

# Isolation of bioactive phytochemicals from *Crinum asiaticum* along with their cytotoxic and TRAIL-resistance abrogating prospect assessment

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## Article

**Keywords:** *Crinum asiaticum*, TRAIL-resistance abrogating, cytotoxicity, molecular docking

**Posted Date:** May 11th, 2023

**DOI:** <https://doi.org/10.21203/rs.3.rs-2820591/v1>

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**Additional Declarations:** No competing interests reported.

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**Version of Record:** A version of this preprint was published at Heliyon on February 1st, 2024. See the published version at <https://doi.org/10.1016/j.heliyon.2024.e25049>.

# Abstract

*Crinum asiaticum* L. (Amaryllidaceae) is a perennial bulbous herb, locally utilized for possessing multifaceted pharmacological properties including anticancer, immune-stimulating, analgesic, antiviral, antimalarial, antibacterial, and antifungal, in addition to their popularity as an aesthetic plant. Separation of MeOH extract of *C. asiaticum* leaves yielded three known compounds as cycloneolitsol (**1**), hippeastrine (**2**) and  $\beta$ -sitosterol (**3**). Among these, compounds **1** and **2** were subjected to the cytotoxic assay and found that **1** decreased cell viability to 45% and 8% against HCT116 cells; 15% and 9% against DU145 cells; 63% and 23% against Huh7 cells at 100  $\mu$ M and 200  $\mu$ M concentrations, respectively. Similarly, **2** decreased cell viability to 10% and 7% against HCT116 cells; 25% and 15% against DU145 cells; 26% and 18% against Huh7 cells at 100  $\mu$ M and 200  $\mu$ M concentrations, respectively. When tested for TRAIL-resistance abrogating activity, **1** (100  $\mu$ M) along with TRAIL (100 ng/mL) showed moderate activity in AGS cells producing 25% more inhibition than the agent alone. Whereas (20 and 30  $\mu$ M) in combination with TRAIL (100 ng/mL) exhibited strong activity in abrogating TRAIL-resistance and caused 34 and 36% more inhibition in AGS cells, respectively. The *in-silico* studies of compounds **1** and **2** revealed high docking hits in the TRAIL and other cancer-associated proteins which indicates a good correlation with the cell-based assay. It is still recommended to conduct further investigations to understand their exact molecular mechanism.

## Introduction

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) or Apo 2 ligand, belongs to the TNF superfamily, can induce selective apoptosis against various tumoral and transformed cells without affecting normal cells and has thus proved as a promising antitumor therapeutic agent in human cancer [1, 2]. Unlike the other members of this superfamily, the *in vivo* administration of TRAIL has been confirmed to be safe because of its capability to induce significant metastasis suppression and inhibition of the progression of cancer in experimental animals without considerable systemic toxicity [1]. The death-receptor (extrinsic) pathway, as well as the mitochondrial (intrinsic) pathway, are two well-known apoptotic pathways where, depending on the cell type, TRAIL can trigger either pathway. During the apoptotic activities, TRAIL binds to the death receptors (DRs) such as DR4 (TRAIL-R1) and DR5 (TRAIL-R2) that contain the cytoplasmic functional death domain. The steps involved in the death-receptor (extrinsic) pathway include the engagement of death receptor (DR), development of DISC (death-inducing signaling complex), activation of caspase-8 and accordingly the stimulation of effector caspase-3 to cause apoptosis [3]. On the other hand, the mitochondrial (intrinsic) pathways become active when Bid is activated by proteolytic caspase-8 and translocates to the mitochondria. The cleaved/truncated Bid, known as tBid, translocates to the mitochondria and activates the mitochondrial pathway. Also, there exists a crosstalk between these two pathways through tBid [4]. But a problem has aroused that a number of highly malignant tumor cells as breast cancer, colon cancer, prostate cancer, gastric cancer, and lung cancer cells are resistant to TRAIL-induced apoptosis [5]. TRAIL resistance may arise at different points in the signaling pathways due to the downregulation of death receptors (DR4 and DR5), over-expression of anti-apoptotic proteins Bcl-2 or Bcl-XL, loss of function of pro-apoptotic proteins Bax or Bak and competition between decoy receptors (DcR1 and DcR2) for TRAIL binding [6]. Therefore, finding the possible mechanism of TRAIL resistance and to overcome this resistance is very important for the successful development of anticancer agents [7, 8]. Several studies showed that combined treatment of TRAIL and various natural products such as luteolin [9], 1-*O*-formylrocagloic acid [10], curcumin [11], fuligocandin B [12], parviflorene F [13] and tunicamycin [14] can restore TRAIL-resistance. The search for bioactive molecules that can up-regulate the expression of death receptors and proapoptotic proteins or that can down-regulate inhibitors of apoptosis (IAP) family proteins and anti-apoptotic proteins may be an effective strategy for re-sensitizing TRAIL-mediated apoptosis against tumor cells.

*C. asiaticum* is a perennial, bulbous and herbaceous plant with attractive leaves which emerged from a large bulb. It is commonly known as poison bulb or giant crinum lily and grows widely across the tropics, subtropics, and temperate zones of the world [15]. Traditionally the plant has important biological and therapeutic effects including anticancer, analgesic, antiviral, antimalarial, antibacterial, antifungal, and anti-inflammatory. Previous phytochemical investigations revealed that various parts of this plant are particularly rich in alkaloids. Alkaloids isolated (6-hydroxycrinamine, lycorine and crinamine) from this plant showed strong inhibitory activity against Hh/GLI1-mediated signaling pathway and caused cytotoxicity against human pancreatic (PANC1) and prostate (DU145) cancer cells [16].

