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Antitumoral activity of different Amaryllidaceae alkaloids: *In vitro* and in silico assays

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ABSTRACT

Ethnopharmacology relevance: The plants of Amaryllidaceae family, such as *Amaryllis belladonna* L., have been used as herbal remedies for thousands of years to address various disorders, including diseases that might today be identified as cancer.
Aim of the study: The objective of this work was to evaluate the potential of three Amaryllidaceae alkaloids against four cancer cell lines.
Material and methods: The alkaloids lycorine, 1-*O*-acetylcaranine, and montanine were evaluated *in vitro* against colon adenocarcinoma cell line (HCT-116) and breast carcinoma cell lines (MCF-7, MDAMB231, and Hs578T). Computational experiments (target prediction and molecular docking) were conducted to gain a deeper comprehension of possible interactions between these alkaloids and potential targets associated with these tumor cells.
Results: Montanine presented the best results against HCT-116, MDAMB231, and Hs578T cell lines, while lycorine was the most active against MCF-7. In alignment with the target prediction outcomes and existing literature, four potential targets were chosen for the molecular docking analysis: CDK8, EGFR, ER-alpha, and dCK. The docking scores revealed two potential targets for the alkaloids with scores similar to co-crystallized inhibitors and substrates: CDK8 and dCK. A visual analysis of the optimal docked configurations indicates that the alkaloids may interact with some key residues in contrast to the other docked compounds. This observation implies their potential to bind effectively to both targets.
Conclusions: In vitro and in silico results corroborate with data literature suggesting the Amaryllidaceae alkaloids as interesting molecules with antitumoral properties, especially montanine, which showed the best *in vitro* results against colorectal and breast carcinoma. More studies are necessary to confirm the targets and pharmaceutical potential of montanine against these cancer cell lines.

1. Introduction

According to the World Health Organization, cancer is an abnormal and uncontrolled cell growth that can occur in almost any organ or tissue of the body. It is globally estimated that 10 million deaths in 2020 were due to cancer, which means 1 in 6 deaths in the world is due to this disease [WHO, 2024]. Breast, lung, colon and rectum, prostate, skin, and stomach cancer, respectively, were the most common cancers, while lung, colon and rectum, liver, stomach, and breast cancer, respectively,

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Abbreviation

AKT1	serine/threonine-specific protein kinase
APEX1	Apurinic/Apyrimidinic Endodeoxyribonuclease 1
BCL2L1	B-cell lymphoma-extra-large like 1
BP	biological process
CC	cell component
CDK8	Cyclin-Dependent Kinase 8
CHUK	Conserved Helix-Loop-Helix Ubiquitous Kinase
CI	confidence interval
dCK	deoxycytidine kinase
DCZ	2′-deoxycytidine
DMEM-F12	Dulbecco's modified Eagle medium F12
DMSO	Dimethyl sulfoxide
EGFR	Epidermal Growth Factor Receptor
ER-alpha	Estrogen Receptor Alpha
FBS	fetal bovine serum
FDR	false discovery rate
GA	Genetic Algorithm
GBM	Glioblastoma Multiforme
GO	gene ontology
HCT-116	colon adenocarcinoma cell line
HIF-1	Hypoxia-Inducible Factor 1
HRAS	Harvey Ras
HSP90AA1	Heat Shock Protein 90 Alpha Family Class A Member 1
Hs578T	breast carcinoma cell line
H1F1A	hypoxia-inducible factor 1-alpha
IGF1	Insulin-Like Growth Factor 1
ITGB1	Integrin Beta-1
IC50	half-maximal inhibitory concentration
KEGG	Kyoto Encyclopedia of Genes and Genomes
KDR	Kinase Insert Domain Receptor
MAPK1	Mitogen-Activated Protein Kinase 1
MAPK8	Mitogen-Activated Protein Kinase 8
MAPK14	Mitogen-Activated Protein Kinase 14
MAP3K1	Mitogen-Activated Protein Kinase Kinase 1
MCF-7	breast carcinoma cell line
MDAMB231	breast carcinoma cell line
MF	molecular function
MMFF	Merck Molecular Force Field
MMP9	Matrix Metallopeptidase 9
MTT	3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide
PDB	protein data bank
NFKB1	Nuclear Factor Kappa B Subunit 1
NOS3	Nitric Oxide Synthase 3
PD-L1	Programmed Death-Ligand 1
PD-1	Programmed Cell Death Protein 1
PIK3CB	catalytic subunit beta of phosphoinositide 3-kinase
PIK3RI	Phosphoinositide-3-Kinase Regulatory Subunit 1
PI3K-Akt	Phosphoinositide 3-kinase (PI3K) and Protein Kinase B (Akt)
PM6	Parametric Method 6
RMSD	root mean square deviation
RPMI	Roswell Park Memorial Institute
SRC	Proto-Oncogene Tyrosine-Protein Kinase Src
STAT1	Signal Transducer and Activator of Transcription 1
TLR4	Toll-like Receptor 4

