Ultrasound-assisted extraction of cytotoxic alkaloids from Caliphruria subedentata: Comparative analysis of bath and high-intensity systems

Sergio Gonzalez-Lopez
Universidad de Antioquia
Lina M. Trujillo Chacón
Universidad de Antioquia
Edison Osorio
edison.osorio@udea.edu.co

Universidad de Antioquía: Universidad de Antioquia https://orcid.org/0000-0002-9821-3420

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Abstract

Cancer remains one of the leading causes of death worldwide, and despite significant therapeutic advances, the discovery of effective and sustainable chemotherapeutic agents remains a major challenge. Alkaloids from the Amaryllidaceae family are well known for their cytotoxic potential, yet their complex phytochemical matrices and low alkaloid contents often lead to low extraction yields and high solvent consumption. This study aimed to optimize the extraction of cytotoxic Amaryllidaceae alkaloids from *Caliphruria subedentata* using unconventional ultrasound-assisted extraction techniques and to evaluate their activity against gastric cancer (AGS) cells. Three extraction approaches were compared: Soxhlet extraction (conventional), bath ultrasound-assisted extraction (BUAE), and high-intensity ultrasound-assisted extraction (HIUAE). Alkaloid profiles were assessed by gas chromatography-mass spectrometry (GC–MS), and cytotoxicity was assessed using the MTT-based colorimetric method. A two-factor, two-level experimental design combined with response surface analysis was applied for optimization. HIUAE provided the highest alkaloid yield and cytotoxic activity, with an inhibitory concentration corresponding to 50% of the maximal effect (IC_{50}) values of 0.42 ± 0.23 µg/ml, 10.30 ± 3.76 µg/ml, and 70.34 ± 3.08 µg/ml for HIUAE, BUAE, and Soxhlet, respectively. Optimal extraction conditions were a methanol: water ratio of 8.28:1.72, a solid-to-liquid ratio of 1:10, and an extraction time of 40 s. The optimized extract exhibited enhanced cytotoxicity consistent with its higher alkaloid content. Overall, HIUAE represents an efficient, rapid, and environmentally friendly method for obtaining bioactive alkaloids from *C. subedentata*, supporting the development of green extraction strategies for potential anticancer agents.

Introduction

Cancer comprises a group of diseases characterized by the uncontrolled proliferation of abnormal cells, which may invade and damage surrounding tissues and organs. According to the Global Cancer Observatory (GCO), cancer remains one of the leading causes of death worldwide, accounting for approximately 9.7 million deaths in 2022 (Bray et al. 2024). Globally, the most common types of cancer by incidence are lung (12.4%), breast (11.6%), colorectal (9.6%), prostate (7.3%), and gastric cancer (4.9%). In terms of mortality, the leading causes are lung (18.7%), colorectal (9.3%), liver (7.8%), breast (6.9%), and gastric cancer (6.8%) (Bray et al. 2024). The main therapeutic approaches used to treat various types of cancer include surgery, radiotherapy, chemotherapy, targeted therapies, immunotherapy, stem cell transplantation, and hormonal therapy (American Cancer Society 2025a). Chemotherapy remains the most widely used treatment because of its versatility and effectiveness when combined with other modalities. It involves the use of cytotoxic agents as chemotherapeutics, which interfere with the growth and division of cancer cells, and are often used in combination with other therapies (Chabner and Roberts 2005; American Cancer Society 2025b). Approximately 25% of the chemotherapeutic agents approved for cancer treatment between 1981 and 2019 are derived from natural products (Huang et al. 2021). Furthermore, reports indicate that out of 121 approved anticancer drugs, 90 originate from plants. Representative examples include alkaloids such as vincristine and vinblastine, which are obtained from

Catharanthus roseus, and taxanes such as paclitaxel, which are isolated from *Taxus* species (Roy et al. 2018).

Plants of the Amaryllidaceae family are recognized as a source of structurally diverse and biologically active alkaloids, many of which have demonstrated cytotoxic activity against various cancer cell lines (Nair and Van Staden 2021a; Tallini et al. 2024). The natural complexity of these plants often results in extracts containing multiple alkaloids (Jin and Yao 2019). This chemical diversity is important not only for its potential bioactivity but also because it poses significant challenges for extraction (Jin and Xu 2013; Jin and Yao 2019). Their isolation is often complicated by structural diversity, varying polarities, and low concentrations, which hinder the selective recovery of individual compounds (Desgagné-Penix 2021). Therefore, the development and comparison of efficient extraction strategies are essential to improve both the yield and reproducibility of these bioactive constituents. In the process of obtaining bioactive compounds, both conventional and unconventional extraction techniques are employed (Veličković et al. 2017; Caldas et al. 2018; Anticona et al. 2020). Conventional methods such as percolation, maceration, and Soxhlet extraction involve prolonged contact between plant material and large volumes of organic solvents (Gomes et al. 2022). In contrast, unconventional techniques such as microwave-assisted extraction, ultrasound-assisted extraction (UAE), and supercritical fluid extraction are generally more efficient, often yielding extracts rich in bioactive compounds in shorter periods and with lower solvent consumption (Lekhak et al. 2022; Pavón-Pérez et al. 2022).