There have been numerous *in vitro* and *in silico* studies aimed at targeting TRAIL to treat cancer because understanding the molecular mechanism of TRAIL resistance and to overcome such resistance is crucial for identifying bioactive compounds to enhance the effectiveness of TRAIL-based cancer therapies [17, 18]. Beside the *in vivo* and *in vitro* approaches, *in silico* studies involving TRAIL typically involve computer simulations and modeling to better understand the protein's structure, interactions with other molecules, and potential therapeutic uses. These studies can help researchers identify potential drug candidates and optimize their properties. With recent developments in the sequencing of wide-ranged genomics and well-established protein sequence databases, bioinformatics has earned immense priority and with that, the appraisal of *in silico* studies is rising with time [19]. A successful molecular contact through molecular docking can activate the ligand to find the enzyme's three-dimensional binding site and establish a connection to the relationship with the ancillary chemical substances [20]. In computational modeling, we performed molecular docking to elucidate the binding affinity of the components with the crystal structures of some typical TRAIL-induced apoptotic pathway related receptors along with some cancer cell linked receptors.

The MeOH extract of *C. asiaticum* leaves three known compounds have been isolated as cycloneolitsol (**1**), hippeastrine (**2**) and  $\beta$ -sitosterol (**3**). In this report we shall present the isolation and structure elucidation of compounds **1–2** along with their cytotoxic activity in different cancer cell lines and TRAIL-resistance abrogating activity against AGS cells (human gastric adenocarcinoma). We also describe the docking potential of **1** and **2** with cancer cell linked receptors (PDB: 3SWZ, 5ZJE and 7T9Q) as well as TRAIL-induced apoptotic pathway related receptors (PDB: 5UUP, 3H13, 6TBT and 7XGF). Here, we report for the first time, the TRAIL-resistance abrogating activity of cycloneolitsol (**1**) and hippeastrine (**2**), and their molecular docking.

## Materials and Methods

### General experimental procedure

Vacuum Liquid Chromatography (VLC) was carried out using Kieselgel 60H (Sigma-Aldrich, USA). Column chromatography was performed using silica gel 60 (Carl Roth GmbH & Co.). Preparative TLC was performed for the compound isolation using Silica gel 60 F 254 (Merck, Germany). NMR spectra were recorded on Bruker (400 MHz) NMR spectrometers using a deuterated solvent. ESIMS were measured on an LCMS 2020 system (Shimadzu Corporation, Japan).

### Plant materials

The leaves of *C. asiaticum* (Amaryllidaceae) were collected from the Mirpur Botanical Garden, Bangladesh in December 2019 and were identified by the experts at Bangladesh National Herbarium, Dhaka where a voucher specimen (DACB 56819) was also deposited. The permission of sample collection was obtained from the authority of Mirpur Botanical Garden only for academic study. To the best of our knowledge and as per the documentation of the National Herbarium, Dhaka, *C. asiaticum* is a very common and widespread plant and is not an endangered plant i.e.,

there are no guidelines with this plant that restricts the plants from cutting down to facilitate conservation purposes. The collection and use of plants in the present study complies with international, national and institutional guidelines.

## Extraction and isolation

Air-dried, powdered leaves of *C. asiaticum* (400 g) were extracted with three liters of MeOH for seven days at room temperature with occasional stirring followed by coarse filtration using fresh cotton plugs and then through Whatman No.1 filter paper. The filtrate thus obtained was then evaporated by using a Buchi Rotary evaporator (Heidolph, UK) to obtain the crude extracts (45 g). About 40 g of the extract was chromatographed on a vacuum liquid chromatography (16.5 × 8.5 cm) using VLC grade Kiesel gel 60H Silica by using n-hexane, dichloromethane, ethyl acetate and MeOH as solvent systems in increasing polarities to obtain different fractions such as 1A-1I. 1C was subjected to silica gel column chromatography (30 cm × 4 cm) with hexane-ethyl acetate solvent system to afford fractions 2A-2H. From fractions 2B and 2C, Compound **1** (3.2 mg) was obtained as a crystal. 1F was subjected to silica gel column chromatography (30 cm × 4 cm) using hexane-ethyl acetate-MeOH solvent system to afford fractions 5A-5J. Fraction 5J was subjected to preparative TLC using the ethyl acetate-MeOH solvent system to obtain Compound **2** (3.5 mg). Compound **3** (7 mg) was obtained from the fraction 1D as crystal.

**Compound 1:** Colorless Crystal;  $^1\text{H-NMR}$  (400MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.32 (1H, d,  $J = 3.6\text{Hz}$ ), 0.54 (1H, d,  $J = 3.6\text{Hz}$ ), 0.798 (3H, s, H-29), 0.85 (3H, d,  $J = 6.4\text{ Hz}$ , H-21), 0.874 (3H, s, H-30), 0.94 (3H, s, H-18), 0.96 (3H, s, H-28), 1.004 (3H, s, H-31), 1.004 (3H, s, H-32), 1.676 (3H, s, H-27), 3.27 (1H, m, H-3), 4.71 (1H, s, H-26), 4.65 (1H, s, H-26).  $^{13}\text{C-NMR}$  (100MHz,  $\text{CDCl}_3$ )  $\delta$ : 14.01 (C-29), 17.99 (C-18), 18.47 (C-21), 19.3 (C-27), 19.4 (C-30), 20.02 (C-9), 21.13 (C-6), 25.45 (C-28), 26.03 (C-11), 26.11 (C-10), 26.3 (C-7), 27.26 (C-32), 27.53 (C-31), 28.13 (C-16), 29.71 (C-19), 30.41 (C-2), 30.76 (C-22), 31.96 (C-1), 32.8 (C-15), 35.89 (C-12), 36.61 (C-20), 37.39 (C-23), 38.74 (C-24), 40.5 (C-4), 45.26 (C-13), 47.14 (C-5), 47.99 (C-8), 48.82 (C-14), 52.16 (C-17), 78.88 (C-3), 109.3 (C-26), 152.4 (C-25). ESIMS  $m/z$ : 455  $[\text{M} + \text{H}]^+$  (Calcd for  $\text{C}_{32}\text{H}_{54}\text{O}$ : 454).