were the most common causes of cancer death [WHO, 2024].

Scientists have devoted many efforts in the search for new drugs for the treatment and prevention of cancers [Lim et al., 2019]. Roughly half of the small molecules-based anticancer drugs that received approval originated either directly or indirectly from natural sources [Newman and Cragg, 2020]. Alkaloids are metabolites, usually found in plants, which present high structural diversity and biological potential, including potent anticancer activity against various cancers [Feher and Schimdt, 2003; Mohan et al., 2012]. Due to the presence of at least one nitrogen group, alkaloids exhibit a diverse array of functional groups (such as amines and amides), which commonly play a crucial role in shaping the molecular structure, charge distribution, and subsequent interactions with biological targets [Korlyukov et al., 2023].

The Amaryllidaceae family, specifically the Amaryllidoideae subfamily, present an exclusive and still expanding group of isoquinoline alkaloids, known as Amaryllidaceae alkaloids, which have fascinating structural features and a large spectrum of biological activities [Bastida et al., 2006]. Members of this subfamily are found in all the continents but predominantly in three distinct geographical locations, including South America, southern Africa, and the Mediterranean [Meerow and Snijman, 1998].

More than 650 different Amaryllidaceae alkaloid structures have been isolated until now [Berkov et al., 2020]. These molecules are usually classified into nine skeleton types: norbelladine, lycorine, homolycorine, crinine, haemanthamine, narciclasine, tazettine, montanine, and galanthamine [Bastida et al., 2006], but a more accurate classification has been performed by Berkov and co-workers [Berkov et al., 2020]. Lycorine was the first Amaryllidaceae alkaloid to be isolated from *Lycoris radiata* in 1877, however, the most known alkaloid of this family is galanthamine, which was isolated in 1955 from *Galanthus woronowii* and approved by the FDA in 2001 for the palliative treatment of mild to moderate Alzheimer's disease symptom [Heinrich and Teoh, 2004; Bastida et al., 2006].

Cultures around the world have long using Amaryllidaceae plants in traditional medicine, harnessing their pharmacological potential which is often linked to the synthesis of specific alkaloids [Kornienko and Evidente, 2008]. Hippocrates of Kos (460-370 BP), considered the father of modern medicine, recommended the oil of *Narcissus* (Amaryllidaceae) species for the treatment of symptoms that today would be recognized as cancer [Goietsenoven et al., 2013]. Furthermore, references to *Narcissus* plants can even be found in the Bible, highlighting the longstanding reputation of Amaryllidaceae species in combating cancer [Duke and Duke, 1983].

Pancratistatin, a narciclasine-type alkaloid firstly isolated from *Pancratium littorale* (Amaryllidaceae) in 1984, has attracted the attention of scientists due to its anticancer proprieties evaluated in various phases of clinical trials [Pettit et al., 1984b; Kornienko and Evidente, 2008]. Initial studies have demonstrated pancratistatin's selective induction of apoptosis in various human cell lines, including Jurkat leukemia, SHSY-5Y neuroblastoma, NT-2 teratocarcinoma, and MCF-7 breast carcinoma cells. Remarkably, minimal effects were observed in nucleated red blood (NRB) and normal human fibroblast (NHF) cells by this alkaloid [Kekre et al., 2005; McLachlan et al., 2005; Pandey et al., 2012].

