libya, were consistent with reference specimens. Ethanolic and aqueous leaf extracts reduced the growth of *E. coli* and *S. aureus* in a turbidity-based microtiter assay, with effects that varied with concentration and incubation time. Inhibition was higher against *E. coli* (up to 42.98% with the ethanolic extract and 39.56% with the aqueous extract) than against *S. aureus* (up to 19.51% and 12.63%, respectively). The assay does not distinguish bacteriostatic from bactericidal action, and no killing effect is claimed. These preliminary results justify further work, including species verification, MIC and MBC determination, viable counts, phytochemical analysis and toxicity assessment.

6. Recommendations for Future Work

- Determine minimum inhibitory and minimum bactericidal concentrations and compare them with standard antibiotics.
- Express extract doses in mg/mL and report extract yield.
- Perform phytochemical screening and chromatographic identification (e.g., HPLC or GC–MS) of the active constituents, in particular alkaloids and phenolics.
- Test additional Gram-positive and Gram-negative species, including reference strains and multidrug-resistant isolates.
- Evaluate the cytotoxicity and safety of the extracts, since *Datura* contains toxic tropane alkaloids, before any therapeutic application is considered.

Safety Considerations

Datura species contain tropane alkaloids (such as hyoscyamine and scopolamine) that are highly toxic to humans and animals if ingested, inhaled or absorbed, and poisonings from plant preparations are well documented. The extracts studied here are laboratory preparations for in vitro screening only; the results are not evidence of safety or efficacy in humans, and the authors do not recommend any self-medication with *Datura*. Plant material and extracts should be handled with gloves, a laboratory coat and eye protection, powdering and solvent evaporation should be carried out in a ventilated area or fume hood, containers should be labelled as toxic and kept from unauthorized access, and waste should be discarded as hazardous laboratory waste. Any further development requires quantification of alkaloid content and toxicity testing.

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Ethical Statement

This study did not involve human participants or experimental animals.

Data Availability

The data supporting the findings of this study are contained within the article.

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