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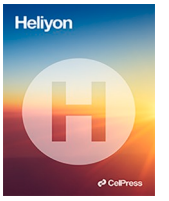

Research article

Antiviral alkaloids from *Crinum jagus*: Extraction, synergistic effects, and activity against dengue virus and human coronavirus OC43

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ABSTRACT

Crinum jagus, a medicinal plant from the Amaryllidaceae family, possesses potent antiviral properties attributed to alkaloids such as cherylline and lycorine. This study evaluated various extraction methods–including continuous shaking, hot solvent, microwave-assisted, ultrasound-assisted, and liquid-liquid extraction using methanol, followed by ethyl acetate and subsequent acid-base to optimize the yield of bioactive compounds. The extraction method significantly influenced phenolic acid and alkaloid precursor content, with liquid-liquid extraction yielding the highest amounts. LC-MS/MS analyses confirmed the presence of major alkaloids in the extracts, notably cherylline and lycorine. The cytotoxic and antiviral properties of *C. jagus* extracts were assessed using a reporter-encoding dengue virus (DENV) vector and the β-coronavirus HCoV-OC43. LLE_E (liquid-liquid extract extract), enriched in phenolic compounds, was the most cytotoxic extract at concentrations above 0.6 μg/mL. Acid-base fractions, enriched in alkaloids, exhibited higher cytotoxicity than the methanol extracts counterparts, with significant cell death at concentrations above 2.5 μg/mL Additionally, the acid-base and LLE_E extracts were also the most efficient in inhibiting the replication of both HCoV-OC43 and DENV, with EC_{50} values ranging from 1 to 2.5 μg/mL. The synergistic antiviral effect of cherylline with other *C. jagus* alkaloids was also evaluated, revealing that a combination of cherylline with gigantellinine strikingly reduced the flavivirus replication. These findings underscore the potential of *C. jagus* as a source of bioactive compounds with antiviral properties and highlight the importance of optimizing extraction methods to enhance specific applications.

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1. Introduction

The medicinal plant family Amaryllidaceae comprises about 75 genera, whose 1600 species are widely distributed worldwide in tropical and subtropical regions. Among them, *Crinum jagus* (J. Thomps.) Dandy, is a tender perennial bulb native to tropical Africa. This geophyte grows primarily in the seasonally dry tropical biome and is characterized by tulip-like white flowers that bloom in clusters during the summer atop leafless stalks typically rising to 2–3′ tall from a clump of strap-shaped green leaves. Some flowers are fragrant, while others have little aroma [1,2]. *C. jagus* extracts show several interesting biological properties and are used in West-African traditional medicines for their emetic, anti-diarrheal, anti-asthma, anti-diabetic, wound-healing, anti-microbial, anti-oxidant anti-drepanocytose properties [3–6]. *C. jagus* also display antiviral activity that has been attributed to the presence of alkaloids cherylline and lycorine [7,8]. Amaryllidaceae alkaloids (AAs) represent a unique class of specialized metabolites found predominantly in the Amaryllidoideae plant subfamily [9]. AAs have been studied since at least 1877, when the isolation of lycorine from *Narcissus pseudonarcissus* was reported [10]. Approximately 650 naturally occurring AAs have been identified, many of which possess various potent biological and pharmacological properties [11–13] Galanthamine, for instance, as an inhibitor od acetylcholinesterase, is a lead molecule currently used to treat symptoms of Alzheimer's disease [14,15]. Lycorine and its derivatives are noteworthy candidates for anti-cancer treatments [16–20]. Other AAs display inhibitory activity specific to the glycogen synthase kinase-3β, an enzyme implicated in bipolar disorder, strokes, and diabetes [21].Lycorine, lycoricidine and cherylline are actively investigated for their antiviral properties, especially against flaviviruses (*i.e.* dengue, Zika, Yellow fever, Japanese encephalitis, and West Nile viruses) [7,22–24] and coronaviruses (such as human coronavirus OC43 and the severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) [25–28]. Cherylline is quasi-exclusively reported in extracts from plants of the *Crinum* genus [29,30], while lycorine is detected in many Amaryllidoideae species.

AA extraction is a critical step for the study and downstream applications of these potent biological compounds. The most commonly utilized extraction methods involve various organic solvents combined with chromatographic and spectrometric techniques [31]. Extraction of AAs from natural sources presents several challenges. The yield of AAs from natural sources is often low or variable, which makes the extraction process inefficient. Traditional extraction methods often involve organic solvents, which can have a significant environmental impact. Further, methods such as Soxhlet and maceration can be costly and energy-intensive [32]. The extraction process can generate significant waste, causing environmental and economic problems. The overharvesting of plants for AA extraction can lead to sustainability issues. To overcome these challenges, researchers are exploring "green" extraction methods, such as ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, and supercritical fluid extraction. These methods are based on mild extraction conditions, environmentally friendly solvents, and lower time, cost, and energy consumption. However, further research and development are needed to improve these methods and make them more efficient and sustainable [33].

The primary objective of this study was to comprehensively investigate the extraction methods of AAs from *C. jagus* and evaluate their antiviral potential. We aim to enhance the yield and purity of these bioactive compounds by improving and comparing various extraction techniques. Furthermore, this research seeks to elucidate the synergistic interactions among the diverse alkaloids within the *C. jagus*, thereby providing deeper insights into their combined antiviral efficacy.

2. Material and methods

2.1. Chemicals

Standards of the alkaloids cherylline, flexinine, gigantelline, gigantellinine and crinine were isolated from *Crinum jagus* as in Ref. [8]. Standards of 3,4-dihydroxybenzaldehyde (97 %), 4-hydroxybenzaldehyde (99 %) and isovanillin (98 %) were purchased from Acros Organics (Massachusetts, USA). Standards of L-tyrosine (99 %) and *p*-coumaric acid (98 %) were purchased from Alfa Aesar (Massachusetts, USA). Standards of caffeic acid (98 %), dopamine (98 %), ferulic acid (99 %), lycorine (98 %), papaverine (98 %), tyramine (99 %) and vanillin (99 %) were procured from Millipore Sigma (Massachusetts, USA). Standard of phenylalanine (98 %) was obtained from MP Biomedicals (California, USA). Analytical LC-MS grade methanol was purchased from Fisher Scientific (New Hampshire, USA). Standards of 4′-*O*-methylnorbelladine and norbelladine were synthetized as described in Girard et al., 2022 [34]. Compounds were dissolved in dimethylsulfoxide (DMSO) as a stock solution at a concentration of 100 mM and stored at −20 °C until further usage. On the day of the assays, they were diluted to the desired concentration in Dulbecco's Modified Eagle's Medium high glucose (DMEM) for Huh7 cells or Roswell Park Memorial Institute medium (RPMI) for HCT8 cells. DMSO was used as a negative control, whereas lycorine was used as positive antiviral control.

2.2. Plant material

Bulbs of *Crinum jagus* (syn. = *Crinum giganteum*) were collected in Senegal, in Montrolland district (14°55′56,22″N and 16°59′38,62″W), in December 2018. A senior scientist from the Herbarium of IFAN of University Cheikh Anta Diop of Dakar taxonomically identified the plant materials [8]. The roots were rinsed with tap water and then distilled to eliminate dust and/or bacterial/fungal residues. The materials were then cut into small pieces using a sterile knife, weighed, divided into sample portions, and promptly stored at −80 °C. These materials were macerated and crushed using liquid nitrogen, mortar, and pestle before being subjected to different extraction methods. All solvents used for extraction and chromatographic analysis were of analytical or HPLC grade (VWR, Canada). Following each extraction, parts of the dried samples were dissolved in methanol for chemical analysis (section

2.3, extracts_E), or in DMSO for biological investigations at a stock concentration of 100 mg/ml. The rest of the dried samples was further extracted with an acid base method (extracts_A, section 2.4), dried and then dissolved in methanol for chemical analysis and in DMSO for biological experiments at 10 mg/ml.

2.3. Preparation of plant extracts

Continuous shaking extraction method (CSE): The macerated plant material (1 g) was placed in 80 % methanol. The plant material and solvent mixture were placed in a shaker, set to 150 rpm and ambient temperature, and shaken continuously overnight. After shaking, the mixture was centrifuged (8000 rpm) at the same temperature for 10 min to separate the solid plant material from the liquid extract. The liquid extract was then concentrated by rotary evaporation under reduced pressure [35,36]. The concentrated extracts were dried (rotary evaporate) to yield solid samples, which were further dissolved in methanol for chemical analysis or in DMSO for subsequential biological experiments (CSE_E). Part of the dried samples were further extracted with an acid base method (CSE_A) as reported in 2.4.