The traditional medicinal aspects, pharmacological activities, and identification of the active principles of Amaryllidaceae plants of the tribe Haemantheae involving *Clivia*, *Cryptostephanus*, *Haemanthus*, *Scadoxus,* and *Gethyllis* species have been recently described by Nair and Van Staden (2022). Among others pathologies, the authors reported the anti-tumoral potential of some of these species and made considerable efforts to delineate the molecular basis to some of these effects (Nair and Van Staden, 2022). Interestingly, several members of Amaryllidaceae family, including *Amaryllis belladonna* L., have been traditionally employed in cancer treatments by indigenous people across different regions. The Sotho, Xhosa, and Zulu people of South Africa, as well as those in Java, have traditionally utilized this species for the treatment of

"swelling," which is presumed to refer to cancer [Pettit et al., 1984a; Nair et al., 2016]. According to Napo et al. (2020), the methanol root extract of *A. belladonna* exhibited remarkable effectiveness, completely inhibiting the growth of the human leukemia K562 cell line at a concentration of 50 μg mL^{-1}, and even at a lower concentration of 25 μg mL^{-1}, it still significantly suppressed cell growth, with over an 80% reduction observed.

Developing new drugs is a long and expensive work [DiMasi et al., 2016]. Studies show it can take an average of 10–15 years and up to $2.6 billion to bring a new drug from initial concept to final approval by the FDA [DiMasi et al., 2016; Pushpakom et al., 2019]. This highlights the urgent need for innovative methods to speed up drug discovery and increase the success rate [Yu et al., 2024]. Notably, the integration of artificial intelligence, deep learning, machine learning, and computational chemistry into drug discovery has significantly improved success rates. Whether employed individually or collaboratively, these methods form innovative strategies that encompass a diverse array of efficient algorithms, enhancing the accuracy and efficiency of predictions [Mak and Pichika, 2019; Selvaraj et al., 2022]. In the realm of successful drug discovery, it is important to identify target proteins directly relevant to the pathophysiology. The meticulous selection of these targets constitutes a crucial step in this process [Selvaraj et al., 2022; Schenone et al., 2013; Singh et al., 2018]. Recognizing the central role of the cell cycle in cellular processes, the pursuit of strategies targeting specific phases of this cycle has become a compelling avenue in cancer chemotherapy. This approach's significance is underscored by the substantial availability of commercially developed anticancer drugs which function through cell cycle intervention, such as paclitaxel and vincristine [Williams and Stoeber, 2012].

In the realm of contemporary approaches to novel drug research and development, computer-aided drug design, *in vitro* drug screening, and *in vivo* evaluation have emerged as fundamental methodologies [Lu et al., 2023]. Given the crucial contribution of natural products to the advancement of innovative antitumoral therapies and the biological potential residing within Amaryllidaceae alkaloids, this investigation embarked on evaluating the anticancer activity of montanine, lycorine, and 1-*O*-acetylcaranine alkaloids within colorectal and breast carcinoma cell lines. Additionally, this study conducted in silico experiments, including comprehensive pharmacological network analysis and molecular docking, to delve into the intricate interactions between these alkaloids and four prospective cancer targets.

2. Materials and methods

2.1. Plant material and alkaloid purification

Bulbs of *Amaryllis belladonna* L., *Hippeastrum aulicum glaucophyllum* (Hook.) Herb., and *Rhodophiala bifida* (Herb.) Traub. were collected in different localities from Brazil (Canela – Rio Grande do Sul, Parque Nacional do Caparaó - Minas Gerais, and coast of Rio Grande do Sul, respectively). A voucher specimen for each species was deposited as detailed by Tallini et al. (2017), Petró-Silveira et al. (2020); Farinon et al. (2017), respectively, according to the following identification: 179860, in the Herbarium of the Universidade Federal do Rio Grande do Sul (UFRGS, Porto Alegre, Brazil); VIES 23438, in the Herbarium VIES from Universidade Federal do Espírito Santo (UFES, Vitória, Brazil); 192333 deposited in the Herbarium of the Universidade Federal do Rio Grande do Sul (Porto Alegre, RS, Brazil), respectively. According to the authors, the samples were dried and the alkaloids were obtained by acid-base extraction, isolated, and purified by different chromatographic tools, and identified by chromatographic and spectroscopic methods as described in the already mentioned papers. The alkaloids 1-*O*-acetylcaranine, lycorine, and montanine (Fig. 1) obtained from these species were evaluated herein against four different cancer cell lines.

