Heliyon 10 (2024) e25049

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

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

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A R T I C L E I N F O

Keywords:
Crinum asiaticum
TRAIL-Resistance abrogating activity
Cytotoxicity
Molecular docking

A B S T R A C T

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 its popularity as an aesthetic plant. Separation of MeOH extract of *C. asiaticum* leaves yielded three known compounds as cycloneolitsol (**1**), hippeastrine (**2**) and β-sitosterol (**3**). Among these, compounds **1** and **2** were subjected to the cytotoxic assay and found that they induced mild effect against HCT116, Huh7 and DU145 cell lines with the IC_{50} values from 73.76 to 132.53 μM. When tested for TRAIL-resistance abrogating activity, **1** (100 μM) along with TRAIL (100 ng/mL) showed moderate activity in AGS cells producing 25 % more inhibition than the agent alone. Whereas **2** (20 and 30 μM) 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 compound **2** revealed high docking hits with the TRAIL-associated anti-apoptotic proteins which give a justification for the regulatory interactions to induce such abrogating activity. It is still recommended to conduct further investigations to understand their exact molecular mechanism.

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https://doi.org/10.1016/j.heliyon.2024.e25049
Received 13 November 2023; Received in revised form 18 January 2024; Accepted 18 January 2024
Available online 19 January 2024

1. 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 thus proved as a promising therapeutic agent against 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 subsequent 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. 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 arisen that a number of highly malignant tumor cells such 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 down-regulation 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.

Crinum asiaticum is a perennial, bulbous and herbaceous plant with attractive leaves which emerge 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. From our earlier studies, 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]. Therefore, we targeted to isolate other alkaloids from this plant with cytotoxic and TRAIL resistance abrogating activity.

In this report, we shall present the isolation and structure elucidation of compounds **1**–**3** from the MeOH extract of *C. asiaticum* leaves with the cytotoxic activity of **1** and **2** in different cancer cell lines. We also report the TRAIL-resistance abrogating activity of **1** and **2** against AGS cells (human gastric adenocarcinoma) and the *in silico* molecular docking with TRAIL-induced apoptotic pathway-related proteins to find out the possible molecular mechanism of such abrogating activity.

2. Materials and methods

2.1. General experimental procedure

Vacuum Liquid Chromatography was carried out using Kiesel gel 60H (Sigma-Aldrich, USA). Column chromatography was performed using silica gel 60 (Carl Roth Gmbh & Co., Germany). 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).

2.2. 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 species *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.

2.3. Extraction and isolation

Air-dried, powdered leaves of *C. asiaticum* (400 g) were extracted with 3 L 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 crystals.

Compound 1: Colorless Crystal; ^{1}H NMR (400 MHz, $CDCl_3$) δ:0.32 (1H, d, $J = 3.6$Hz), 0.54 (1H, d, $J = 3.6$Hz), 0.798 (3H, s, H-29), 0.85 (3H, d, $J = 6.4$ 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}C NMR (100 MHz, $CDCl_3$) δ: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 $[M+H]^+$ (Calcd for $C_{32}H_{54}O$: 454).

Compound 2: Colorless Solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: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}C NMR (100 MHz, $CDCl_3$) δ: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 $[M+H]^+$ (Calcd for $C_{17}H_{17}NO_5$: 315).

Compound 3: Colorless Crystal; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.68 (3H, s), 0.80 (3H, d, $J = 7.6$Hz), 0.82 (3H, d, $J = 7.6$Hz), 0.84 (3H, t, $J = 7.6$Hz), 0.91 (3H, d, $J = 6.4$Hz), 1.01 (3H, s), 3.51 (1H, m), 5.34 (1H, d, $J = 4.4$Hz).

2.4. 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, 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., Japan) with 10 % FBS and 1 % PS. Cultures were maintained in a humidifier incubator at 37 °C in 5 % CO_2/95 % air.

2.5. 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 [17]. The cells were seeded (1.5×10^3 cells/well) in a 96-well black microplate with 200 μL of DMEM and were incubated for 24 h at 37 °C. The medium was removed and 200 μL of DMEM containing the test sample (dissolved in 0.1 % DMSO) 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 μL PBS, and 200 μL FDA solution (3.5 μg/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 ± standard deviation of three independent experiments. DMSO (0.1 %) was used as the negative control.

2.6. 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) [17]. TRAIL-resistant human gastric adenocarcinoma (AGS) cells were seeded in a 96-well culture plate at a density of 6×10^3 cells/well with 200 μL 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 μL of FDA (Fluorescein Diacetate) solution (10 μg/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 μM was used as the positive control.