In Amaryllidaceae species, several studies have explored the application of modern extraction techniques to isolate alkaloids, acknowledging the challenges posed by their structural diversity and low natural abundance (Mroczek and Mazurek 2009; Takla et al. 2018). Among these techniques, UAE has received particular attention for its ability to improve mass transfer and preserve thermolabile compounds. However, limited comparative research has examined the performance of different UAE modalities, such as high-intensity ultrasound and ultrasound bath systems, for the extraction of Amaryllidaceae alkaloids. This gap highlights the need to systematically evaluate and optimize these methods to improve their selectivity, efficiency, and reproducibility in alkaloid extraction. UAE is especially suitable for alkaloid extraction, as it enhances cell wall disruption and mass transfer while operating at relatively low temperatures, which is an advantage for thermolabile compounds. Compared with methods such as microwave-assisted or supercritical fluid extraction, UAE offers a simpler, more cost-effective, and scalable alternative, particularly for natural products with complex matrices such as those found in Amaryllidaceae plants. Optimizing the UAE parameters is therefore essential to enhance the recovery of alkaloids from these species and enable further evaluation of their cytotoxic potential. In this study, alkaloid-rich extracts were tested against gastric cancer cells, a disease with high incidence and mortality rates worldwide. Despite advances in treatment, the management of gastric cancer still relies heavily on chemotherapy, underscoring the importance of identifying new natural compounds with potential antitumor activity, such as Amaryllidaceae alkaloids.

Materials and methods

Plant material

Specimens of *C. subedentata* were collected from the nursery of the Universidad Católica de Oriente (UCO), located in Rionegro, Antioquia, Colombia (GPS coordinates: 6.150816, -75.365797). The plant material was identified by the botanist Fernando Alzate and supported by a voucher specimen housed in the Herbarium of the University of Antioquia (HUA) under accession number 5400 Alzate. The bulbs were washed, chopped into small pieces, and dried in an oven at 40.0°C for 48 h. The dried material was subsequently ground and preserved at room temperature under conditions free from moisture and light exposure.

Chemicals and reagents

A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to obtain deionized water. Methanol, chloroform, n-hexane, dimethyl sulfoxide (DMSO), ammonium hydroxide, sulfuric acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and C7–C40 alkane mixtures, fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, 10x trypsin-EDTA solution, and Dulbecco´s Modified Eagle Medium-high glucose (DMEM), were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Extraction techniques

Conventional extraction: The conventional extraction process was conducted using a Soxhlet apparatus with methanol as the solvent at a liquid-to-solid ratio of 16.67:1 for 24 h. The extract was filtered and adjusted to pH ≈ 2.0, and nonpolar compounds were extracted with n-hexane. The aqueous phase was subsequently adjusted to approximately pH 10.0 with 25% ammonium hydroxide. Finally, the alkaloids were extracted through successive extractions with chloroform.

Bath ultrasound-assisted extraction (BUAE): The extraction was conducted out following the method proposed by Dary et al., 2017 (Dary et al. 2017a). An ultrasound bath (Elma Schmidbauer GmbH, Elmasonic P60H, Singen, Germany) was used with the following fixed operating conditions: a frequency of 37 kHz, a power output of 180 Watts, a maximum temperature of 40.0°C, and a solid-to-liquid ratio of 1:10. Two factors were evaluated in the experimental design: solvent type and extraction time, each with two levels.

High-intensity ultrasound-assisted extraction (HIUAE): The extraction was performed via a probe-type ultrasonic processor (Sonomechanics, LSP-500, Miami, FL, USA) with a frequency of 20 kHz, a power output of 500 Watts at 100% amplitude, and a 21 mm sonotrode immersed 4 cm into the solution. The process was conducted at a maximum temperature of 40.0°C. Two factors were evaluated as part of an experimental design: solvent type and extraction time, each with two levels.

Alkaloid profiling by GC–MS

Analysis of Amaryllidaceae alkaloids from *C. subedentata* via GC–MS was carried out following a previously reported method (Trujillo-Chacón et al. 2019). Approximately 1 µL of the alkaloid extract was injected into an Agilent 7890 gas chromatograph equipped with a 5975C mass selective detector. The detector was operated in electron impact (EI) mode at 70 eV in splitless mode and acquired data over an *m/z* range of 40–400. Alkaloid separation was achieved via an HP-1 MS capillary column (30 m × 0.25 mm × 0.25 µm) with helium as the carrier gas at a flow rate of 1 ml/min. The temperature ramp was as follows: 100.0–180.0°C at 15.0°C/min, 180.0–300.0°C at 5.0°C/min, and hold for 10 min at 300.0°C. The injector temperature was maintained at 250.0°C.

Data processing and quantitative analysis

Mass spectra fragments of each compound were compared with the Amaryllidaceae Alkaloid Spectroteca database from Agro Bio Institute (Bulgaria)" and with data reported in the scientific literature. The Kovats retention index (RI) for the compounds was calculated using a calibration mixture of *n*-alkanes (C7-C40). The percentage of total ionic current (TIC) was determined for each alkaloid, and its abundance was calculated from a galanthamine curve (200–1000 µg/ml) as an external standard and reported as µg of galanthamine per gram of alkaloid extract (µg Gal/g AE).