**Compound 2:** Colorless Solid;  $^1\text{H-NMR}$  (400MHz,  $\text{CDCl}_3$ )  $\delta$ : 2.06 (3H, s, H-1), 2.55 (1H, d, H-3), 2.67 (1H, d, H-16), 2.87 (1H, dd, H-15), 3.18 (2H, t, H-2), 4.25 (1H, dd, H-6), 4.56 (1H, bs, H-7), 5.66 (1H, s, H-5), 6.09 (2H, s, H-17), 7.04 (1H, s, H-13), 7.4 (1H, s, H-10).  $^{13}\text{C-NMR}$  (100MHz,  $\text{CDCl}_3$ )  $\delta$ : 27.21 (C-3), 39.19 (C-15), 42.13 (C-1), 55.76 (C-2), 66.5 (C-6), 67 (C-16), 82.68 (C-7), 102.5 (C-17), 108.4 (C-13), 109 (C-10), 118 (C-14), 119 (C-5), 139.2 (C-9), 143.6 (C-4), 148.3 (C-12), 152.4 (C-11), 165.1 (C-8). ESIMS  $m/z$ : 316  $[\text{M} + \text{H}]^+$  (Calcd for  $\text{C}_{17}\text{H}_{17}\text{NO}_5$ : 315).

**Compound 3:** Colorless Crystal;  $^1\text{H-NMR}$  (400MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.68 (3H, s), 0.80 (3H, d,  $J = 7.6\text{Hz}$ ), 0.82 (3H, d,  $J = 7.6\text{Hz}$ ), 0.84 (3H, t,  $J = 7.6\text{Hz}$ ), 0.91 (3H, d,  $J = 6.4\text{Hz}$ ), 1.01 (3H, s), 3.51 (1H, m), 5.34 (1H, d,  $J = 4.4\text{Hz}$ ).

## Cell cultures

AGS cells were derived from the Institute of Development, Aging and Cancer, Tohoku University, Japan. HCT116 and DU145 were purchased from American Type Culture Collection, USA. Huh7 were purchased from Health Science Research Resources Bank, Osaka, Japan. AGS cells were cultured in RPMI-1640 (Roswell Park Memorial Institute) medium (Wako, Japan) with 10% FBS (Fetal Bovine Serum; Biowest, France) and 1% penicillin-streptomycin, PS (Sigma, USA). DU145, HCT116 and Huh7 cells were cultured in DMEM (Dulbecco's Modified Eagle Medium, DS Pharma Biomedical Co., Ltd. Osaka, Japan) with 10% FBS and 1% PS. Cultures were maintained in a humidifier incubator at 37°C in 5%  $\text{CO}_2$ /95% air.

## Cytotoxicity assay

Three human carcinoma cell lines as DU145 (human prostate cancer cell lines), HCT116 (human colon carcinoma cell lines), and Huh7 (human hepatocellular carcinoma cell lines) were used to evaluate cytotoxic activity using the FMCA method [21]. The cells were seeded ( $1.5 \times 10^3$  cells/well) in a 96-well black microplate with 200  $\mu$ L of DMEM and were incubated for 24 h at 37°C. The medium was removed and 200  $\mu$ L of DMEM containing the test sample at an appropriate concentration was added to each well. The cells were then again incubated 72 h. After removing the medium, the cells were washed with 200  $\mu$ L PBS, and 200  $\mu$ L FDA solution (3.5  $\mu$ g/mL) was added to each well. The plates were then kept in an incubator for 1 h at 37°C, and fluorescence was measured at 538 nm with excitation at 485 nm using a Fluoroskan Ascent. All the Data were presented as the mean  $\pm$  standard deviation of three independent experiments. DMSO (0.1%) was used as the negative control.

## TRAIL resistance-abrogating activity assay

The TRAIL resistance-abrogating activity was determined by comparing cell growth inhibitory activity in the presence and absence of TRAIL using fluorometric microculture cytotoxicity assay (FMCA) [21]. TRAIL-resistant human gastric adenocarcinoma (AGS) cells were seeded in a 96-well culture plate at a density of  $6 \times 10^3$  cells/well with 200  $\mu$ L of RPMI medium containing 10% FBS. After incubation for 24 h at 37°C, test samples at different doses with or without 100 ng/mL of TRAIL were added to each well. After another 24 h incubation, the cells were washed with PBS (Phosphate-Buffered Saline), and 200  $\mu$ L of FDA (Fluorescein Diacetate) solution (10  $\mu$ g/mL) was added to each well. The plates were then kept in an incubator for 1 h at 37°C, and fluorescence was measured at 538 nm with excitation at 485 nm using a Fluoroskan Ascent (Thermo Fisher Scientific, USA). 0.1% DMSO was used as the negative control and Luteolin at 17.5  $\mu$ M was used as the positive control.

## Molecular Docking

### Ligand Preparation

The 2D structure of the cycloneolitsol (PubChem CID: 101306728) and hippeastrine (PubChem CID: 441594) were attained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in SDF format. Aiming to determine the best hit for these targets, they were created as ligands and reduced using PyRx. The default settings for the virtual screening program PyRx from MGL-Tools (<https://ccsb.scripps.edu/mgltools/>) have been kept [22].