2.2. Cell culture and MTT assay

The colon adenocarcinoma cell line (HCT-116) was grown in RPMI, while the breast carcinoma cell lines (MCF-7, MDAMB231, and Hs578T) were grown in DMEM-F12. Both media were supplemented with 10% of FBS and 1% antibiotics (penicillin and streptomycin). The cells were kept in an incubator with 5% of CO_2 at 37 °C. For MTT assay, the cells were seeded on 96-well plates at a density of 0.6 × 104 per well for HCT-116 cell lines and 1.0 x 104 per well for MCF-7, MDAMB231 and Hs578T with 200 μL of culture medium. After 24h, montanine, lycorine, and 1-*O*-acetylcaranine (0.0032–50 μM) were added to the cultures and incubated for 72 h. DMSO (0.05%) was used as negative control and the antineoplastic compound doxorubicin (0.00064–10 μM) was used as positive control. Three hours before the end of the experiment, culture media were replaced by fresh media containing MTT solution (0.5 mg mL^{-1}) and incubated. The MTT solution was removed, and the formazan product was solubilized in 150 μL DMSO. The absorbance was obtained at 570 nm. The IC_{50} values and their 95% confidence interval were calculated by sigmoidal nonlinear regression using GraphPad Prism 8.0 software.

2.3. Computational experiments

2.3.1. Target prediction and enrichment analysis

The chemical structures of montanine and lycorine were first drawn into ChemSketch and converted as SMILES notation. Then, the compounds had their target predicted in three web servers considering *Homo sapiens* as reference specie: STITCH [Kuhn et al., 2008], SuperPred [Nickel et al., 2014], and Swiss Target Prediction [Daina et al., 2019]. All targets were identified by their gene name using the UniProt database (UniProt Consortium, 2019). The duplicated data was removed, the targets were manually combined, and the result was plotted on a Venn diagram using InteractiVenn [Heberle et al., 2015].

The potential targets related to breast and colorectal cancers were identified using two gene databases: GeneCards [Stelzer et al., 2016] and DisGeNET [Piñero et al., 2019]. The following keywords were used to address the targets: "breast carcinoma"; "adenocarcinoma of colon" or "colorectal adenocarcinoma" or "adenocarcinoma of large intestine".

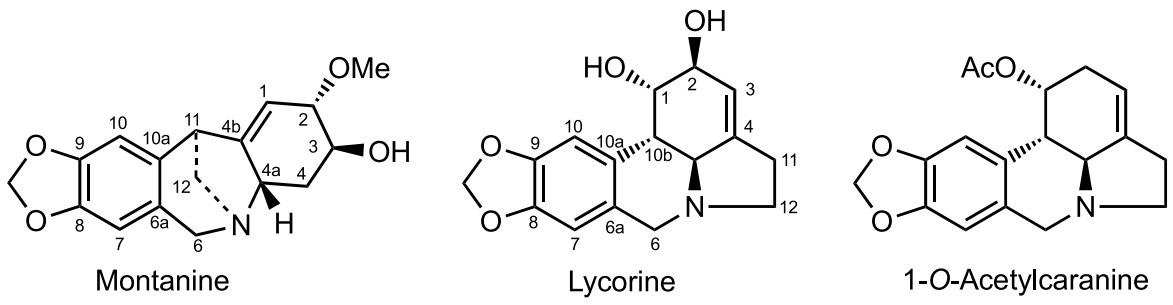

Fig. 1. Chemical structures of the Amaryllidaceae alkaloids montanine, lycorine, and 1-*O*-acetylcaranine.

The same data removal pre-treatment was performed in order to exclude duplicated data.