Experimental design and optimization strategy

Two-factor experimental design

A two-factor, two-level experimental design was developed to identify the most efficient extraction methodology by evaluating the effects of various factors on alkaloid yield. The alkaloid content (mg) in the extract was taken as the response variable. Table S1 (Supplementary Material) presents the experimental design for the BUAE and HIUAE, whereas Table S2 (Supplementary Material) provides the conditions used in both extraction techniques. Soxhlet extraction was not included in the factorial design, as it was conducted under fixed conventional conditions and was included solely as a comparative reference.

HIUAE Optimization via response surface methodology (RSM)

Based on the results from the two-factor, two-level experimental design, an extraction method was selected for optimization via RSM. Among the different designs, the two-factor factorial design with three levels was selected because of its superior efficiency in comparison to the others. The two factors selected were the methanol percentage and the solid–liquid ratio, studied at three levels (high = + 1, medium = 0, and low = -1). Table S3 (Supplementary Material) presents the matrix design consisting of 30 experimental runs, and Table S4 (Supplementary Material) details the experimental conditions evaluated.

Cytotoxic activity assessment in gastric cancer cells

Cell Culture

The viability of alkaloidal fractions from *C. subedentata* was evaluated in AGS gastric cancer cells (ATCC® CRL-1739™). HaCaT keratinocytes (ATCC® PCS-200-011™) were used as nontumorigenic controls. Cells were maintained under standard culture conditions at 37.0°C; 5.0% CO_2, using high-glucose DMEM supplemented with 10% heat-inactivated FBS, 100 µg/ml streptomycin, 100 IU/ml penicillin, and 2 mM L-glutamine. The morphology of the cells was monitored using a Nikon Eclipse TS100 inverted phase-contrast microscope. Experiments were performed when the cells reached 75–80% confluency. The adherent cells were released by treatment with 0.25% trypsin–EDTA (1 mM EDTA).

Cell viability assay

Cell viability was determined via the MTT colorimetric assay, as described by Trujillo et al., 2019 (Trujillo-Chacón et al. 2019). Alkaloid fractions, along with the isolated alkaloids lycorine and haemantamine, were evaluated in AGS and HaCaT cells at concentrations of 50, 30, 20, 10, 5, 1, 0.5, and 0.1 µg/ml. Cells were incubated at 37.0°C; 5.0% CO_2 for 48 h following treatment. As a positive control, doxorubicin was tested at concentrations of 10, 5, 2.5, 1.25, 0.62, and 0.31 µg/ml. Subsequently, 50 µL of MTT solution (1.0 mg/ml in phosphate-buffered saline) was added to each well. Following incubation for 4 h at 37.0°C, the MTT-containing medium was carefully removed and replaced with 150 µL of DMSO to dissolve the resulting formazan. The plates were maintained under gentle agitation in the dark for an additional 2 h, after which absorbance was read at 570 nm with a Synergy HT microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Statistical analysis

All the statistical analyses and graphics were generated using Python 3.12, GraphPad Prism 5.0, and Statgraphics 19.4.04. Cell viability experiments were performed in triplicate, each with six technical replicates. The results were evaluated via one-way analysis of variance (ANOVA) followed by the Newman—Keuls multiple comparison test. IC_{50} values were calculated and expressed as the mean ± standard deviation (SD).

Results and discussion

Two-factor experimental design

The effects of extraction time and solvent composition on alkaloid content obtained by BUAE and HIUAE are illustrated in Fig. 1. In BUAE, the highest alkaloid yields were recorded for combinations (− 1, − 1) and (1, 1), with 2.19 ± 0.02 mg and 1.91 ± 0.28 mg, respectively, whereas the remaining conditions yielded between 1.05 ± 0.18 mg and 1.50 ± 0.02 mg. In contrast, HIUAE consistently produced higher yields under all experimental conditions, reaching a maximum of 7.20 ± 0.17 mg at (− 1, 1) and remaining above 4.30 ± 0.17 mg even in the least favorable combination. These results reveal a clear effect of

ultrasonic power intensity on the extraction performance: the probe-based HIUAE system yielded nearly twice the alkaloid content of BUAE, even under equivalent solvent and time settings.

This superior efficiency can be attributed to the higher energy density and localized acoustic power of HIUAE, which enhances cavitation and microjet formation, promoting more effective cell wall rupture and solvent penetration. Such mechanisms increase the release of intracellular alkaloids and improve mass transfer rates, as reported for other thermolabile phytochemicals extracted under similar high-intensity sonication conditions (Šic Žlabur et al. 2022). The pronounced differences between both techniques confirm that ultrasound intensity, rather than exposure time alone, plays a decisive role in optimizing the recovery of Amaryllidaceae alkaloids.

Selection of HIUAE for optimization via the RSM

Based on the two-factor experimental design, HIUAE exhibited markedly higher efficiency than BUAE, reaching an alkaloid yield of 7.20 ± 0.17 mg under conditions (− 1, 1), compared with 2.19 ± 0.02 mg obtained with BUAE at (− 1, − 1). When the alkaloidal content from the optimal conditions of each technique was compared (Table 1), HIUAE yielded 1.44 mg/g, followed by BUAE with 0.44 mg/g, and conventional Soxhlet extraction with 2.30 mg/g, the latter requiring considerably higher solvent volumes, longer extraction times, and elevated temperatures. These differences highlight the superior extraction performance of HIUAE under milder and more sustainable conditions.