### Protein Preparation

To screen the anticancer molecular docking analysis of the isolated compounds (**1–2**), the 3D crystal structures of the cancer cell-related receptors (PDB: 3SWZ, 5ZJE and 7T9Q) and TRAIL-apoptosis related proteins (PDB: 5UUP, 3H13, 6TBT and 7XGF) were derived from the protein data bank (PDB) (<https://www.rcsb.org/structure>) in PDB format. Here, Human Cytochrome P450 17A1 in complex with TOK-001 (PDB: 3SWZ) is a target for the treatment of breast and prostate cancers that proliferate in response to estrogens and androgens [23]. Lactate dehydrogenase A (PDB: 5ZJE) expression is responsible for cancer growth and energy metabolism in various cancers via the aerobic glycolytic pathway [24]. Human Ornithine Aminotransferase (PDB: 7T9Q) is associated with Hepatocellular carcinoma [25]. Atypical production of the cell death-inhibiting BCL2 protein (PDB: 5UUP) has been linked to a wide range of human diseases, including cancer [26]. Again, a transcriptional repressor known as B cell lymphoma 6 (BCL6) (PDB: 6TBT) is frequently dysregulated in diffuse large B cell lymphoma, making it an important therapeutic target [27].

Overexpression of anti-apoptotic Bcl-2 family proteins; BCLxL (PDB: 7XGF) [28] and c-FLIPL protease (PDB: 3H13) [29] promotes cancer progression and confers chemotherapy resistance [30]. All water and heteroatoms have been taken out of proteins using Discovery Studio 2020. To prepare proteins, the Gasteiger charge and nonpolar hydrogens were left at their default configuration. Additionally, all proteins were processed for additional analysis utilizing normal residues in AMBER ff14sB and other residues in Gasteiger mode, with all proteins being brought to a minimal energy level using UCSF Chimera [31].

## Protein-Ligand interactions

To aid the docking of the selected protein-ligand complexes, PyRx AutoDock Vina has been executed [32]. For the docking study, a semi-rigid docking system was used. PyRx AutoDock Vina was used to compress the size of the protein and the phytochemicals used here were converted to PDBQT format. Both the protein's stiffness and the ligand's adaptability were conserved in this study. The ligand molecules have been given 10 degrees of freedom. AutoDock outlines the steps to be taken to automatically convert the molecules to the pdbqt format, down to the molecule type, box type, grid box construction, etc. The grid box was constructed around a functional location. Besides, the process of identifying optimal docking places in BIOVIA Discovery Studio Visualizer 2020 was accelerated [33].

## ADMET prediction

ADMET stands for absorption, digestion, metabolism, elimination, and toxicity. The online admetSAR server (<http://lmmd.ecust.edu.cn/admetSar2/>) is also utilized to forecast the pharmacokinetics and toxicological characteristics of two isolated substances [34]. The canonical SMILES of hippeastrine and cycloneolitsol were derived from the PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) database and transmit the canonical SMILES to the admetSAR server and predicted their ADMET (Lipinski's rules) properties for drug discovery [35].

## Results and Discussion

### Isolated phytochemicals from *C. asiaticum*

The MeOH extract of *C. asiaticum* leaves was fractionated sequentially using *n*-hexane, dichloromethane, ethyl acetate (EtOAc), and MeOH through vacuum liquid chromatography (VLC) technique. Further separation of the fractions using different chromatographic techniques yielded three known compounds (**1–3**) (Fig. 1). Using 1D and 2D NMR spectroscopy along with mass spectrometric technique, these compounds were identified as cycloneolitsol (**1**) [36], hippeastrine (**2**) [37] and  $\beta$ -Sitosterol (**3**) [38]. Compound **1** was first isolation from this plant.

## Cytotoxic activity of the isolated compounds

The cytotoxic activity of isolated compound **1** (cycloneolitsol) and compound **2** (hippeastrine) was determined against the human prostate cancer cell line (DU145), human colon carcinoma cell line (HCT116), and human hepatocellular carcinoma cell line (Huh7). The result was evaluated after 72 hrs. As shown in Fig. 2, treatment with compound **1** showed cytotoxic effect and decreased cell viability to 45% and 8% against HCT116 cells; 15% and 9% against DU145 cells; 63% and 23% against Huh7 cells at 100  $\mu$ M and 200  $\mu$ M concentrations, respectively. Similarly, compound **2** decreased cell viability to 10% and 7% against HCT116 cells; 25% and 15% against DU145 cells; 26% and 18% against Huh7 cells at 100  $\mu$ M and 200  $\mu$ M concentrations, respectively.

# TRAIL resistance abrogating activity of isolated compounds

TRAIL-resistance abrogating activity is assessed by comparing cell viability both in the presence and absence of TRAIL against TRAIL-resistant cancer cell lines [39]. Plant extracts or compounds producing more than 25% of difference in cell viability are considered as active in abrogating TRAIL-resistance [2]. Compounds **1** and **2** were tested for their activity in abrogating TRAIL-resistance in AGS (human gastric adenocarcinoma) cells. Recently, this cell line has been used extensively as a representative model for evaluating apoptosis in cancer cells and is claimed to be resistant to TRAIL-induced apoptosis [40]. Treating cells with 10, 50, and 100 µM of compound **1** in presence of TRAIL (100 ng/mL) resulted in 5, 14, and 25% more inhibition than the agent alone indicating its mild TRAIL-resistance abrogating activity. Whereas, in combination with TRAIL (100 ng/mL), **2** (10, 20, and 30 µM) caused 17, 34, and 36% more inhibition than the agent alone. It was evident from the above results that compound **2** exhibited more potent TRAIL-resistance abrogating activity in AGS cells (Fig. 3). Luteolin (17.5 µM), used as a positive control, produced 49% more inhibition in combination with TRAIL (100 ng/mL) than the agent alone.

## Molecular docking simulation

The results of molecular docking analysis unveiled the binding affinity of the cancer cell associated receptors and the apoptosis related receptors with the compounds (**1–2**) from *C. asiaticum*. Computer aided assessment of the binding affinity of a chemical substance to a receptor provides prospective insights into the regulation candidacy of that chemical i. e. interactions of isolated phytochemicals towards the proteins related to TRAIL-induced apoptosis validated the lab findings of TRAIL-resistance overcoming property of the isolated phytochemicals.