Hot solvent extraction (HE): Plant sample was placed in 80 % methanol and incubated in a 70 °C water bath. The heat facilitates the solubilization of the desired compounds into the solvent. The mixture was continuously stirred to ensure uniform heating and to maximize the contact between the plant material and the solvent. After 1 h of heating, the mixture was centrifuged to separate the solid plant material from the liquid extract. Like the above, the extract was concentrated and dried for further use (HE_E) [35,37].

Microwave-assisted extraction (MAE): The plant material 80 % methanol mixture was exposed to microwave radiation. The extraction process was carefully controlled considering several parameters. These included the system's temperature (250 °C), the microwaves' power level, and the irradiation time (5 min). Each of these parameters was optimized to ensure efficient extraction of the bioactive compounds. To remove debris, samples were centrifuged. The supernatant was collected and concentrated to gain yield for further experiments (MAE_E).

Ultrasound-assisted extraction (UAE): This method couples high-power, low-frequency ultrasound waves into macerated plant material slurry in methanol. The ultrasound waves were combined via a probe-type ultrasonic processor into the slurry. The extraction process took place at room temperature and was carefully monitored considering the ultrasonic power, extraction time (15 min), and solvent-to-solid ratio (10:1). Each of these parameters was optimized to ensure efficient extraction of the bioactive compounds [38]. As before, centrifugation was used to remove debris, and the sample was concentrated, dried and dissolved (UAE_E).

Liquid-liquid extraction (LLE): The method separates compounds based on their relative solubilities in two different immiscible liquids, usually water (polar) and an organic solvent (less-polar). This method suspended the initial methanol extracted and dried plant material in water. The mixture was then subjected to a process where there was a net transfer of one or more species from aqueous to organic (*n*-hexane and ethyl acetate step wisely). *n*-Hexane assisted in the removal of apolar lipids. The ethyl acetate fraction was concentrated and dried for further experiments [39]. This LLE fraction is referred to as the LLE extracts (LLE_E).

2.4. Acid-base extraction

Following each extraction method described in 2.4, part of the dried material were subjected to an alkaloid extraction procedure (acid-base extraction); the dried crude extracts were dissolved in 20 mL of 2 % H_2SO_4, then an equal amount of petroleum ether was added. The separated aqueous layer was basified (pH 9.5–10) with 25 % liquid ammonia. Ethyl acetate was used to extract the alkaloid fraction [40], which was then evaporated and dissolved in a known volume of methanol for chemical analysis and in DMSO at 10 mg/ml for biological assays. The obtained fractions are hereafter referred to as acid-base extracts (i.e., CSE_A, HE_A, MAE_A, UAE_A, and LLE_A). All samples were treated uniformly.

2.5. Total alkaloid content (TAC)

The total alkaloid content (TAC) was calculated for each extract using an established method [41]. In summary, plant extracts were treated with 1 mL of 2 N HCl, filtered, then combined with an equal amount of bromocresol green and sodium phosphate buffer. The resulting solution was then boiled and diluted in distilled water. The reaction mixture was then extracted using chloroform, and the absorbance of the chloroform layer was measured at a wavelength of 470 nm. Strychnine standards of varying concentrations (20–120 μg) were utilized, and the results were reported as milligrams of strychnine equivalent (SE) per gram of extract.

2.6. Total phenolic content (TPC)

The extracts' total phenolic content (TPC) was calculated using the Folin–Ciocalteu method [41]. For this, 200 μL of the extract (mg mL^{-1}) was combined with the Folin–Ciocalteu reagent and mixed by agitation. Sodium carbonate (7.5 % w/v) was added following an incubation of 60 min at room temperature. The absorbance was then measured at a wavelength of 765 nm. A calibration curve was plotted using gallic acid as a standard (20–200 μg mL^{-1}). The TPC was then reported as milligrams of the extract's gallic acid equivalent per gram (GAE g^{-1}).

2.7. Total flavanoid content (TFC)

The total flavonoid content (TFC) was calculated using the aluminium chloride colorimetric method [42]. The plant extract or a

catechin standard solution (with concentrations ranging from 50 to 250 μg mL^{-1}) was placed into a test tube. The volume was then adjusted using distilled water, and 75 μl of a 5 % (w/v) sodium nitrite solution was added. After incubating for 6 min, 150 μl of a 10 % (w/v) aluminium trichloride solution was added, and the mixture was left to stand at room temperature for 5 min. The absorbance was then measured at a wavelength of 510 nm immediately after adding 0.5 mL of a 1 M sodium hydroxide solution. The TFC was then reported as the extract's milligrams of catechin equivalent per gram (CE g^{-1}).

2.8. Total tannin content (TTC)

The total tannin content (TTC) was measured using the vanillin-HCl method [43]. The sample extract or a catechin standard solution (50–300 μg mL^{-1}) was mixed with a reagent solution (4 % vanillin in methanol and 8 % concentrated HCl in methanol at a 1:1 ratio). Following a 20 min incubation at room temperature, the developed colour was read at a wavelength of 500 nm with a spectrophotometer. The TTC was then reported as milligrams of catechin equivalent per gram (CE g^{-1}) of the extract.

2.9. Standard preparation, LC-MS^n analysis

All standards (*i.e.* dopamine, tyramine, lycorine, L-tyrosine, norbelladine, phenylalanine, cherylline, flexinine, crinine, gigantellinine, gigantelline, 4′-O-methylnorbelladine, 3,4-dihydroxybenzaldehyde, papaverine, 4-hydroxybenzaldehyde, caffeic acid, isovanillin, vanillin, *p*-coumaric acid and ferulic acid) were first prepared individually as stock solutions at 100 mg/L in LC-MS grade methanol. From these stocks, a working solution was prepared containing all analytes at 10 mg/L each in LC-MS grade methanol. This solution was further diluted to prepare calibration solutions with the following concentrations in triplicate in mobile phase (*i.e.* Milli-Q water and LC-MS grade methanol, both containing formic acid 0.1 % v/v (90:10)): 0, 12.5, 25, 50, 100, 200, 1000 and 5000 ng/mL. A quality control was also prepared in triplicate at 500 ng/mL to validate different analytical parameters. An internal standard, papaverine, was used to normalize the signals obtained on the LC-MS/MS and was added in each calibration solution and quality control at a final concentration of 1000 ng/mL. All solutions, including mixed standards, samples, and spiked solutions, were filtered using a 0.22 μm nylon syringe filter and preserved at −20 °C until injection. Calibration solutions were injected into the HPLC-MS/MS system and used to generate calibration curve regressions. Calibration curves were obtained by plotting the peak area ratio (i.e. analyte's peak area divided by papaverine internal standard's peak area) as a function of the analyte's concentration, which allowed targeted compounds' quantification.

Targeted metabolite analysis for AA precursor molecules and AAs were conducted using a high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) (Agilent, QC, Canada) system equipped with an Agilent Jet Stream ionization source, a Kinetex EVO C18 column (150 × 4.6 mm, 5 μm, 100 Å; Phenomenex, Torrance, USA), a binary pump, an autosampler set at 4 °C and a column compartment. Five microliters of each sample were injected into the column that was set at 30 °C. A gradient made of (A) formic acid 0.1 % v/v in Milli-Q water and (B) formic acid 0.1 % v/v in methanol, with a flow rate of 0.4 mL/min, was used to achieve chromatographic separation. The HPLC elution program was as follows: 0–10 min, 10 % B; 10–20 min, 100 % B; 20–25 min, 100 % B; 25–26 min, 10 % B. The total run time was 30 min per sample to allow the reconditioning of the column prior the next injection. The parameters used for the MS/MS source were set as follows: gas flow rate 10 L/min, gas temperature 300 °C, nebulizer 45 psi, sheath gas flow 11 L/min, sheath gas temperature 300 °C, capillary voltage 4000 V in ESI+ and 3500 V in ESI– and nozzle voltage 500 V. Agilent MassHunter Data Acquisition (version 1.2) and MassHunter Qualitative and Quantitative Analysis (version 10.0) softwares were used for data acquisition and processing, respectively. Sample analyses were carried out in Multiple Reaction Monitoring (MRM) acquisition mode allowing compounds identification using authentic standards. Table 1 shows selected

Table 1
MRM transitions and corresponding parameters for selected alkaloids and precursors.