Table 1
Comparison of extraction methods

Extraction method	Conditions				Alkaloid content mg/g DW ($\bar{X}$ ± RSD, n = 3)
	Solvent*	Volume (ml)	Time (s)	T (°C)	
BUAE	1: 1	50.0	600	40.0	0.44 ± 0.02
HIUAE	1: 0	50.0	40	40.0	1.44 ± 0.17
Conventional	1: 0	250.0	3600	65.0	2.32 ± 0.04
* Methanol: Water (v/v)					

This enhanced efficiency is attributed to the high-intensity probe system, which generates localized cavitation and intense microstreaming, facilitating solvent penetration and efficient cell disruption that accelerates alkaloid release. Although ultrasound-assisted extraction can potentially cause degradation of thermolabile metabolites, the short exposure times and the use of cooling systems in HIUAE minimize this risk, as reported by Dary et al. (Dary et al. 2017b). Consequently, HIUAE represents the most suitable technique for optimization through response surface methodology (RSM), offering an optimal balance between efficiency, processing speed, and environmental sustainability.

HIUAE condition optimization

A factorial design with two factors and three levels was applied to optimize the HIUAE conditions to maximize alkaloid yield. The factor level selection was based on results from the previous two-factor experimental design. As shown in Figure S1 (Supplementary Material), no statistically significant differences were observed between the two levels tested for extraction time; therefore, this variable was fixed at its lowest level. In contrast, solvent composition exhibited significant effects on alkaloid recovery, prompting the construction of a new design matrix comprising 30 experimental runs (Table S3, Supplementary Material).

The absence of a significant time effect indicates that the extraction process rapidly reaches equilibrium under high-intensity sonication, emphasizing that energy density and solvent polarity exert a stronger influence than prolonged exposure. The pronounced effect of solvent composition confirms that solvent–matrix interactions govern the solubilization and release of Amaryllidaceae alkaloids. By expanding the design to 30 experimental runs, a more comprehensive mapping of the factor space was achieved, providing a solid foundation for subsequent response surface modeling to determine the true optimal extraction parameters.

Response surface methodology analysis

Figure 2 shows the results obtained from the response surface design described in Table S3 (Supplementary Material). Alkaloid content increased progressively along the solvent axis, reaching a maximum between the coded levels of − 0.2 and 0.2, while the solid–liquid ratio displayed a nearly constant response across the evaluated range. This pattern indicates that variations in solvent polarity have a far greater impact on extraction efficiency than changes in solid loading. Accordingly, and considering the objective of minimizing solvent use, the lowest solid–liquid ratio (− 1) was selected.

The response surface analysis identified optimal coded values of − 0.14 for solvent composition and − 1 for the solid–liquid ratio, corresponding to a ratio of 8.28:1.72 (methanol: water) and a solid-liquid ratio of 1:10, confirming that solvent composition was the dominant variable influencing alkaloid yield. The observation of maximal recovery at intermediate solvent levels suggests the existence of an optimal polarity window in which both alkaloid solubility and cavitation efficiency are balanced. When the methanol proportion exceeds this range, the decreased cavitation intensity reduces cell disruption and mass transfer, leading to lower extraction performance, as previously reported by Dary et al. (Dary et al. 2017b). These findings demonstrate that a precise balance between solvent polarity and ultrasonic energy transmission is essential to maximize efficiency while maintaining the sustainability of the extraction process.

Alkaloids identified in the optimized fraction

GC–MS analysis of alkaloids from *C. subedentata* bulbs revealed eleven compounds belonging to the lycorine, haemanthamine, tazettine, narciclasine, and galanthamine structural groups (Fig. 3). Among these, galanthamine- and lycorine-type alkaloids were the most abundant in the HIUAE extract, reaching 4.12 and 1.86 µg galanthamine equivalents per gram of alkaloid extract (µg Gal/g AE), respectively. Other constituents included tazettine (0.89 µg Gal/g AE), 8-O-demethylmaritidine (0.53 µg Gal/g AE),

haemanthamine (0.68 µg Gal/g AE), and trisphaeridine (0.52 µg Gal/g AE), indicating a chemically diverse alkaloidal composition.

The detection of multiple Amaryllidaceae-type skeletons highlights the metabolic richness of *C. subedentata*. The predominance of galanthamine- and lycorine-related alkaloids in the HIUAE extract suggests that high-intensity ultrasound not only enhances overall yield but may also promote selective recovery of moderately polar compounds. This selectivity likely results from the efficient microstreaming and cavitation effects of the ultrasonic probe, which facilitate intracellular diffusion and solvent–matrix contact while minimizing degradation. The enrichment of galanthamine- and lycorine-type alkaloids is particularly relevant given their known anticholinesterase and cytotoxic properties (Cortes et al. 2018; Trujillo et al. 2023), reinforcing the pharmacological potential of *C. subedentata* extracts obtained under optimized HIUAE conditions.

Cell viability in gastric cancer cells

Consistent with its higher alkaloid yield and the predominance of lycorine- and galanthamine-type compounds, the HIUAE extract displayed the most potent cytotoxic activity against AGS gastric cancer cells (Table 2). It showed an IC_{50} value of 0.42 ± 0.23 µg/ml, approximately 25 times lower than that of BUAE (10.30 ± 3.76 µg/ml) and more than 160 times lower than that of Soxhlet (70.34 ± 3.08 µg/ml). Doxorubicin, used as a positive control, presented an IC_{50} of 1.09 ± 0.10 µg/ml. A similar pattern was observed in non-tumorigenic HaCaT cells, where HIUAE again exhibited the greatest potency but also the highest selectivity index (SI), indicating a stronger cytotoxic preference toward cancer cells.