2.7. Molecular docking

2.7.1. Ligand preparation

The 2D structure of **2** (PubChem CID: 441594) was attained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Aiming to determine the best hit for this target, it was 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 [18].

2.7.2. Protein preparation

To screen the molecular docking analysis of **2**, we select TRAIL apoptosis-related proteins such as BFL-1 (PDB: 5UUP), BCL-xL (PDB:

7XGF) and c-FLIP protease (PDB: 3H13). 3D crystal structures of these proteins were derived from the protein data bank (PDB) (https://www.rcsb.org/structure) in PDB format. 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 [19].

2.7.3. Protein-ligand interactions

To aid the docking of the selected protein-ligand complexes, a semi-rigid docking system was used. The PyRx AutoDock Vina has been executed to compress the size of the proteins as well as the ligand (compound **2**) and converted to PDBQT format [20]. Both the protein's stiffness and the ligand's adaptability were conserved in this study. The ligand molecule has 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 [21].

2.7.4. 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 characteristics of **2** [22]. The canonical SMILES of **2** was derived from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database and transmitted the canonical SMILEs to the admetSAR server and predicted their ADMET (Lipinski's rules) properties for drug discovery [23].

2.7.5. Statistical analysis

Three independent experiments were performed to evaluate the cytotoxicity and TRAIL resistance-abrogating activity assay, and the obtained results in the study were presented as mean ± standard deviation (SD). All the statistical analyses were obtained using GraphPad Prism version 10.1.2 and Microsoft Excel 2019 version.

3. Results and discussion

3.1. 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. 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**) [24], hippeastrine (**2**) [25] and β-Sitosterol (**3**) [26]. Compound **1** was first time isolated from this plant.

The ^{1}H NMR spectrum of compound **1** displayed the characteristic cycloartane type methylene signals at δ_H 0.54(1H, d, $J = 3.6$Hz) and 0.32 (1H, d, $J = 3.6$Hz) and two olefinic protons at δ_H 4.71 (s) and 4.65 (s) in addition to an oxymethine proton signal at δ_H 3.27 ppm. It also showed the presence of eight methyl groups at δ_H 0.798 (3H, s), 0.96 (3H, s), 0.94 (3H, s), 0.874 (3H, s), 0.85 (3H, d, $J =$ 6.4 Hz), 1.004 (3H, s), 1.004 (3H, s) and 1.676 (3H, s) ppm. A total of 32 carbons including eight sp3 methyl, eleven sp3 methylene, one sp2 methylene, five sp3 methine and seven tertiary carbons were observed in the ^{13}C NMR spectrum. Considering the above information along with COSY and HMBC correlations, compound **1** was identified as cycloneolitsol which was further confirmed by comparison with the published data [24].

The ^{1}H NMR spectrum of compound **2** displayed two singlets in the aromatic region at δ_H 7.4 (s) and 7.04 (s) ppm; an intense singlet at δ_H 6.09 (s) ppm corresponding to the methylenedioxy protons and a broad singlet at δ_H 5.66 (s) ppm assigned as an olefinic proton. Two coupled alkyl protons were observed at δ_H 4.56 and 4.25 ppm due to the deshielding effects of nearby oxygen groups. The ^{13}C NMR spectrum expressed seventeen carbon signals, of which six were assigned as aromatic carbons at δ_C 152.5, 148.3, 139.3, 118.0, 109.0, and 108.4 ppm; two olefinic carbons at δ_C 143.6 and 119.0 ppm; one sp3 methylenedioxy carbon at δ_C 102.56 ppm; two oxygenated methines at δ_C 82.68 and 66.5 ppm; two sp3 methines at δ_C 67.0 and 39.2 ppm; two sp3 methylenes at δ_C 55.8 and 27.2 ppm; one lactone carbonyl at δ_C 165.1 ppm; and one N-methyl carbon at δ_C 42.1 ppm. Considering the above information along with

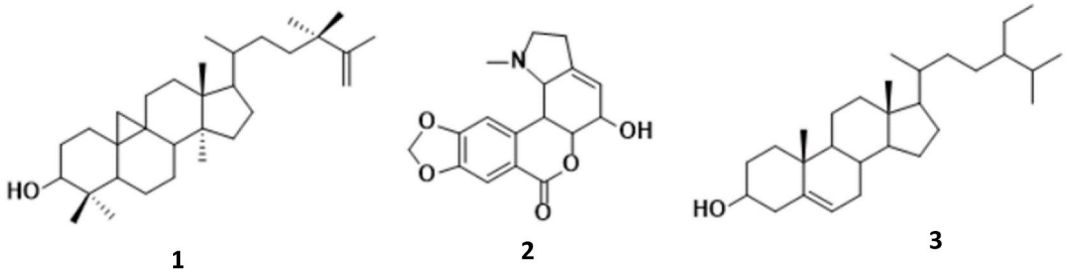

Fig. 1. Structures of isolated compounds (**1–3**) from *C. asiaticum*.

the COSY and HMBC correlations, compound **2** was identified as hippeastrine which was further confirmed by comparison with literature data [25].