Both **1** and **2** showed a very prominent binding affinity with the tested cancer cell associated receptors. Compound **2** bound to the human cytochrome P450 17A1 receptor (PDB: 3SWZ) through serine 475 and proline 474 residue and compound **1** stuck through glu 191 and asp194 pockets. The active sticking in between complex of Lactate dehydrogenase A (PDB: 5ZJE) and **2** is ser 104, asp 194, leu 106, ile 325 and this complex formed a binding affinity of -6.2 Kcal/mol. Where, the identical receptor binds to compound **1** via leu 265 and his 264. Again, 13.87 Kcal/mol energy yielded owing to the formation of human ornithine aminotransferase (PDB: 7T9Q) – **2** complexes. The formation of interaction hits in tyr 55, tyr 85 and glu 235 pockets. This complex consists of disulfide bond formation and the sticking formation between this receptor and compound **1** happened in tyr 85 pocket of the receptor (**Table: 1** and **Figure: 4**).

Table 1  
Docking scores of **1** and **2** with some cancer cell-associated receptors

| Molecular Docking score (binding affinity) |              |                                      |                                   |                                            |
|--------------------------------------------|--------------|--------------------------------------|-----------------------------------|--------------------------------------------|
| Compounds                                  | Compound CID | Receptors                            |                                   |                                            |
|                                            |              | 3SWZ<br>(Human Cytochrome P450 17A1) | 5ZJE<br>(Lactate dehydrogenase A) | 7T9Q<br>(Human Ornithine Aminotransferase) |
| <b>1</b>                                   | 101306728    | -7.6                                 | -6.1                              | -7.8                                       |
| <b>2</b>                                   | 441594       | -8.2                                 | -6.2                              | -13.8                                      |

Moreover, the interaction revealed that hippastrine (**2**) is comparatively a better substrate than cycloneolitsol (**1**) as it showed the highest affinity to most of the TRAIL-apoptosis related proteins and yielded the best docking results. The

interaction of **2** with 5UUP (human Bfl-1), 3H13 (c-FLIP L protease), 6TBT (BCL-6) and 7XGF (crystal structure of BCL-xL) was found very prominent, and the binding energy exerted by these complexes are – 6.0 kcal/mol, -5.3 kcal/mol, -6.0 kcal/mol and – 7.2 kcal/mol, respectively (**Table: 2** and **Figure: 5**). Compound **2** bound to the 5UUP through a series of amino acid residues such as phe 125, asn 129 and glu 76. The complex of 7xgf and hippeastrine is made up of arg 148 and thr 21 residues. The formation of 3H13 and hippeastrine complex were yielded by the molecular hitting in the pockets of val 361 and gln 319. Similarly, hippeastrine hits the pockets (asn 23, leu 20, arg 94 and leu 19) of the B cell lymphoma 6 (6TBT) receptor. As Bfl-1, c-FLIP, BCL-6, and BCL-xL are known as apoptosis inhibitors, the TRAIL-resistance abrogating effect of **2** may be due to its regulatory interactions with the aforementioned anti-apoptotic proteins related receptors.

Table 2

Docking score of Hippeastrine (**2**) with Human Bfl-1, c-FLIPL protease, B cell lymphoma and crystal structure of BCL-xL receptors.

| Molecular Docking score (binding affinity) |                       |                            |                             |                                       |
|--------------------------------------------|-----------------------|----------------------------|-----------------------------|---------------------------------------|
| Compound CID                               | Receptors             |                            |                             |                                       |
|                                            | 5UUP<br>(Human Bfl-1) | 3H13<br>(c-FLIPL protease) | 6TBT<br>(B cell lymphoma 6) | 7XGF<br>(crystal structure of BCL-xL) |
| 441594                                     | -6.0                  | -5.3                       | -6.0                        | -7.2                                  |

## ADMET analysis

Analyses were conducted according to the drug likelihood scale and the five principles of Lipinski. Compounds that comply with at least four out of five of Lipinski's guidelines are more likely to qualify as drug candidates. In this experiment, isolated compounds were found to adhere to Lipinski's criteria (**Table: 3**)

Table 3

ADMET properties prediction for drug bioavailability

| Compound                                                                       | Molecular Weight | HBD | HBA | AlogP | Human oral bioavailability | Carcinogenicity (binary) | Hepatotoxicity | Acute Oral Toxicity |
|--------------------------------------------------------------------------------|------------------|-----|-----|-------|----------------------------|--------------------------|----------------|---------------------|
| <b>1</b>                                                                       | 454.78           | 1   | 1   | 8.81  | 0.6000                     | 0.9300                   | 0.7593         | 0.7744              |
| <b>2</b>                                                                       | 315.33           | 1   | 6   | 1.04  | 0.5429                     | 0.9300                   | 0.6750         | 0.9900              |
| HBD = Hydrogen bond donor; HBA = Hydrogen Bond Acceptor; AlogP = Lipophilicity |                  |     |     |       |                            |                          |                |                     |

## Conclusions

In this study, separation of *C. asiaticum* (Amaryllidaceae) leaves led to the isolation of three known compounds (**1–3**), of which **1** was first isolation from this plant. Both **1** and **2** showed cytotoxic activity against DU145, HCT116 and Huh7 cancer cell lines. Among the isolates, **2** showed strong TRAIL-resistance overcoming activity against AGS cells at 20 and 30 µM, respectively. Molecular docking analysis showed that both compounds **1** and **2** interact with the cancer cell-associated receptors (PDB: 3SWZ, 5ZJE, 7T9Q). Compound **2** interacts with the TRAIL pathway-associated receptors (PDB: 5UUP, 3H13, 6TBT, 7XGF) and thus regulated these receptors. These results correlated well with the

cell-based assay, and thus tends to suggest that the TRAIL-resistance abrogating effect of **2** may be due to its regulatory interactions with the aforementioned anti-apoptotic proteins related receptors.