Analyte	Retention time (min)	Precursor ion (Q1, *m/z*)	Product ion (Q3, *m/z*)		Dwell time (ms)	Collision energies (V)
			Quantifier	Qualifiers		
Lycorine	4.011	288	147	270, 177, 119	5	28, 20, 20, 40
Phenylalanine	6.246	166	120	103, 77	5	9, 29, 40
Norbelladine	7.383	260	138	123, 121	5	5, 20, 17
Cherylline	7.739	286	243	165, 137	5	18, 40, 18
Flexinine	8.455	288	147	242, 149	5	30, 26, 34
Crinine	10.069	272	136	226, 196	5	26, 26, 34
Gigantellinine	13.710	316	137	273, 192	5	20, 20, 20
Gigantelline	15.599	300	257	194, 107	5	20, 20, 40
4′-*O*-methylnorbelladine	15.766	274	137	122, 94	5	18, 40, 40
3,4-dihydroxybenzaldehyde	15.888	137	108	119, 92	5	26, 20, 26
Papaverine	18.055	340	202	324, 171	5	27, 40, 40
4-hydroxybenzaldehyde	18.086	121	92	120, 65	5	26, 18, 30
Caffeic acid	18.406	181	163	135, 117	5	5, 10, 21
Isovanillin	18.604	153	93	125, 65	5	13, 10, 25
Vanillin	18.783	153	93	125, 65	5	13, 10, 25
p-Coumaric acid	19.449	165	147	119, 91	5	5, 17, 20
Ferulic acid	19.610	195	177	145, 117	5	5, 20, 21

MRM transitions and MS/MS parameters used for targeted compounds identification.

2.10. Biological assays

2.10.1. Cell culture

Huh7 cells (RRID:CVCL_0336) were grown in DMEM supplemented with 10 % fetal bovine serum and 1 % penicillin/streptomycin solution (PS) (all from GIBCO, Fisher Scientifics). HCT-8 cells (HRT-18, CCL24 from ATCC) were maintained in RPMI medium supplemented with 10 % horse serum (GIBCO, Fisher Scientifics) and 1 % PS. Cell lines were grown at 37 °C with 5 % CO_2. Infection assays were carried out at 33 °C and 5 % CO_2.

2.10.2. Cytotoxic assays of extracts

The cytotoxic activity of *C. jagus* extracts was measured at concentrations ranging from 0.039 to 10 μg/mL with the MTT-based colorimetric assay (Cell proliferation Kit I, Roche, Millipore Sigma) on both HCT-8 and Huh7 cells, as described in Ref. [44]. After a 72 h incubation at 33 °C and 5 % CO_2, the MTT labeling reagent was added at a final concentration 0.5 mg/mL, 4 h later the solubilizing solution was added, and cells were incubated overnight. Spectrophotometrical absorbance of the formazan product was read at 575 nm, using 700 nm as reference wavelength on a microplate spectrophotometer (Synergy H1, Biotek, QC, Canada). The % of viable cells was calculated as the ratio of absorbance in cells in presence of extract over absorbance in cells in absence of extract (maximal viability).

2.10.3. Cytotoxic assays of alkaloids

The alkaloids individual and synergistic (0.42–100 μM, 4-fold dilutions) cytotoxicity was assessed using the Cell-Titer GLO assay kit (Promega, Madison, WI, USA), as in Ref. [7] on both Huh7 and HCT8 following a 72 h incubation of at 33 °C and 5 % CO_2. DMSO was used as negative control (solvent) and lycorine as positive control. The room temperature reagent was added to the plates also equilibrated to room temperature, then plates were shaken orbitally for 2 min and rested for 10 min at room temperature. Luminescence was recorded with a microplate spectrophotometer. Viability percentages were obtained as the ratio of the signal corresponding to each alkaloid concentration to the signal of the equimolar DMSO control.

2.10.4. Human coronavirus-OC43 (HCOV-OC43) infection

Briefly, 2×10^4 HCT-8 cells were seeded in 96-well plates, treated with *C. jagus* extracts, alkaloids or solvent controls (same concentration range as for the cytotoxic assay), and infected with wild type human coronavirus (HCoV)-OC43 (Betacoronavirus 1, VR1558, ATCC), at an MOI = 0.01 (titer 1.6×10^6 $TCID_{50}$/mL). Cells were incubated for 4–5 days at 33 °C in a humidified 5 % CO_2, detached using trypsin (Fisher Scientifics), washed twice with PBS containing 0.5 % bovine serum albumin (BSA) at 180×*g* for 5 min, fixed with 4 % formaldehyde (Biobasic), washed twice, and permeabilized with 0.1 % Triton X-100. Cells were washed twice again and stained with a monoclonal mouse antibody specific for the HCoV-OC43 nucleoprotein (1:1000; clone 542-7D; Sigma-Aldrich) for 1 h at room temperature followed by an overnight incubation at 4°C, washed, and incubated with chicken anti-mouse immunoglobulin conjugated to CF™ 488 (1:400; Millipore Sigma) for 60 min at room temperature. Antibodies were diluted in PBS buffer containing 0.5 % BSA. After a last wash, the fluorescent cells were analyzed on a Beckman Cytoflex S flow cytometer (at least 10,000 events were acquired), or using a Axio Observer microscope (Carl Zeiss, Inc., Toronto, ON, Canada). In the case of microscopy analysis, the trypsinization step was omitted from the staining process and images were analyzed with ImageJ (NIH) [45]. Flow cytometry data analysis was performed using Flowjo software (BD, FlowJo LLC, Ashland, OR, USA). Fold inhibition of viral infection was calculated as the ratio between the % of untreated infected cells (maximal infection) and the % of infected cells in presence of extract.

2.10.5. Dengue virus infection

We used the green fluorescent protein (GFP) dengue virus propagative vector ($DENV_{GFP}$) encoded by pFK-DVs-G2A kindly provided by Ralf Bartenschlager (Heidelberg University, Germany) and Laurent Chatel-Chaix (Institut national de la recherche scientifique, Québec, Canada) [46]. Viral titer (UI/ml) was measured by flow cytometry, using the formula [(% infected cells - % mock) x (number of seeded cells))/(100 x volume of virus inoculated), as described in Ref. [47]. For antiviral assays, 1×10^4 Huh7 cells/well were plated to 96-well plates and incubated at 37 °C overnight, treated with plant extracts, alkaloids and solvent controls, and infected at MOI of 0.1. Cells were incubated continuously with treatment and virus at 37 °C and 5 % CO_2 for 72 h, detached with trypsin for flow cytometry, and fixed in 4 % formaldehyde. Infection was assessed using a fluorescence microscope and the percentage of infected cells was measured by flow cytometry.

2.11. Statistical analyses

All experiments were conducted in triplicates. The results are presented as the average value ± standard error. A one-way analysis of variance was utilized to compare the average values of each treatment using SPSS. Duncan's test was used to identify significant differences between the averages. Statistical analyses and related figures were computed using GraphPad Prism version 10.0.2 (GraphPad Software, San Diego, California USA).

3. Results

3.1. Chemical composition

Initially, five extraction methods were investigated for their chemical properties. The recovery rates expressed as a percentage of fresh weight, varied among the techniques employed. The continuous shaking extract (CSE) and hot extract (HE) methods resulted in the highest yields, 11.59 ± 0.32 % and 11.21 ± 0.26 %, respectively. The microwave-assisted extract (MAE) reached 9.97 ± 0.41 %, while the ultrasonic-assisted extract (UAE) and the liquid-liquid extract (LLE) demonstrated similar efficiencies, at 10.21 ± 0.07 % and 10.18 ± 0.06 %, respectively.

The liquid-liquid extraction (LLE) method proved most effective for the extraction of compounds with high polarity or solubility in specific solvents, resulting in the highest total alkaloid content (TAC) of 6.5 ± 0.3 mg strychnine equivalent (SE) g^{-1} extract. The MAE method followed closely with 6.2 ± 0.2 mg SE g^{-1} extract, while UAE yielded 5.75 ± 0.1 mg SE g^{-1} extract. TheHE and CSE methods yielded lower TAC values of 4.5 ± 0.4 mg SE g^{-1} extract and 3.75 ± 0.1 mg SE g^{-1} extract, respectively.