Structure of compound **3** was elucidated as β-sitosterol based on the characteristic peaks appeared at δ_H 3.52 and 5.34 ppm along with six methyl signals at δ_H 0.68–1.01 ppm. Finally, the structure was confirmed by compared with the published data [26].

3.2. Cytotoxic activity of the isolated compounds

The cytotoxic activity of isolated compounds **1** and **2** were determined against the human colon carcinoma cell line (HCT116), human hepatocellular carcinoma cell line (Huh7) and human prostate cancer cell line (DU145). The result was evaluated after 72 h. As shown in Table 1, treatment with compound **1** showed low cytotoxic effect with the IC_{50} values of 104.52 μM against HCT116, 132.53 μM against Huh7 and 85.42 μM against DU145 cell lines. Whereas compound **2** induced a moderate cytotoxic effect against HCT116, Huh7 and DU145 cells with the IC_{50} values 73.76, 101.39 and 87.05 μM, respectively.

3.3. 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 [27]. 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 [28]. 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 **2** (10, 20, and 30 μM) in combination with TRAIL (100 ng/mL) 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. 2) which suggested the probable synergistic effect of **2** with TRAIL. Thus, we assume it may serve as a drug candidate. Luteolin (17.5 μM), used as a positive control, produced 49 % more inhibition in combination with TRAIL (100 ng/mL) than the agent alone.

3.4. Mechanism of TRAIL-resistance abrogating activity of compound 2 via molecular docking simulation

Nowadays, the docking approach is widely used as a common computational tool to identify new active compounds and their receptor-specific binding affinities. The examination of the topology and the interaction between ligands and receptors provides us with valuable information on the dimensions, configuration, electrical distribution, and the existence of certain amino acids inside the binding site. These factors ultimately dictate the strength of the binding and the selectivity of the ligand. To be more precise, the docking mechanism anticipates the optimal alignment of the ligand when it attaches to the protein, resulting in the formation of a stable complex. An illustrative graphic depicting the pocket and highlighting crucial structural and functional characteristics of the protein might serve as a helpful aid in comprehending the docking outcomes. The binding pocket, neighboring amino acids, and significant characteristics like hydrophobic areas, charged residues, and hydrogen bond donors or acceptors are usually highlighted in this diagram. The diagram's visual representation helps clarify the reasons why certain chemicals bind more efficiently than others and provides insight into the molecular processes that govern these interactions.

Here, we checked the molecular interaction of **2** with some TRAIL-apoptosis pathway-related proteins as BFL-1 (PDB: 5UUP), BCL-xL (PDB: 7XGF) and c-FLIP protease (PDB: 3H13) using *in-silico* approaches to find out the possible mechanism of action of **2**. The over-expression of anti-apoptotic BCL-2 family proteins including human BFL-1 (PDB: 5UUP) and BCL-xL (PDB: 7XGF) have been identified as a potential resistance factor against several cancer therapies such as leukemia, breast cancer, pancreatic and hepatocellular carcinoma etc. [29–31]. Again, c-FLIP (PDB: 3H13) is considered as a master anti-apoptotic regulator which develops resistance via suppressing tumor necrosis factor-α, Fas-L and TRAIL-induced apoptosis as well as the apoptosis triggered by chemotherapeutic agents [32]. Therefore, we selected and hypothesized that the molecular interaction of **2** with these proteins (BFL-1, BCL-xL and c-FLIP) may be responsible for the sensitizing effect to TRAIL.

From the molecular docking study, the interaction of **2** with 5UUP, 7XGF and 3H13 was found very prominent, and the binding energy exerted by these complexes were −6.0, −7.2 and −5.3 kcal/mol, respectively (Table 2 and Fig. 3). Compound **2** bound to the 5UUP through a series of amino acid residues such as ASN129 and GLU76 [Fig. 3 (a-b)]. The complex of 7XGF and **2** was made up of ARG148 and THR21 residues [Fig. 3 (c-d)]. The formation of 3H13 and **2** complexes was yielded by the molecular hitting in the pockets of VAL361 and GLN319 [Fig. 3 (e-f)]. As BFL-1, BCL-xL and c-FLIP are known as apoptosis inhibitors, the TRAIL-resistance abrogating

Table 1
IC_{50} values of compounds **1–2** against HCT116, Huh7 and DU145 cells.