The targets related to the compounds (alkaloids) and diseases (breast and colorectal cancer) were manually curated and mapped using the STRING database [Szklarczyk et al., 2020] regarding multiple proteins based on the *Homo sapiens* organism. The STRING database builds a protein-protein interaction (PPI) network based on the uploaded protein list. The potential targets were selected and used for the enrichment analysis. Furthermore, Cytoscape v 3.9.1 [Shannon et al., 2003] was used for network visualization and for topological parameter calculation (degree, betweenness, closeness, and shortest path length), in order to highlight important genes in the network.

The enrichment analyses of the common targets were performed using gene name notation at ShinyGO 0.77 webserver [Ge et al., 2020] in order to obtain their GO and the related pathway, which was recovered from the KEGG. The top 20 KEGG pathways were selected based on the false discovery rate (FDR <0.05) and sorted by fold enrichment values. The potential targets within the best KEGG pathways were selected for molecular docking analyses. Moreover, the GO annotation was obtained through STRING module, which classified the data into BP, CC, MF. The top 10 annotations were selected according to the FDR cutoff (<0.05) and ranked according to the number of genes.

2.3.2. Molecular docking simulations

The alkaloids' structures were obtained from Pubchem [Kim et al., 2018] in structure data file extension (.sdf). Then, the hydrogens were added based on protonation rules using openbabel 2.3.2 [O'Boyle et al., 2011] considering pH equal to 7.4. After that the compounds had their energy minimized by MMFF followed by PM6 methods through Spartan'14 (Spartan'14Wavefunction).

The crystallography models of the proteins were obtained from the PDB (Barman et al., 2000) under the following identification codes (PDBID) and resolution (Supporting information Table 1). The hydrogens were added based on protonation rules from propka module embedded into pdb2pqr webserver (Unni et al., 2011) considering a pH equal to 7.4 and AMBER forcefield.

The molecular docking simulations were performed by GOLD v 5.2 [Jones et al., 1997] considering the following parameters: (a) number of GA runs equal to 10; (b) solvated water molecules were deleted; (c) scoring function was set as ChemPLP with disallowed early termination; (d) search efficiency set as default; (e) grid location and size as described in Supporting information Table 1. ChemPLP is a dimensionless scoring function, therefore a rescore of the obtained poses were done using Autodock Vina [Trott and Olson, 2010; Eberhardt et al., 2021] and a new rank was done using a rank-by-rank consensus score. The redocking procedure was accomplished to validate the docking protocol using the RMSD of the atomic distance of the co-crystallized ligand cut-off equal to 2.0 Å [Caroli et al., 2014]. In addition, other co-crystallized or literature reported ligands were docked into the predicted targets in order to compare their docking score with the alkaloids. The best ranked pose of each ligand was selected and visually inspected according to the key residue interactions described as important for the target inhibition in the literature and in a frequency comparison of the crystallographic complexes(Supporting information Table 2).

3. Results and discussion

3.1. Cytotoxic activity

Results of tumor growth inhibition obtained from MTT assay showed the sensitivity of colorectal (HCT-116), and breast (MCF-7, MDAMB231 and Hs578T) carcinoma cell lines for montanine, lycorine, and 1-*O*-acetylcaranine (Table 1). The half-maximum inhibitory concentration (IC_{50}) of montanine and lycorine showed considerable cytotoxic activity against HCT-116, MCF7, and MDAMB231 cell lines. Both compounds were less cytotoxic against the cell line Hs578T. The alkaloid 1-*O*-acetylcaranine did not present any cytotoxic activity against the cell lines tested, showing IC_{50} values over 50 μM. Possible explanations for the lack of activity of 1-*O*-acetylcaranine towards the different cancer cell lines may be associated to two chemical reasons: (a) the acetylation of hydroxyl substituent linked to C1 and/or (b) the absence of a (*S*)-hydroxyl substituent attached to C2, when compared to lycorine. Either the absence of a (*S*)-hydroxyl group at C2 or the presence of an acetyl group at C1 may hamper some important hydrogen bonds, in the last case the acetyl substituent may lead to steric hindrance in the binding site.