Table 3
Cytotoxic potential in AGS and HaCaT cells

IC_{50} (µg/ml)			
	AGS	**HaCaT**	**Selectivity index**
Soxhlet	70.34 ± 3.08	36.94 ± 4.72	0.53
BUAE	10.30 ± 3.76	1.47 ± 0.34	0.14
HIUAE	0.42 ± 0.23	0.87 ± 0.48	2.06
Doxorubicin	1.09 ± 0.10	1.55 ± 0.11	1.42

The enhanced biological activity of the HIUAE extract correlates with its higher relative abundance of lycorine-type alkaloids (4.11 µg Gal/g AE), which was more than twice that obtained with BUAE (1.47 µg Gal/g AE) and over 55 times greater than that from Soxhlet (0.07 µg Gal/g AE) (Figure S5, Supplementary Material). This enrichment likely underlies the markedly lower IC_{50} values, since lycorine-type alkaloids are known for their strong antiproliferative effects in diverse cancer models (Nair and van Staden 2014). The presence of additional haemanthamine- and tazettine-type derivatives, previously reported to exert

cytotoxic effects against gastric and other cancer cell lines (Nair and Van Staden 2021b; Cahlíková et al. 2021), may contribute additively or synergistically to the observed activity.

Pearson correlation analysis (Fig. 4) further revealed that galanthamine, narwedine, 8-O-demethylmaritidine, trisphaeridine, and 11,12-dehydrolycorene exhibited strong inverse correlations with IC_{50} values ($r > -0.94$) in both AGS and HaCaT cells, suggesting that these compounds are principal contributors to the cytotoxic response. However, the negative correlations observed in both cell lines also indicate potential off-target or nonselective toxicity for some constituents, underscoring the importance of future mechanistic validation.

Interestingly, lycorine and haemanthamine—although well documented for their potent cytotoxicity as isolated compounds (Havelek et al. 2017)—showed weaker correlations in the extract mixtures. This attenuation could stem from antagonistic interactions among co-occurring alkaloids, competition for molecular targets, or restricted uptake within complex matrices (Caesar and Cech 2019). Further studies using combination-index analysis, bio-guided fractionation, and mechanistic assays will be necessary to clarify whether these interactions are synergistic or antagonistic and to identify the specific compounds driving the cytotoxic effects of *C. subedentata* extracts.

Conclusions

HIUAE represents a superior and sustainable alternative to conventional extraction methods for recovering bioactive alkaloids from *C. subedentata*. The ultrasonic intensity, rather than extraction time alone, is the determining factor in alkaloid yield and cytotoxic activity, with solvent polarity playing a critical role in optimizing cavitation efficiency. The chemical diversity of *C. subedentata* alkaloids, particularly the enrichment of lycorine-type and galanthamine-type compounds under optimized HIUAE conditions, underlies the marked cytotoxic selectivity toward gastric cancer cells over non-tumorigenic cells. These findings support the use of HIUAE as a green and scalable extraction strategy for Amaryllidaceae alkaloids and position *C. subedentata* as a promising botanical source for the development of potential anticancer agents. Future studies should focus on mechanistic validation, bio-guided fractionation, and in vivo evaluation to further characterize the pharmacological potential of this species.

Abbreviations

HIUAE
high-intensity ultrasound-assisted extraction BUAE,bath ultrasound-assisted extraction
GC–MS
gas chromatography coupled with mass spectrometry
AGS
gastric adenocarcinoma cell line
GCO

Global Cancer Observatory
UAE
ultrasound-assisted extraction
UCO
Universidad Católica de Oriente
HUA
Herbarium of the University of Antioquia
EDTA
ethylenediaminetetraacetic acid
DMSO
dimethyl sulfoxide
MTT
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltriazolium bromide
FBS
fetal bovine serum
DMEM
Dulbecco´s Modified Eagle Medium-high glucose
EI
electron impact
RI
Kovats retention index
TIC
total ionic current
RSM
response surface methodology
HaCaT
immortalized human keratinocyte cell line
ANOVA
one-way analysis of variance.

Declarations

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Competing interests

The authors have no relevant financial or nonfinancial interests to disclose.

CRediT authorship contribution statement: Sergio Gonzalez-Lopez: conceptualization, data curation, formal analysis, investigation, visualization, methodology, project administration, Writing – original draft, Writing – review & editing. **Lina M. Trujillo Chacon**: conceptualization, resources, formal analysis, supervision, validation, investigation, methodology, Writing – review & editing. **Edison Osorio**: conceptualization, resources, supervision, methodology, project administration, Writing – review & editing.