Compound	IC_{50} (μM)		
	HCT116	Huh7	DU145
1	104.52	132.53	85.42
2	73.76	101.39	87.05

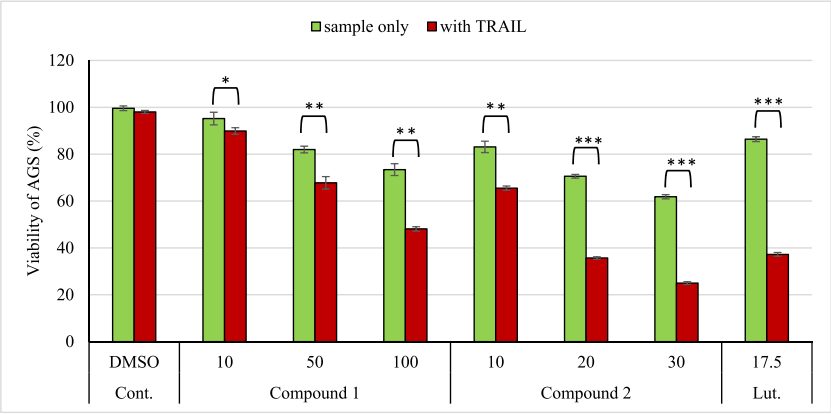

Fig. 2. Effects of compounds **1** and **2** against AGS cells, luteolin (Lut.), and DMSO (negative control: cont.) in the presence or absence of TRAIL on the viability of AGS cells. Bars represent the mean ± SD (n = 3). Significance was determined with Tukey's test, *p < 0.05, **p < 0.01 and ***p < 0.001.

Table 2
Docking score of **2** with BFL-1, BCL-xL and c-FLIPL protease proteins.

Molecular Docking score/binding affinity (kcal/mol)			
Compound CID	Proteins		
	5UUP (Human BFL-1)	7XGF (BCL-xL)	3H13 (c-FLIPL protease)
441594	−6.0	−7.2	−5.3

effect of **2** may be due to its regulatory interactions with the afore-mentioned anti-apoptotic proteins.

3.5. ADMET analysis

Analyses were conducted according to the drug likelihood scale and the well-known five principles of Lipinski. According to the Lipinski rule, the solubility and permeability of any molecule depend on the specific physicochemical properties including the molecular weight (≤500 Da), Hydrogen bond donor (≤5), Hydrogen bond acceptor (≤10) and lipophilicity/partition co-efficient (≤5) [33]. 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 compound **2** was found to adhere to Lipinski's criteria (Table 3).

4. Conclusion

In this study, the separation of *C. asiaticum* (Amaryllidaceae) leaves led to the isolation of three compounds (**1**–**3**), of which **1** was first isolated from this plant. Both **1** and **2** showed mild to moderate cytotoxic activity against HCT116, Huh7 and DU145 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 **2** interacts with the TRAIL pathway-associated proteins (PDB: 5UUP, 3H13 and 7XGF) and thus regulates these proteins. Therefore, we suggest that the TRAIL-resistance abrogating effect of **2** may be due to its regulatory interactions with the aforementioned anti-apoptotic related proteins.

Data availability statement

The dataset utilized in this study is not currently stored in a public data repository. However, necessary data generated or analyzed during this study are included in this published article [and its supporting information files]. Data will be made available on request.

Author contribution statement

Sharmin Ahmed Rakhi: Methodology, Investigation, Formal analysis. **Yasumasa Hara:** Methodology, Investigation. **Md Saiful Islam:** Writing – review & editing, Writing – original draft, Methodology. **Teruhisa Manome:** Methodology, Investigation. **Safaet Alam:** Writing – original draft, Software, Methodology. **Nazim Uddin Emon:** Software, Methodology. **Muhammad Abdullah Al-Mansur:** Formal analysis, Data curation. **Md Ruhul Kuddus:** Visualization, Funding acquisition, Conceptualization. **Md Raihan Sarkar:** Funding acquisition, Conceptualization. **Masami Ishibashi:** Supervision. **Firoj Ahmed:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