In terms of total phenolic content (TPC), the MAE method demonstrated the highest extraction output, yielding 8.75 ± 0.4 mg gallic acid equivalent (GAE) g^{-1} extract. The UAE method also proved effective in preserving phenolic compounds, with a TPC value of 6.77 ± 0.2 mg GAE g^{-1} extract. The LLE, CSE, and HE methods resulted in lower TPC values of 5.64 ± 0.32, 5.01 ± 0.32 and 4.61 ± 0.32 mg GAE g^{-1} extract, respectively.

The total flavonoid content (TFC) and total tannin content (TTC) were lower compared to TAC and TPC. The LLE method resulted in the highest TFC (4.25 ± 0.2 catechin equivalent (CE) g^{-1}) but the lowest TTC (1.12 ± 0.34 CE g^{-1}). The MAE and UAE methods exhibited moderate TFC and TTC values, consistent with their performance in TAC and TPC extraction. The HE and CSE methods consistently yielded the lowest values for all measured parameters.

3.2. LC-MS^n detects alkaloid metabolites and precursors

In analyzing the metabolite yield from different extraction methods, distinct patterns emerged for each compound (Fig. 1a). Notably, LLE_E consistently outperformed other methods, particularly for cherylline and lycorine. For gigantelline, all methods except LLE yielded similar results, suggesting that alternative extraction method had little impact. Lycorine yield varied significantly across methods, with CSE_E yielding the least. Flexinine and vittatine/crinine were present in consistently low concentrations, regardless of the method. AAs were enriched several-fold following the acid-base extraction procedure across all extracts (Figs. 1b and 2). LLE acid-base extracts (LLE_A) proved the most efficient method for recovering all six targeted alkaloids from *C. jagus* bulb (Fig. 1b and c, and Fig. 2). Significant differences ($p < 0.0001$) were observed between LLE_A and the other extraction methods (CSE_A, HE_A, MAE_A, UAE_A) in the yields of cherylline, gigantelline, gigantellinine, and lycorine. Notably, lycorine yield was affected by the extraction method ($p < 0.0001$), with the highest recovery achieved through LLE_A. In the case of gigantelline, a statistically significant difference

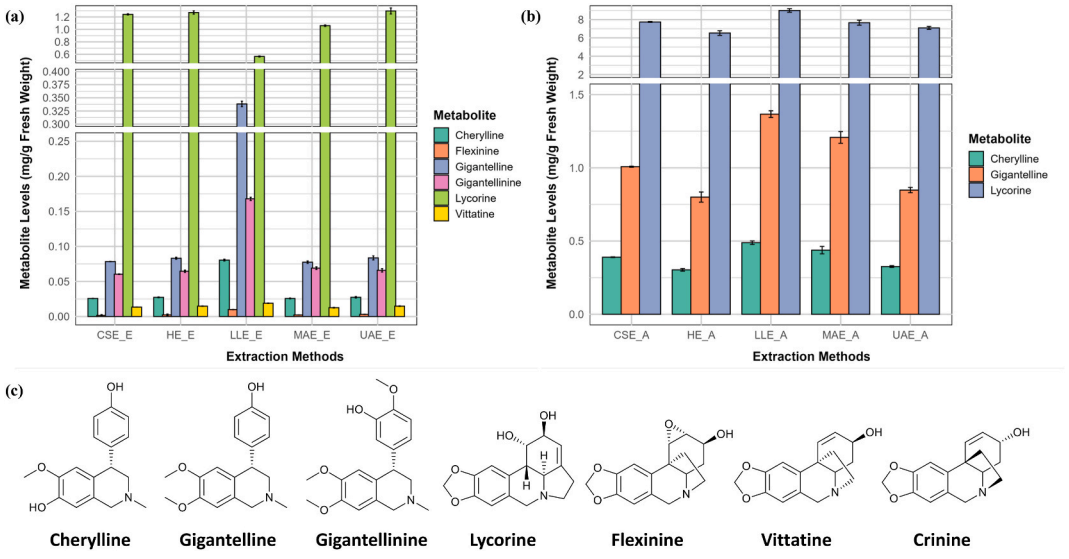

Fig. 1. Alkaloid content and chemical structures of *Crinum jagus* extracts prepared using various extraction methods. (a) and (b) show the alkaloid content of extracts obtained using continuous shaking extraction (CSE), hot extraction (HE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and liquid-liquid extraction (LLE). Methanol extraction is represented by E and subsequent acid-base extraction are denoted by A. (c) shows the chemical structures of the alkaloids cherylline, gigantelline, gigantellinine, lycorine, flexinine, vittatine, and crinine. Data represent the mean ± standard deviation (n = 3). Different letters above the bars indicate significant differences ($p < 0.05$) according to Turkey's test. FW: fresh weight.

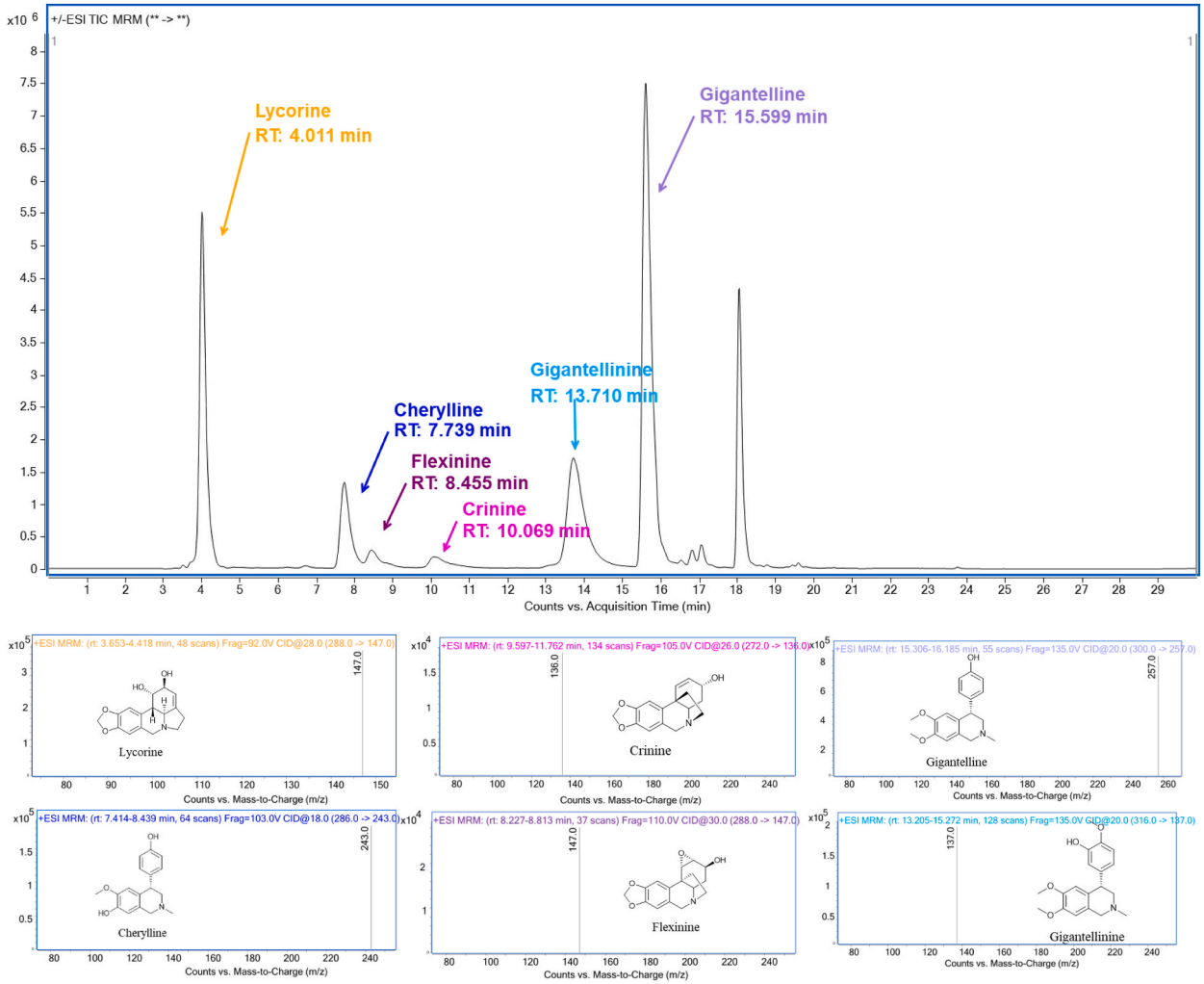

Fig. 2. Total Ion Chromatogram (TIC) of *Crinum jagus* extract analyzed by HPLC-MS/MS and MS of selected alkaloids. The TIC shows the relative abundance of ions detected over time, with peaks corresponding to individual compounds. The major alkaloids identified in the extract are labeled: cherylline, flexinine, crinine, lycorine, gigantellinine, and gigantelline. The retention time of each compound is indicated on the x-axis. MS is provided indicating its mass ion peak and structure.

was observed between CSE_A and HE_A ($p < 0.05$), with HE_A resulting in higher yields.