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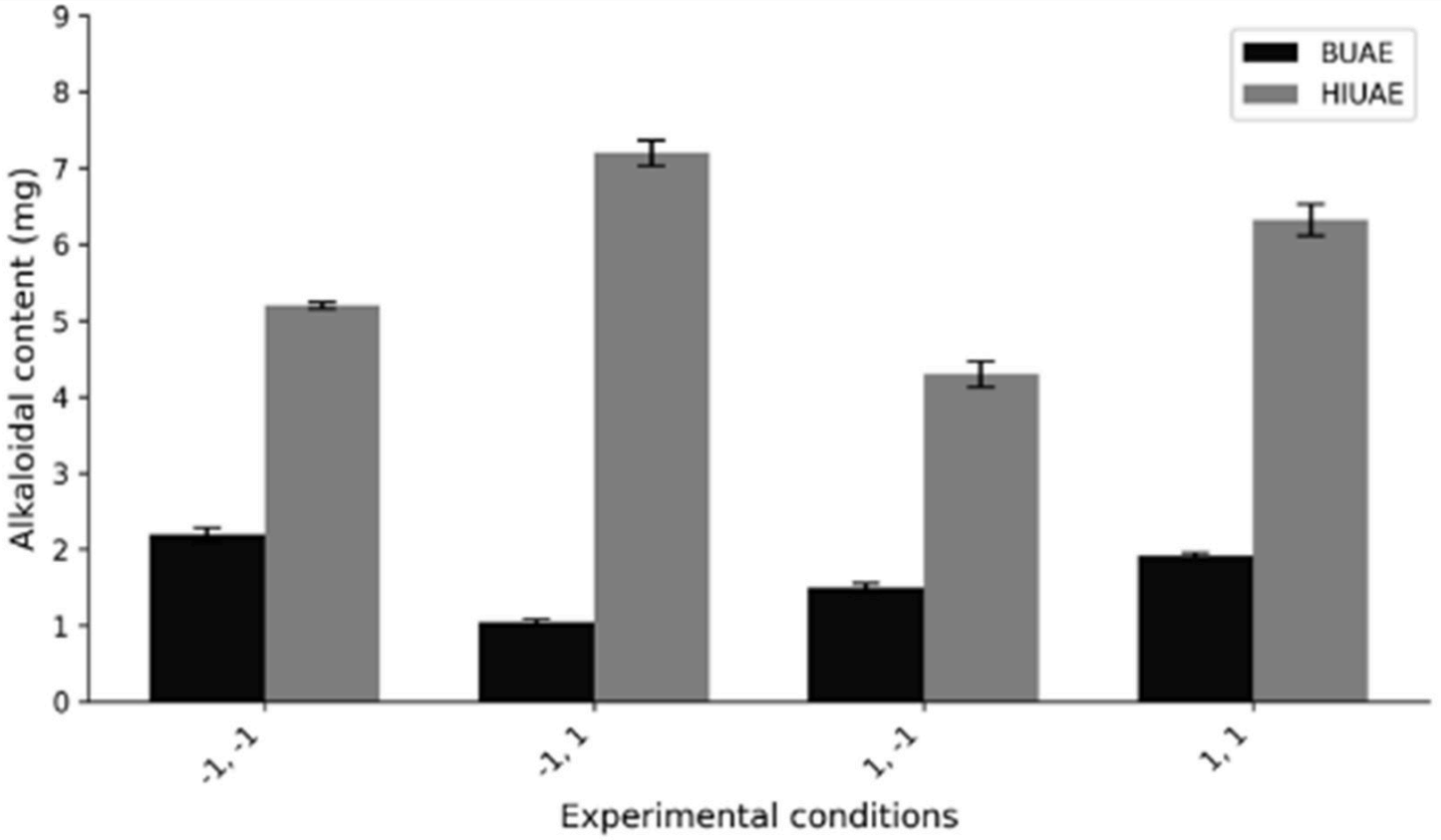
Table 2

Table 2 is not available with this version

Figures



Factors	Level	BUAE[a]	HIUAE[b]
Extraction time (min)	1	10	7
	-1	60	13
Solvent [c]	1	1: 0	1: 0
	-1	1: 1	1: 1

[a] BUAE, Bath Ultrasound Assisted Extraction. [b] HIUAE, High Intensity Ultrasound Assisted Extraction. [c] Methanol: Water (v/v)