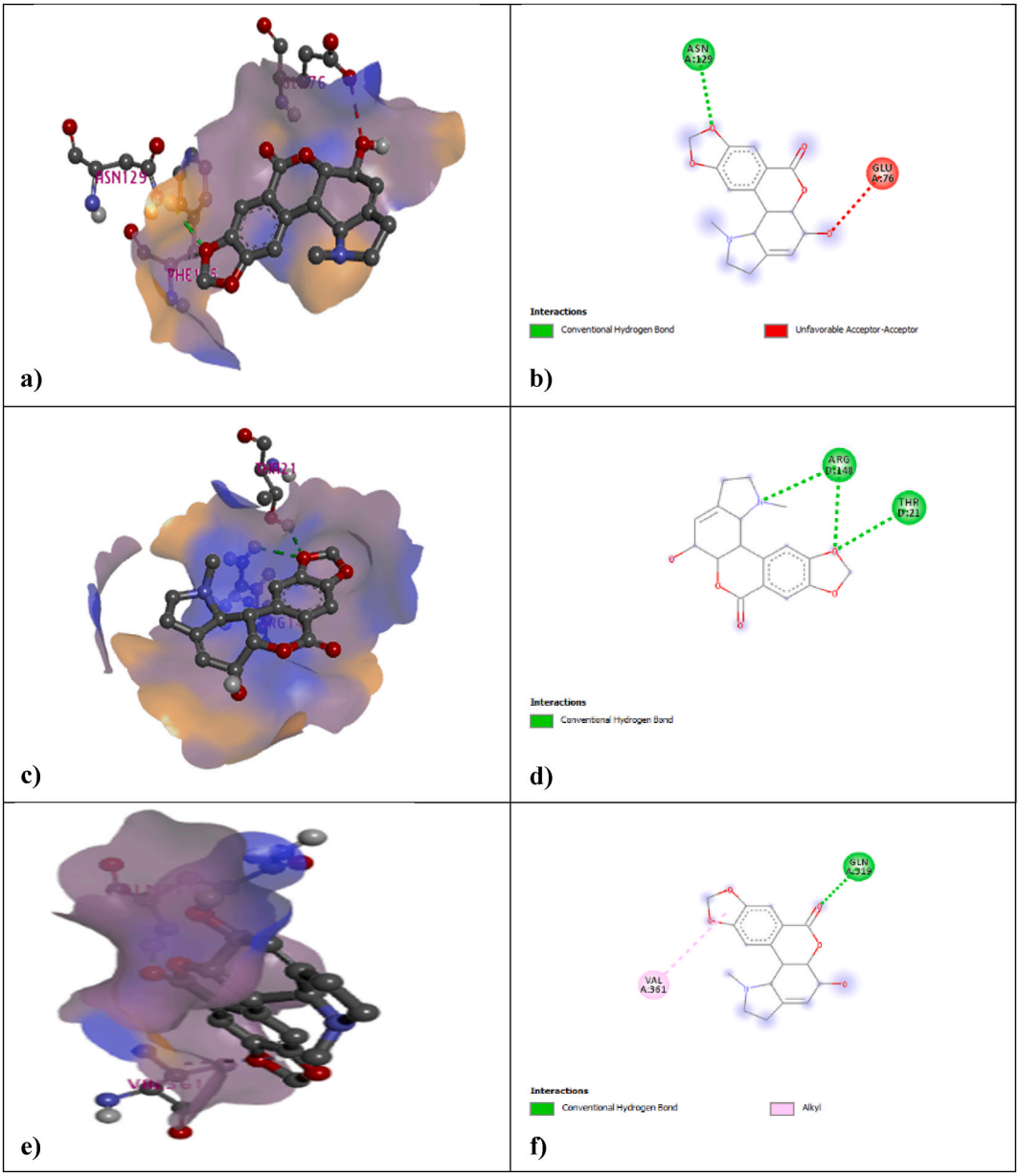

Fig. 3. 3D and 2D illusion of hitting sites of compound **2** with the apoptosis proteins. **a-b) 2 -** 5UUP **c-d) 2 -** 7XGF and **e-f) 2 -** 3H13. Here, 5UUP = Human BFL-1; 7XGF = Crystal structure of BCL-xL (protein) and 3H13 = c-FLIPL protease.

Table 3
ADMET properties prediction for drug bioavailability.

Compound	Molecular Weight	HBD	HBA	AlogP	Human oral bioavailability	AOT	Mice LD 50 (mol/kg)
2	315.33	1	6	1.04	0.5429	3	2.8079

HBD = Hydrogen bond donor; HBA = Hydrogen Bond Acceptor; AlogP = Lipophilicity; AOT = Acute Oral Toxicity.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Firoj Ahmed reports financial support was provided by Dhaka University Centennial Research Grant-2021, University of Dhaka. Firoj Ahmed reports a relationship with University of Dhaka that includes: employment. 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.

Acknowledgment

This study was supported by Centennial Research Grant-2021 (Reg/Ad-3/47852), University of Dhaka, Dhaka-1000, Bangladesh.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25049.

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