The concentrations of phenylpropanoid precursors (caffeic acid, ferulic acid, 4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, vanillin, isovanillin) varied depending on the extraction method (Fig. 3) and were only detectable following LLE_E total extraction (Fig. 3a). 3,4-DHBA was the most abundant compound extracted in LLE_E (Fig. 3b), while only 4′-*O*-methylnorbelladine was detected following UAE_E extraction method (Fig. 3b). Subsequent acid-base extraction resulted in the detection of 4′-*O*-methylnorbelladine and vanillin in samples derived from most extraction methods. LLE-A rather contained 3,4-DHBA together with vanillin. Thus, the acid-base method helped enrich in the protoalkaloid structure 4′-*O*-methylnorbelladine and in vanillin in most extracts.

3.3. *Cytotoxicity of Crinum jagus extracts towards adenocarcinoma HCT-8 and hepatocarcinoma Huh7 cells*

Following the characterization of the chemical properties of the extracts, we compared their cytotoxic activity, using lycorine and DMSO as positive and negative controls, respectively. Adenocarcinoma HCT-8 and hepatocarcinoma Huh7 cell lines were studied. The cytotoxicity of lycorine was visible at all concentrations in HCT-8 cells (0.039–10 μg/mL, 40–66 % viable cells) after 72 h treatment (Fig. 4b). Extracts were less cytotoxic to Huh7 (CC_{50} was not reached at 10 μg/mL) than HCT-8 cells, but the same trends were observed. LLE_E was the most active against both cell lines. In Huh7 cells, the acid base extracts UAE_A, LLE_A, MAE_A, CSE_A, and HE_A were more toxic (ranked from most to less cytotoxic) thanthe total extracts counterparts (Fig. 4a–Table 2). In HCT-8 cells, total extracts (i.e. not acid-base) were weakly cytotoxic, except for LLE_E and CSE_E (CC_{50} = 1.7 and 9.7 μg/mL, respectively)

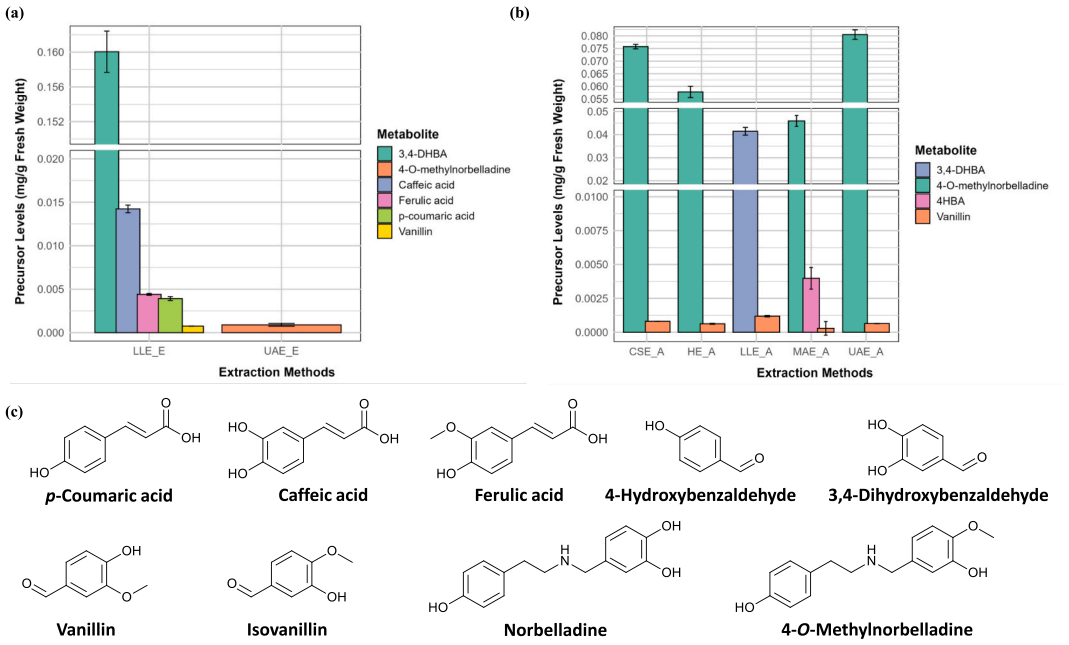

Fig. 3. Phenolic acid and alkaloid precursor content in *Crinum jagus* extracts. (a) and (b) Quantification of phenolic acids (*p*-coumaric acid, caffeic acid, ferulic acid) and alkaloid precursors (4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde (3,4.DHBA), vanillin, isovanillin, norbelladine, 4-*O*-methylnorbelladine) using various extraction methods: cold solvent extraction (CSE), hot solvent extraction (HE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and liquid-liquid extraction (LLE) with ethanol (E) and acid-base (A) as solvents. Data are presented as mean ± standard deviation (n = 3). Asterisks indicate significant differences among extraction methods for each compound ($p < 0.05$, Tukey's test). FW: fresh weight. (b) Chemical structures of the identified phenolic acids and alkaloid precursors.

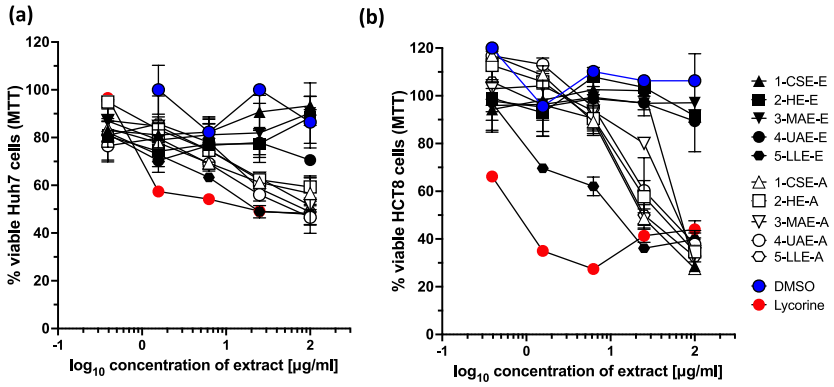

Fig. 4. Cytotoxic potential of *Crinum jagus* extracts in Huh7 cells (a) **and HCT-8** (b). After a 72 h treatment with each extract fractions, viability of HCT8 and Huh7 cells was assessed by MTT assay. CSE: Continuous shaking extraction method, HE: Hot solvent extraction, MAE: Microwave-assisted extraction, UAE: Ultrasound-assisted extraction, LLE: Liquid-liquid extraction (LLE), E: total extract, A: acid base Shown are means of biological triplicates ± SD. When SD is smaller than the symbol, it is not visible.

(Fig. 4a–Table 2). Acid-base extractions were more cytotoxic with LLE_A > CSE_A > HE_A > UAE_A > MAE_A (Table 2). Overall, the higher cytotoxicity of acid-base extracts suggests that the enrichment in alkaloids contributed to this effect. However, in both cellular contexts, LLE_E was the most cytotoxic, suggesting a role for phenylpropanoid compounds.