## Declarations

### Data availability

All data generated or analyzed during this study are included in this published article [and its Supplementary information files].

**Acknowledgments** This study was supported by Centennial Research Grant-2021 (Reg/Ad-3/47852), University of Dhaka, Bangladesh.

### Author contributions

Sharmin Ahmed Rakhi and Firoj Ahmed collected the plant and designed the research; Md. Saiful Islam, Safaet Alam and Firoj Ahmed prepared the manuscript; Yasumasa Hara, Teruhisa Manome and Masami Ishibashi conducted cytotoxic activity study, TRAIL-resistance abrogating activity study and recorded MS data; Nazim Uddin Emon and Safaet Alam conducted *in-silico* experiments; Sharmin Ahmed Rakhi, Muhammad Abdullah Al-Mansur and Firoj Ahmed worked on isolation and structure determination; Md Ruhul Kuddus, Md. Raihan Sarkar and Firoj Ahmed conceptualized and managed funds for the study.

### Competing interests

The authors declare no competing interests.

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## Figures

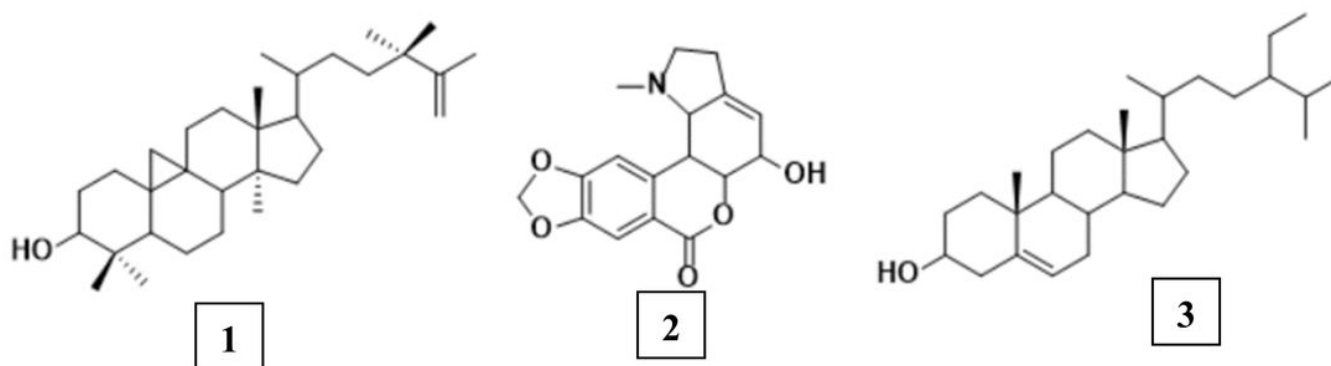


Figure 1

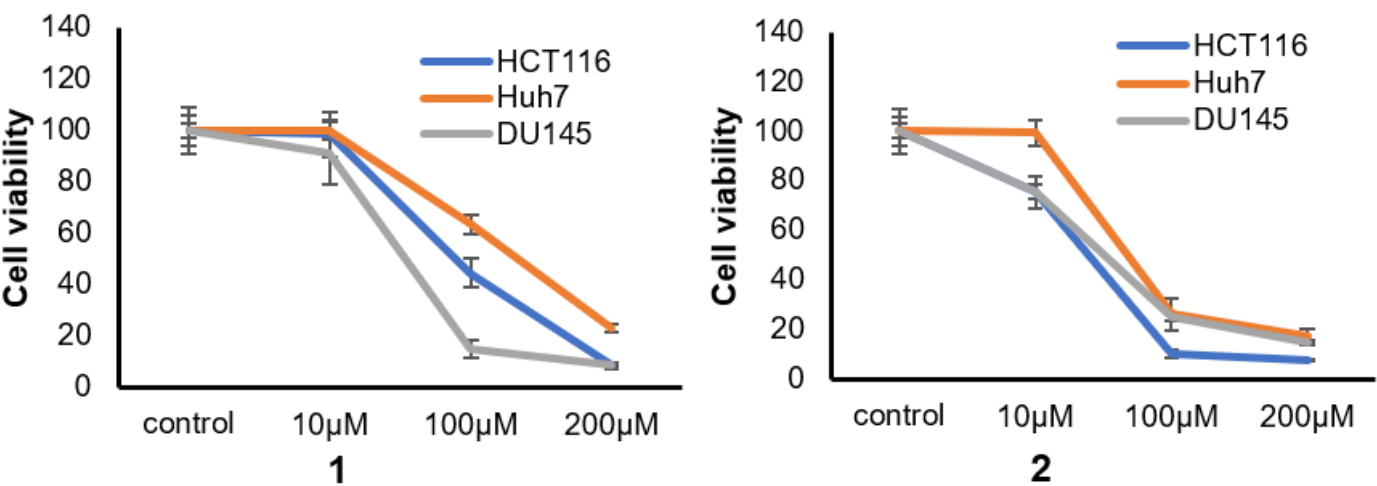


Figure 2

Cell viability was measured using the fluorometric microculture cytotoxicity assay (FMCA).