Figure 1

Effect of experimental conditions on the extraction of the alkaloidal extract

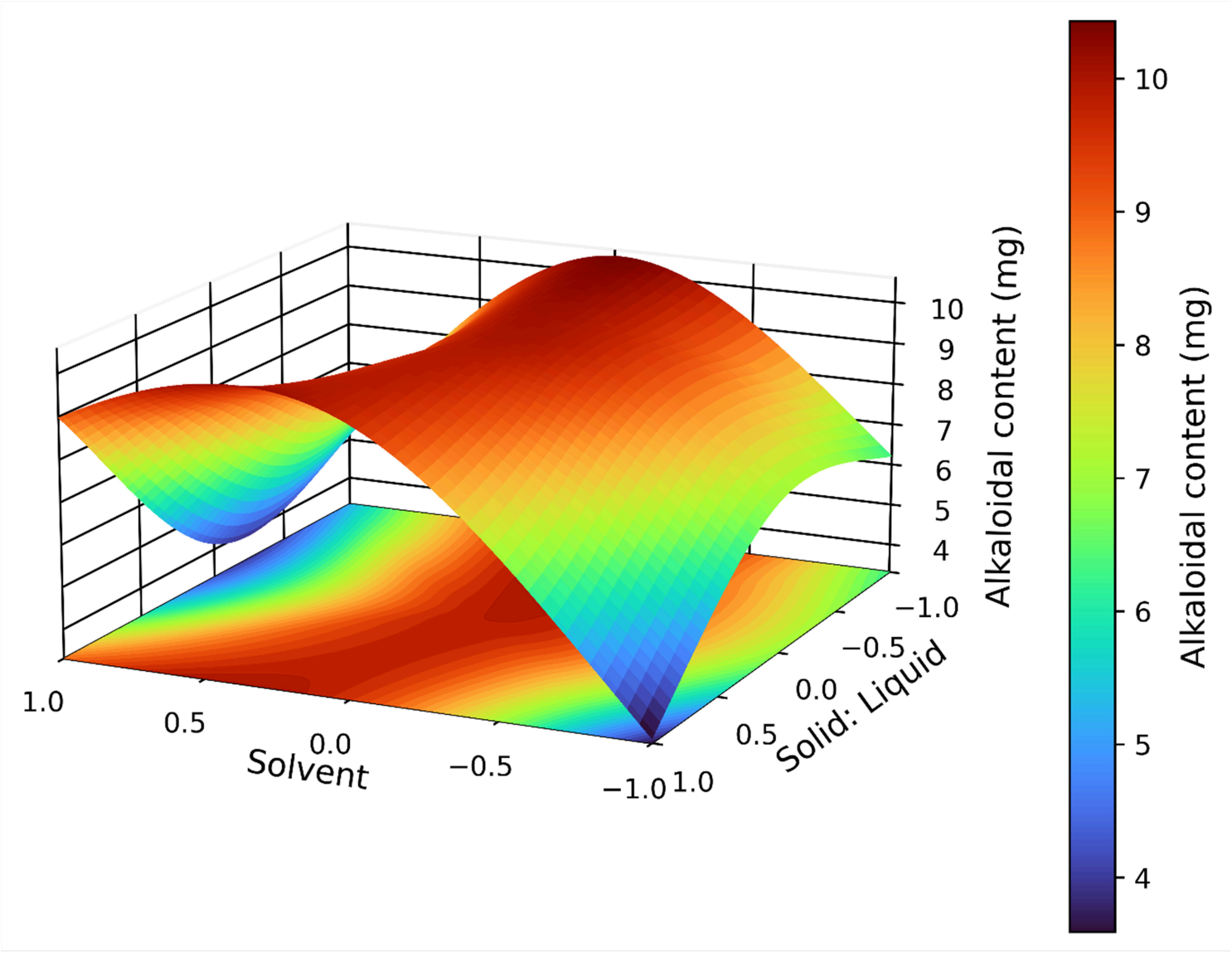


Figure 2

Response surface plots representing the effects of the solvent and solid: liquid ratio on the alkaloidal extract yield