3.4. Antiviral properties of Crinum jagus extracts

C. jagus crude extracts were previously shown to display anti-flaviviral properties. We tested which of the extraction methods could lead to maximal antiviral activity. We used $DENV_{GFP}$ as representative of the flavivirus genus and observed that acid-base extracts and LLE_E displayed anti-DENV properties. As the CC_{50} was not reached at the highest tested concentration (10 µg ml^{-1}), the selectivity index (SI) could not be precisely estimated. Nonetheless, the most selective extracts were LLE_E, UAE_A and CSE_A with EC_{50} ranging

Table 2
Cytotoxic and antiviral properties of *C. jagus* methanol and acid-base extracts.

	CC_{50} (μg/mL)		EC_{50} (μg/mL)		SI	
	HCT-8	**Huh7**	**OC-43**	**DENV**	**OC-43**	**DENV**
CSE-E	9.7	>10	>10	>10	na	na
CSE-A	3.1	>10	0.57	0.19	5.43	>52.63
HE-E	>10	>10	>10	>10	na	na
HE-A	4.4	>10	0.36	0.41	12.2	>24.4
MAE-E	>10	>10	>10	>10	na	na
MAE-A	8.3	>10	0.53	0.40	15.7	>25
UAE-E	>10	>10	>10	>10	na	na
UAE-A	6.5	>10	0.62	0.15	10.5	>66.7
LLE-E	1.7	>10	0.63	0.15	2.70	>66.7
LLE-A	3.0	>10	0.48	0.31	6.25	>32.3

CC_{50}: concentration associated with 50 % of cell death, EC_{50}: concentration associated with 50 % of viral inhibition, SI: selectivity index was calculated as the ratio of CC_{50}/EC_{50}, OC43: human coronavirus OC43, DENV: dengue virus, CSE: Continuous shaking extraction method, HE: Hot solvent extraction, MAE: Microwave-assisted extraction, UAE: Ultrasound-assisted extraction, LLE: Liquid-liquid extraction (LLE), E: total extract, A: acid base, na: not applicable because the EC_{50} was not reached at the highest concentration tested.

between 0.15 and 0.19 μg/mL and SI > 50 (Fig. 5a and Table 2).

Second, as the extracts contain lycorine, we hypothesized that they could also target coronaviruses replication [7,25]. We used HCoV-OC43, a β-coronavirus responsible for mild upper tract respiratory symptoms, and genetically closely related to life-threatening coronaviruses, such as SARS-CoV-2 (Fig. 5b). Acid-base extracts and LLE_E were the most potent mixtures with EC_{50} ranging from 0.36 to 0.63 μg/mL, MAE_A, HE_A and UAE_A being the most selective (Table 2). These results show that *C. jagus* extracts possess anti-coronaviral and anti-flaviviral properties.

3.5. Antiviral activity of Crinum jagus metabolites

First, we assessed the antiviral activity of individual metabolites detected in *C. jagus* extracts, including 3,4-DHBA, as well as AAs cherylline, gigantelline, gigantellinine, flexinine, and lycorine specifically against $DENV_{GFP}$ and HCoV-OC43. Tested concentrations were chosen based on cytotoxicity (Fig. 6a and b) and on inhibition of flaviviral replication from previous report [7]. The anti-$DENV_{GFP}$ effect was observed by measuring the decrease in GFP fluorescence using microscopy imaging. Maximal infection was measured following DMSO treatment. Cells treated with 8 and 16 μM of cherylline display a dose-dependent decrease in GFP confirming the anti-DENV activity of cherylline (Fig. 7a). Gigantelline (150 μM), gigantellinine (150 μM), and lycorine (0.25 μM) also displayed anti-DENV activity, while the effect of Flexinine (150 μM) and 3,4-DHBA (25 μM) was not clear.

HCoV-OC43 infection levels were measured by flow cytometry following intracellular staining with an anti-nucleocapsid antibody. Cherylline treatment (8 and 16 μM) led to a drop in the frequency of HCoV-OC43 infected cells from 7.58 to 2.27 and 1.27 %, respectively, showing that cherylline inhibits coronavirus infection in addition to flavivirus' (Fig. 7 b). Flexinine (150 μM), gigantelline (150 μM), gigantellinine (150 μM), 3,4-DHBA (25 μM), and lycorine (0.25 μM) also decreased infection in comparison to DMSO treatment (Fig. 7b). Gigantellinine led to a 9-fold decrease in HCoV-OC43 infected cells (0.88 %), 3,4-DHBA (25 μM, 2.08 %) decreased the frequency of HCoV-OC43 infected cells by a 4-fold magnitude, while others lead to a 2-fold decrease. Overall, these results show that several alkaloids present in *C. jagus* extracts display antiviral activities.

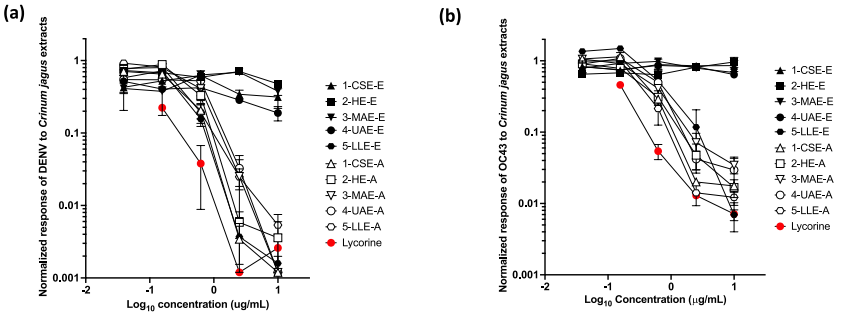

Fig. 5. Antiviral properties of *Crinum jagus* extracts. (a) Anti-flavivirus (DENV) activity in Huh7 cells. (b). Anti-betacoronavirus (HCOV-OC43) activity in HCT8 cells. Cells were treated with the fractions and infected with the corresponding virus at on MOI of 0.1 (DENV) or 0.01 (OC43). Huh7 cells were trypsinized, fixed and analyzed by flow cytometry 72 h post-infection. HCT8 cells were trypsinized, fixed, permeabilized, and stained with an anti-nucleocapsid antibody (green), secondary antibody and with hoechst33642 96 h post-infection. The % of infected cells was assessed by flow cytometry and normalized to the % of untreated control to yield the relative infection levels. Shown are means of biological triplicates ± SD.

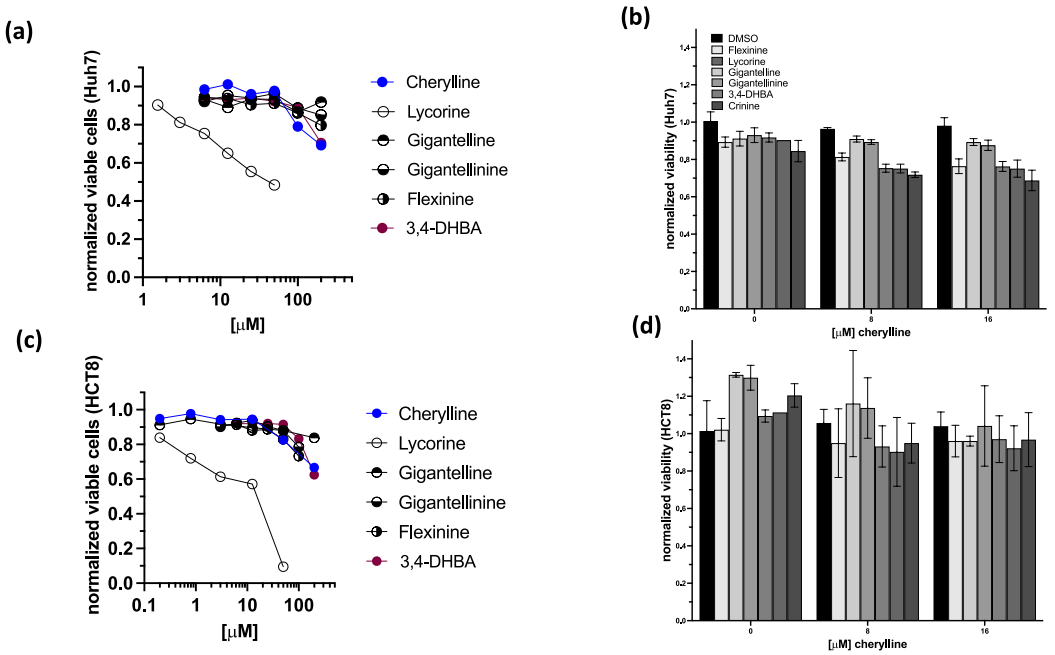

Fig. 6. Viability of Huh7 (a) and (b) and HCT8 (c) and (d) cells exposed to alkaloids alone (a) and (c) or in combination (b) and (d) was assessed using the Cell-Titer GLO assay as in Ref. [7].