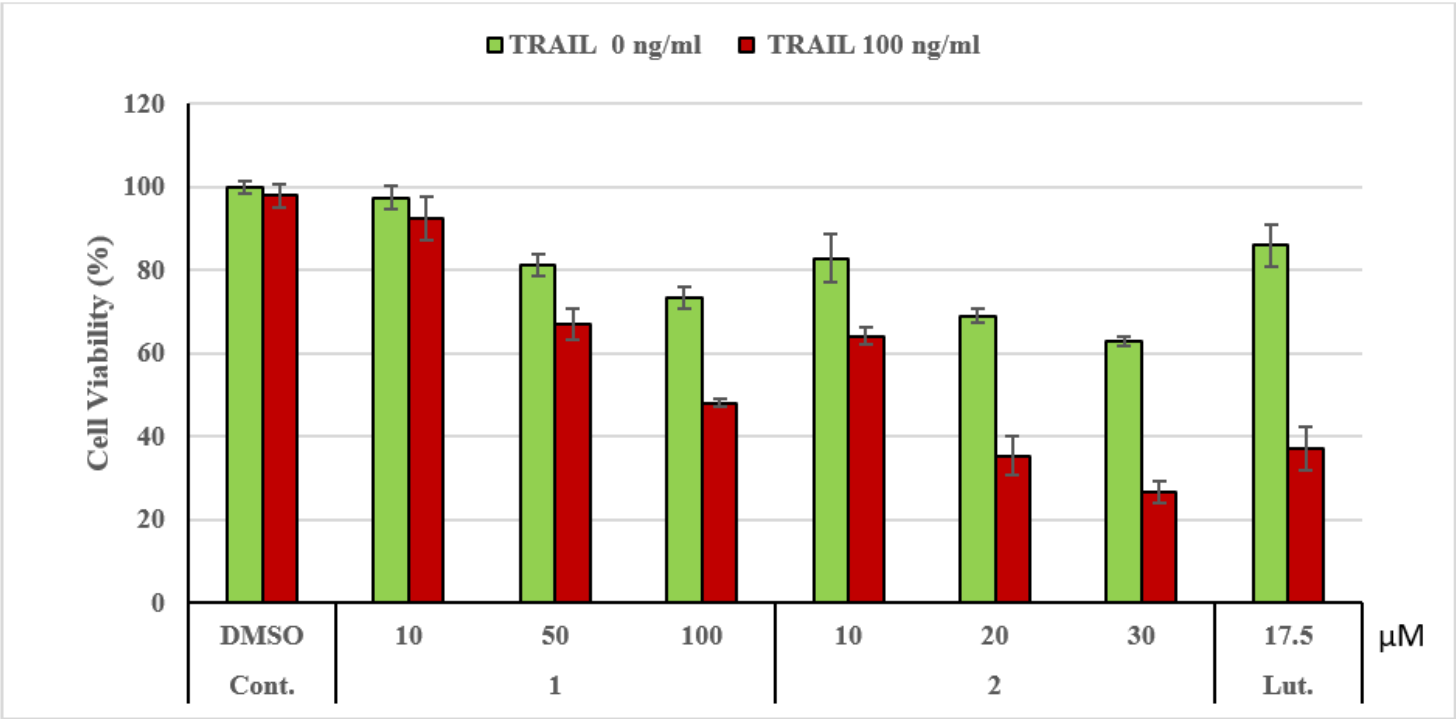
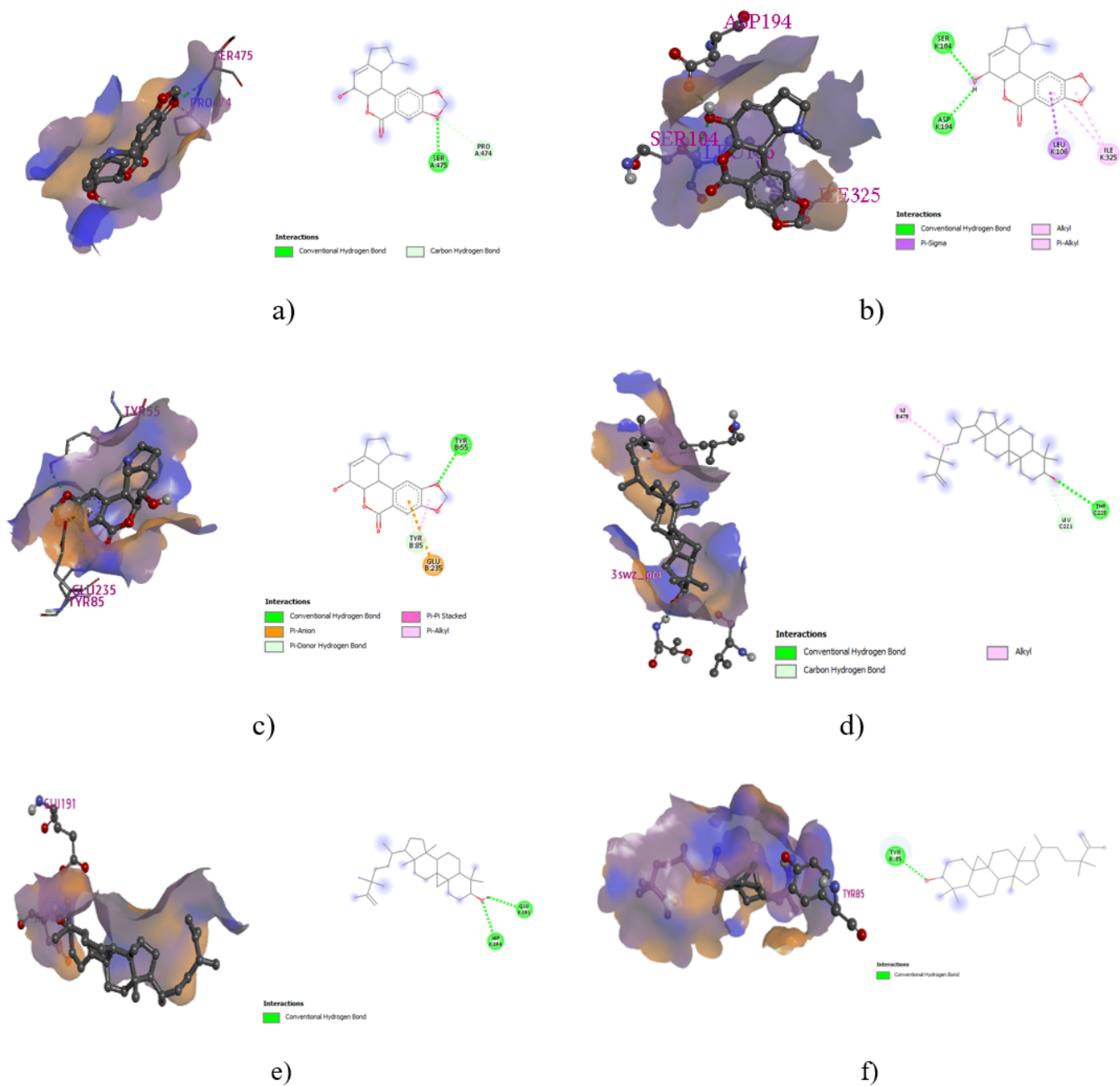