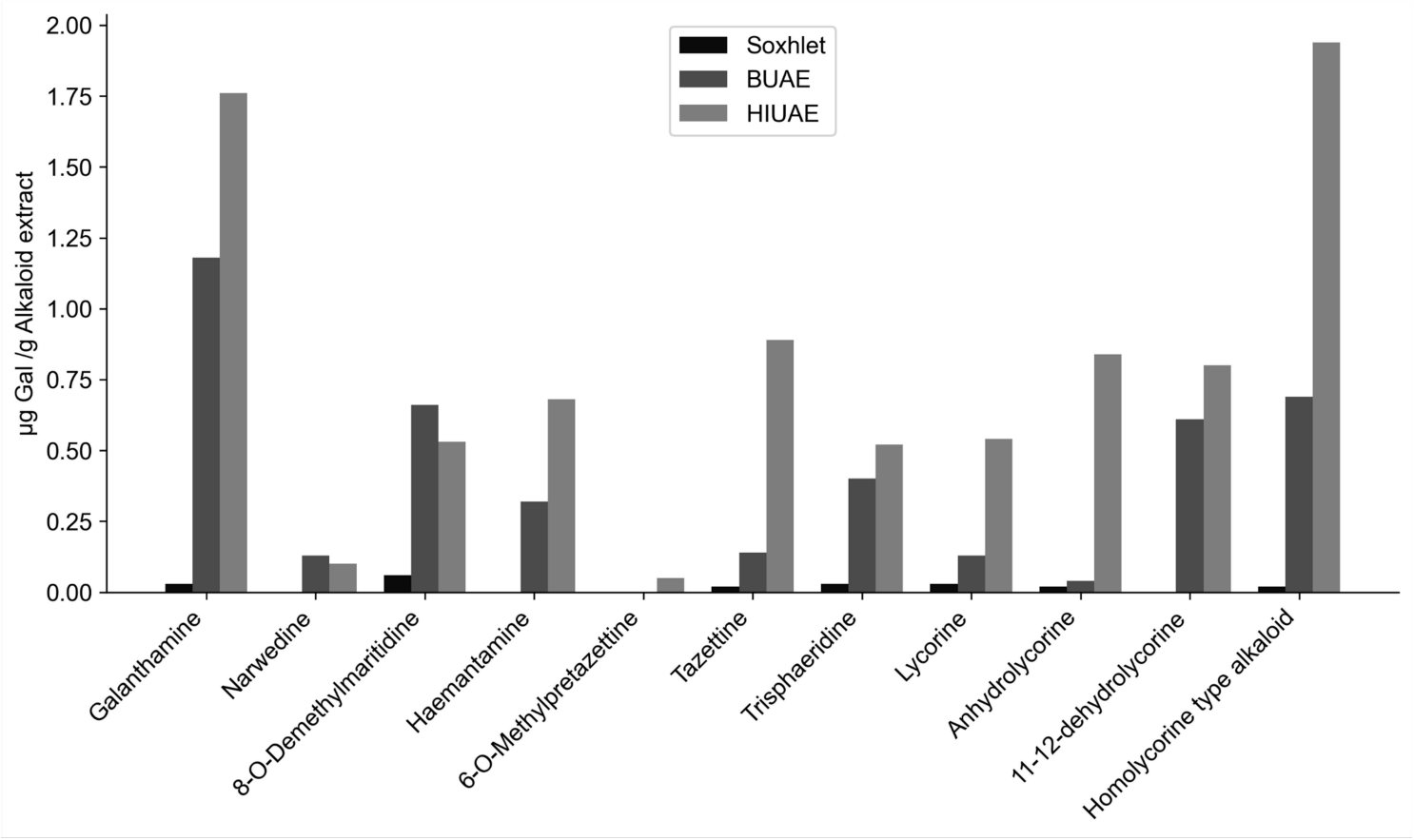


Figure 3

Alkaloids identifiedin bulbs of *C. subedentata* via Soxhlet, BUAE, and HIUAE methods

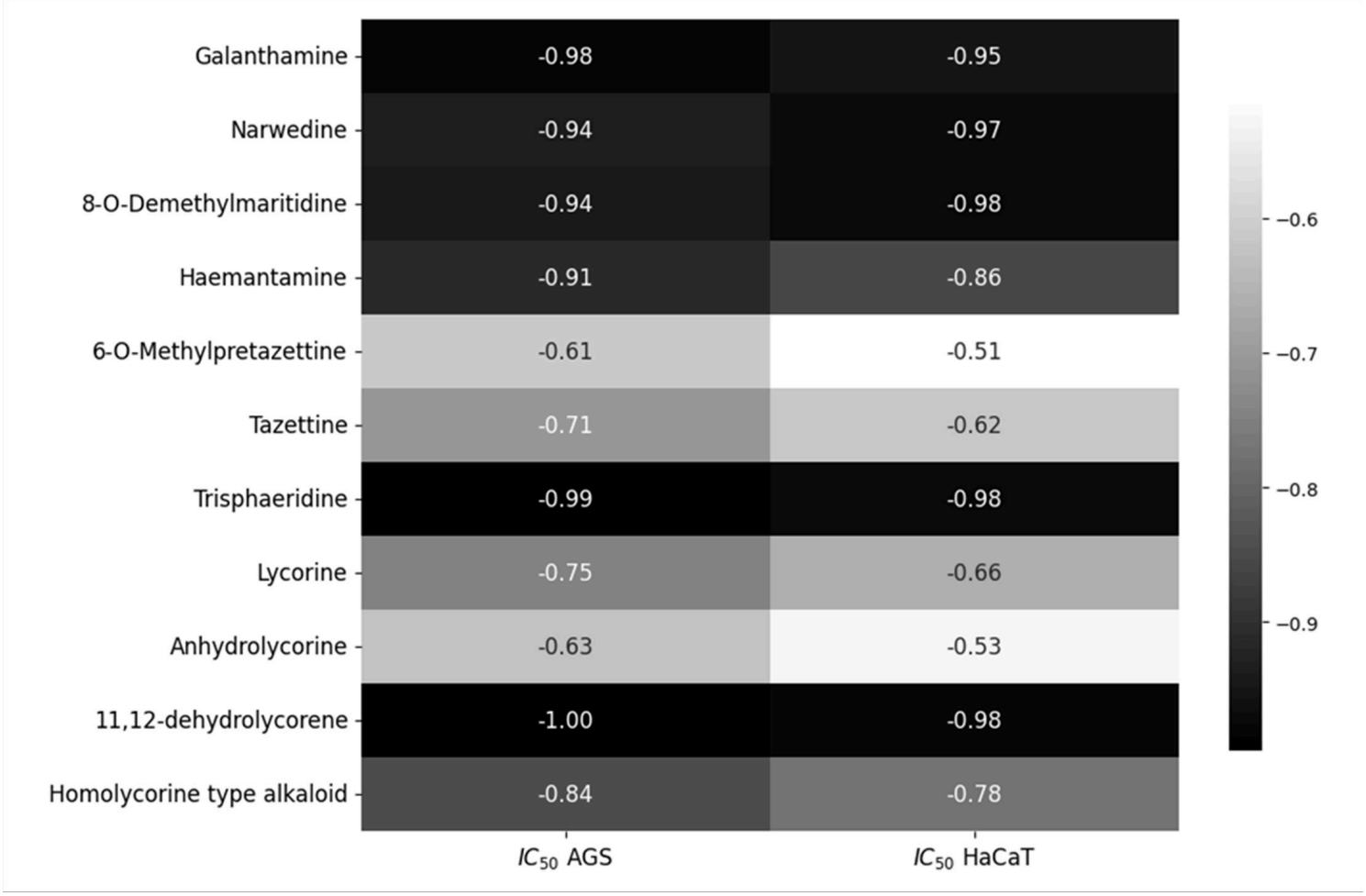


Figure 4

Pearson correlation heatmap between identified alkaloids and their cytotoxic potential in AGS and HaCaT cells (IC_{50} µg/ml)

Supplementary Files

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