3.6. Crinum jagus isolated AA synergistic antiviral activity

The relative abundance of lycorine or cherylline alone could not explain the differences between each extract's antiviral activity. Thus, we explored the possibility of a synergistic effect between cherylline and other metabolites present in the extract, *i.e.* 3,4-DHBA and AAs flexinine, gigantelline, gigantellinine, and lycorine. Combined inhibition was measured by flow cytometry and microscopy (Fig. 7) following assessment of cytotoxicity (Fig. 6b and d). Fold inhibition was computed as the ratio of the % of virus-infected cells in absence of treatment over the % of virus-infected cells in presence of treatment. A synergistic effect is indicated by an inhibition superior to the combined (additive) effect of the compounds separately. Combining 8 μM of cherylline (3-fold inhibition) with gigantellinine (4-fold inhibition) led to a striking enhancement of DENV inhibition (21-fold), much superior to a simple additive effect (which would be 7-fold, Fig. 7c). Combining 16 μM of cherylline (7-fold inhibition) with gigantellinine (4-fold) led to an even greater enhancement of DENV-inhibition (60-fold), suggesting synergism between the two cherylline-type alkaloids in antiviral activity. A combination of flexinine (2-fold), gigantelline (1.5-fold), or lycorine (2-fold) with cherylline at 16 μM also led to significant leaps in fold inhibition, i.e. 17- , 13- and 28-fold inhibition, respectively, while the combination with 3,4-DHBA did not result in any synergistic effect. While an additive outcome was noted, such synergistic effects between metabolites could not be observed in cells infected with HCoV-OC43 (Figs. 6c and 7d).

4. Discussion

Our investigation into the extraction of bioactive compounds from *C. jagus* bulbs revealed a nuanced interplay between yield and phytochemical composition, echoing previous findings in the field [48]. While traditional methods like CSE and HE offered higher overall yields, they fell short of retaining specific compounds of interest. This resonates with studies in other plant species where harsher extraction techniques led to the degradation of heat-sensitive antioxidants [49]. Interestingly, our results demonstrate the superiority of LLE for isolating alkaloid-rich fractions, aligning with its established efficacy in extracting polar compounds [50]. The rapid and efficient MAE method [51], proved most effective in retaining phenolic compounds, possibly due to its ability to disrupt cell walls without extensive heat exposure. The UAE method, known for enhancing mass transfer and cell disruption [52], struck a balance between yield and compound preservation, suggesting its potential as a versatile technique for *C. jagus* extraction. These findings emphasize the need for a tailored approach to extraction, where the chosen method is carefully matched to the desired phytochemical profile.

Subsequent experiments showed that LLE using methanol (LLE_E) consistently outperformed other methods, particularly for cherylline and lycorine, suggesting that these compounds were preferentially extracted with these specific solvent conditions. Interestingly, all methods except LLE yielded similar results for gigantelline, indicating that the choice of extraction method might not be critical for this particular compound. This observation warrants further investigation into the specific properties (the position of its methyl group), of gigantelline that might contribute to its consistent yield across various extraction methods. The yield of lycorine

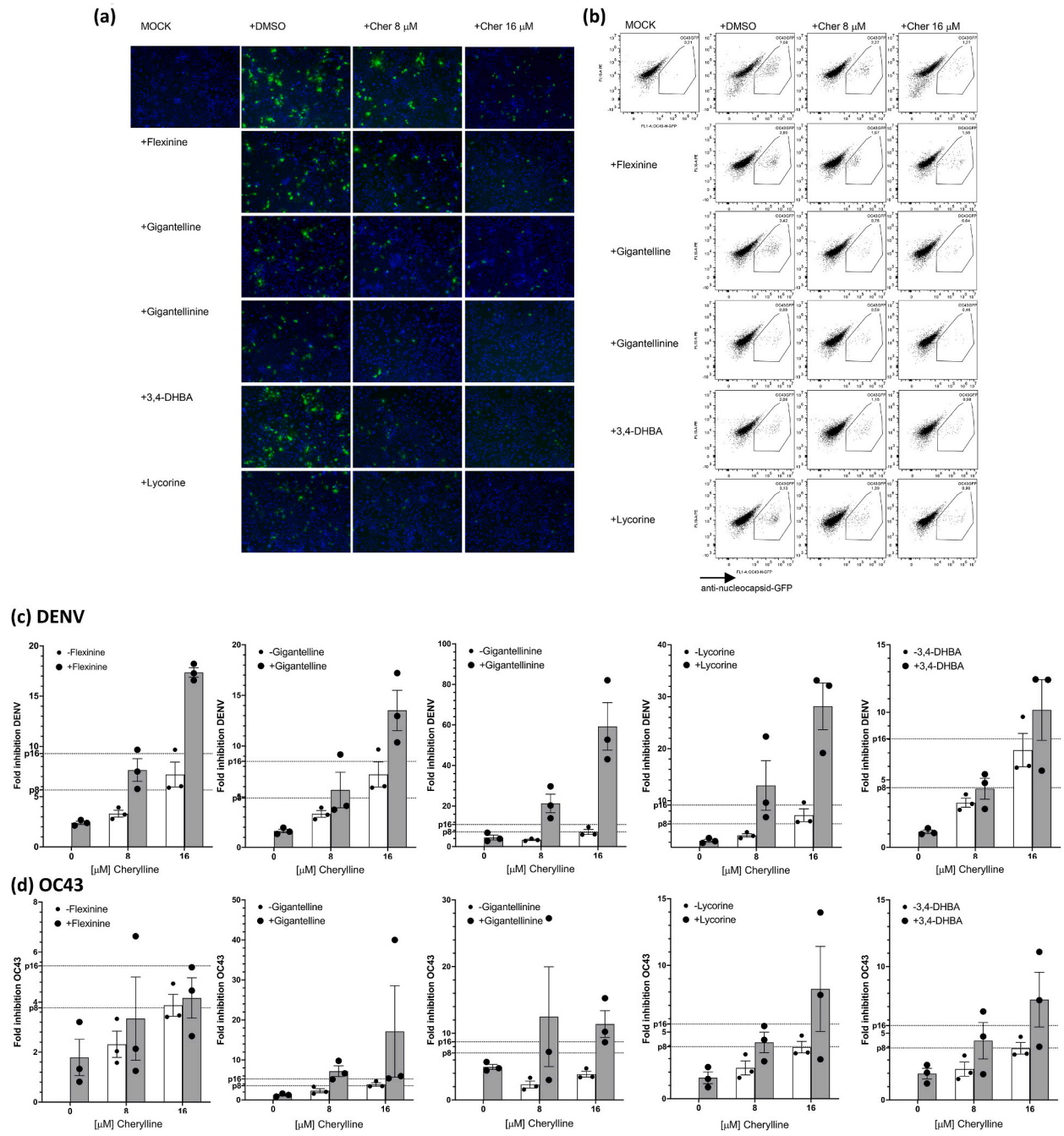

Fig. 7. Antiviral activity of *Crinum jagus* specialized metabolites. (a): Anti-flavivirus activity of single and combination of AAs extracted. Inverted microscope merged images taken at 5X of Huh7 cells stained with Hoechsst33642 (nucleus, blue) and infected with $DENV_{GFP}$ (green). (b) Anti-coronavirus activity of single or combined AAs and 3,4-DHBA. Flow cytometry dot plots of HCT8 cells infected with HCOV-OC43 treated with a combination of DMSO (0.1 %) or cherylline (8 or 16 μM), and flexinine (150 μM), gigantelline (150 μM), gigantellinine (150 μM), 3,4-DHBA (25 μM), or lycorine (0.25 μM). Cells were trypsinized, fixed, permeabilized, and stained with an anti-nucleocapsid antibody (green) and a secondary antibody, and with hoechsst33642 to gate on intact cells. Shown are representative pictures and dot plots on three biological replicates. (c) *C. jagus* AA synergestic effects on DENV inhibition. The % of infected cells was assessed by flow cytometry 72 h post-infection. (d) Combination of metabolites inhibitory effect against anti-betacoronavirus (HCoV-OC43). HCT-8 were used as target of OC43 infection, while Huh7 were used for DENV. Shown are means with SEM.

varied significantly depending on the extraction method, with continuous shaking extraction (CSE_E) yielding the least amount. This finding suggests that lycorine extraction might be influenced by the specific agitation and solvent exposure conditions employed in different methods. In analyzing the phenylpropanoid precursors, the results revealed distinct patterns depending on the extraction method. 3,4-DHBA was the most abundant compound extracted, with LLE_E again yielding the highest concentrations. The remaining phenylpropanoids were either not detected or present at very low concentrations across all methods except for LLE_E. This observation might be attributed to the specific solvent polarity and extraction conditions employed in LLE_E, which could have favoured the recovery of these compounds.