Figure 3

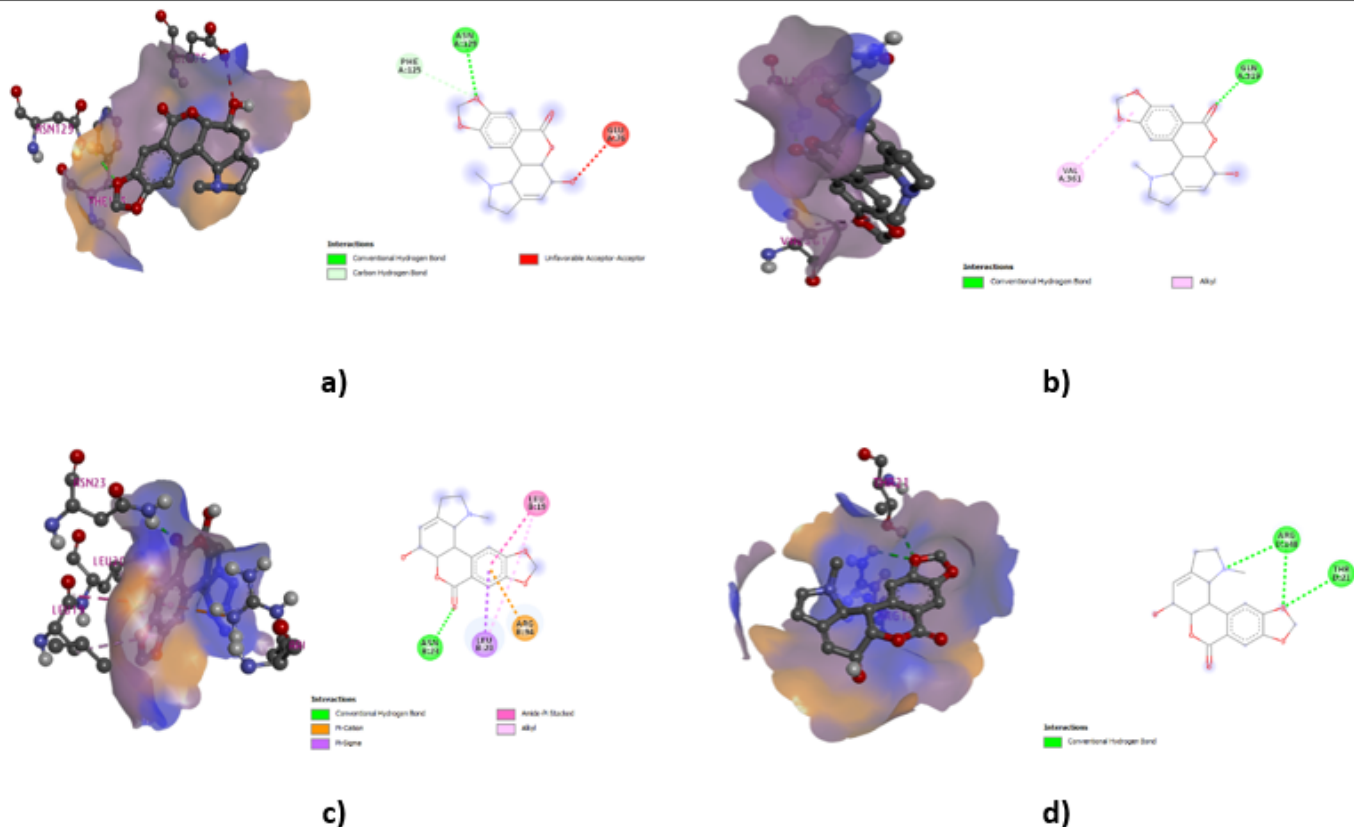
Effects of compounds 1 and 2, luteolin (Lut.), and DMSO (negative control: cont.) in the presence or absence of TRAIL on the viability of AGS cells. Bars represent the mean  $\pm$  SD ( $n = 3$ ). \* $p < 0.05$ ; \*\* $p < 0.01$ , Tukey's test



**Figure 4**

3D and 2D illustration of hitting sites of compounds **1** and **2** and cancer cell-related receptors. a) 3SWZ-Hippeastrine (**2**) b) 5ZJE-Hippeastrine (**2**) c) 7T9Q-Hippeastrine (**2**) d) 3SWZ-cycloneolitsol (**1**) e) 5ZJE-cycloneolitsol (**1**) f) 7T9Q-cycloneolitsol (**1**)

3SWZ = Human Cytochrome P450 17A1; 5ZJE = Lactate dehydrogenase A; 7T9Q = Human Ornithine Aminotransferase



**Figure 5**

3D and 2D illustration of hitting sites of compound **2** with the apoptosis receptors. a) 5UUP – Hippeastrine (**2**) b) 3H13 – Hippeastrine (**2**) c) 6TBT – Hippeastrine (**2**) d) 7XGF – Hippeastrine (**2**)

5UUP = Human Bfl-1; 3H13 = c-FLIPL protease; 6TBT = Crystal structure of the B cell lymphoma 6; 7XGF = Crystal structure of BCL-xL (receptor)

## Supplementary Files

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