A subsequent acid-base extraction led to an increase in the amount of all alkaloids. Notably, LLE using acid-base partitioning (LLE_A) proved to be the most efficient method for recovering alkaloids, in agreement to Evidente et al. (1984) [53], underscoring the importance of pH adjustment in optimizing the extraction of alkaloid compounds. LLE performed better for alkaloids due to its ability to separate compounds based on their pKa and solubility in aqueous versus organic phases. The sequential use of methanol, ethyl acetate, and acid-base treatments optimizes the partitioning of alkaloids, enriching fractions with bioactive compounds like cherylline and lycorine. MAE leverages high temperatures and localized heating to enhance the release of phenolic compounds from the plant matrix. However, these conditions may degrade thermolabile compounds, explaining the variable yield of alkaloids compared to phenolics.

The alkaloids extracted from *C. jagus* exhibit diverse chemical structures, with various number and a diverse arrangement of exposed hydroxyl and methyl groups, influencing their isolation and purification. Cherylline and lycorine, with their polar hydroxyl groups, are effectively extracted using polar solvents like methanol in LLE. Gigantelline, also polar due to its hydroxyl groups, shows consistent recovery across various methods, suggesting its robustness to different extraction conditions. Gigantellinine, a structural isomer of gigantelline, likely shares similar polarity and extraction behaviour. Vittatine, the least polar due to the absence of hydroxyl groups, predominantly resides in the organic phase. By adjusting the pH to acidic conditions, the alkaloids are protonated, becoming more water-soluble inorganic salts and thus shifting towards the aqueous phase. This allows, by modulation of the pH of the medium, for selective extraction of the more polar alkaloids like lycorine, while the less polar ones like vittatine remain in the organic phase. Subsequent basification of the aqueous phase deprotonates the alkaloids, rendering them less water-soluble and enabling their recovery into a fresh organic solvent. This refined acid-base extraction, coupled with careful solvent selection based on polarity, enables efficient fractionation and isolation of individual AAs from complex plant extracts [54].

Previous studies have shown that the ethnopharmacology of *C. jagus* extracts included anti-microbial (*Shigella flexneri*) [3], and anti-oxidant [4] properties. Here, the differences in chemical composition were associated with distinct biological activities. We show that LLE-E extracts, with their diversified profile of alkaloids, phenylpropanoids and with the highest total flavonoid content, were the most cytotoxic extracts of this study. Indeed, a comparison of the extracts deeper content in AAs indicated that lycorine alone was not sufficient to explain enhanced cytotoxicity towards adenocarcinoma and hepatocarcinoma cells, since it was present in comparable amounts in studied samples. The presence of precursors of the phenylpropanoid pathway, such as 3,4-DHBA, vanillin, and of 4′-*O*-methylnorbelladine and norbelladine in LLE_E likely contributes to its enhanced cytotoxicity. In the context of RNA virus infections, several studies have shown that extracts possessed antiviral activity, against flaviviruses [40], coronaviruses and other families of virus [7,40], and that alkaloids play an important role in that antiviral effect [7,22,27,28,55–59].

C. jagus extracts also efficiently inhibited DENV and HCoV-OC43 replication. Acid-base extracts were more selective towards virus inhibition compared to cancer cell line toxicity. The most selectively antiviral extracts were UAE_A and CSE_A towards the flavivirus, and MAE_A, HE_A and UAE_A for the β-coronavirus. This suggests that subtle differences in alkaloid contents impacted the activity of the extracts (cytotoxic towards cancer cell line, *vs.* antiviral).

To investigate further, we measured the antiviral and cytotoxic activity of isolated and combined of metabolites. We confirmed that the tested AA displayed anti-flaviviral activity and detected that they possessed anti-coronavirus potential. Lycorine is cytotoxic at μM concentrations [17], while cherylline is more selective [7], and much less characterized. We propose that a combination of cherylline with other AA could lead to the synergistic effect that supports the strong antiviral activity of *C. jagus* extracts. Indeed, combining cherylline with other AAs at non-cytotoxic doses resulted in a synergistic anti-DENV activity in all cases, supporting a key role of this AA in the plant antiviral properties. Such synergistic effects could not be observed in cells infected with the coronavirus. This is possibly related to the method of detection of HCoV-OC43^{+} cells, which requires intracellular staining and led to more variation, compared to direct evaluation of GFP as a surrogate viral protein for DENV. Alternatively, there could be no detectable synergistic effect between the tested AA in the coronavirus infection context. At the present time, the limited accessibility of these natural products precluded a deeper characterization of their synergistic potential.

To date, the mechanism of actions of Amaryllidaceae alkaloids is not clear to date. While lycorine has been shown to target, specific viral RNA structure [60], the proteases [61]as well as the polymerase [22,23]of coronaviruses and flaviviruses, it was also shown to interact with the ribosome and blocks translation [62,63]. Other studies suggested that AAs trigger the integrated stress response [28], or like cherylline rather target viral RNA replication step [7,27]. Elucidating their target(s) will be crucial to prevent the emergence of variants, decrease their cytotoxicity and optimize their synergistic effects.

Overall, these results show that *C. jagus* extracts possess anti-coronaviral and anti-flaviviral properties and strengthen the key role of alkaloids as antiviral specialized metabolites in plant extracts. Although DENV [64,65] and HCoV-OC43 [66,67] are considered adequate surrogate for other viruses in their genus, the antiviral activity against other flavivirus (such as Zika virus) and other coronaviruses (such as SARS-CoV-2) should be confirmed in future studies.

5. Conclusion

This study highlights the antiviral properties of *Crinum jagus* and the importance of effective extraction methods for yielding bioactive compounds. Liquid-liquid extraction resulted in higher levels of phenolic acids and alkaloids compared to ultrasound- and microwave-assisted, hot solvent and continuous extracts, with LC-MS/MS analyses confirming the presence of key antiviral compounds like cherylline and lycorine. This extract was also the most cytotoxic. Subsequent acid-base extraction yielded in alkaloid-rich phenolic-deprived fractions regardless of the initial extraction procedure. These alsoyielded in an increased ability to block the replication of both the β-coronavirus and flavivirus used in this study. These fractions were also less cytotoxic compared to the initial liquid-liquid ethanol extracts, suggesting that phenolic compounds contribute to this effect. Additionally, a combination of cherylline and gigantellinine exhibited a strong synergistic effect, reducing dengue virus replication. These results underscore the potential of *Crinum jagus* as a valuable source of antiviral compounds and point to the need for further refinement of extraction methods. Future studies should aim to improve these techniques and investigate the clinical potential of these alkaloids in antiviral treatments, particularly for emerging viral diseases. Finally, despite their promising pharmacological potential, the complete biological characterization and clinical development of AAs is hindered by limited commercial availability. A better understanding of AA biosynthesis and metabolic regulation is crucial in order to take advantage of new metabolic engineering technologies for improving the efficiency and sustainability of AA production.

CRediT authorship contribution statement

Thilina U. Jayawardena: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Natacha Merindol:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nuwan Sameera Liyanage:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Sarah-Eve Gélinas:** Writing – review & editing, Investigation, Formal analysis. **Berthoux Lionel:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Ka Seydou:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **Matar Seck:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Antonio Evidente:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Isabel Desgagné-Penix:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Isabel Desgagne-Penix reports financial support was provided by Canada Research Chair. Isabel Desgagne-Penix reports a relationship with Canada Research Chairs Program that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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