Among the alkaloids tested, montanine presented the best activity against colorectal (HCT-116) and breast carcinoma (MDAMB231 and Hs578T) cell lines (see Table 1). This alkaloid presented an interesting activity against colorectal carcinoma cells and it has been the first report about its potential use against HCT-116 cell line.

Indeed, many studies have demonstrated the cytotoxic activity of this alkaloid with similar potency as described herein. Masi and co-workers isolated montanine from *Haemanthus humilis* Jacq (Amaryllidaceae) [Masi et al., 2019]. The antitumoral potential of this com-pound was examined against MCF-7, MDAMB231, and Hs578T cell lines, revealing IC_{50} values of 4.4 ± 0.4, 3.4 ± 0.9, and 3.6 ± 1.7 μM, respectively [Masi et al., 2019]. Similarly, Silva and co-authors isolated montanine from *Hippeastrum vittatum* (Amaryllidaceae). They evaluated this compound against MCF-7 cell line of breast cancer and obtained IC_{50} values of 0.74 ± 0.02 μg mL^{-1} [Silva et al., 2008]. In a previous study, Govindaraju and co-workers synthetized various analogs of montanine-type alkaloids. They verified that manthine, an analogue of montanine with a methoxy group at C3, presented IC_{50} values of 4 μM against MCF-7 cell line [Govindaraju, 2018]. Recently, some researchers evaluated the potential of montanine against eight cancer cell lines and one non-cancer cell line, and revealed that this alkaloid showed the most potent antiproliferative activity against Jurkat and A549 cell lines, with IC_{50} values of 1.04 and 1.09 μM, respectively, and IC_{50} values of 1.39 μM against MCF-7 cells line [Koutova et al., 2023]. This authors also revealed that montanine triggered apoptosis of acute lymphoblastic leukemia (MOLT-4 cells line) via caspase activation, mitochondrial depolarization and Annexin V/PI double staining, and showed that the protein levels of Chk1 Ser345 were upregulated with increased montanine concentration [Koutova et al., 2023].

Different biological activities have been attributed to montanine, including the potential of this alkaloid in autoimmune diseases, such as rheumatoid arthritis using different animal models [Farinon et al., 2017]. Previous studies have shown the montanine inhibitory impact on the growth of diverse bacterial strains [Castilhos et al., 2007]. Silva and co-authors suggested that montanine has psychopharmacological

Table 1
Mean inhibitory concentration (IC_{50}) and confidence interval (C.I. 95%) of the Amaryllidaceae alkaloids montanine, lycorine and 1-*O*-acetylcaranine and the positive control doxorubicin in colorectal carcinoma (HCT 116) and breast carcinoma (MCF-7, MDAMB231, and Hs578T) cell lines (n = 3). Values expressed as μM.

	HCT-116		MCF-7		MDAMB231		Hs578T	
	IC_{50}	C.I. 95%	IC_{50}	C.I. 95%	IC_{50}	C.I. 95%	IC_{50}	C.I. 95%
Montanine	1.3	0.94–1.8	4.3	2.8–6.6	3.9	2.4–6.5	25.2	13.8–57.2
Lycorine	2.6	1.4–5.6	2.5	1.8–3.4	6.2	3.6–11	32.8	18.7–69.3
1-*O*-Acetylcaranine	>50	–	>50	–	>50	–	>50	–
Doxorubicin	0.2	0.08–0.45	0.5	0.2–1.4	0.7	0.4–1.1	2.0	1.3–3.3

Table 2
Docking score of the best poses for the four predicted targets found by GOLD using the ChemPLP scoring function. The alkaloids were marked in bold letters.

Docking CDK8						Docking ER-alpha					
Name	ChemPLP[a]	Rank CPLP	Vina	Rank Vina	Consensus	Name	ChemPLP[a]	Rank CPLP	Vina	Rank Vina	Consensus
C1I	82.5417	1	−8.818	1	1	Raloxifene	111.61	1	−10.4936	1	1
50R	63.047	2	−4.73401	2	2	5FB	72.8712	2	−7.18626	4	3
Lycorine	59.7393	3	−3.06933	4	3.5	Estradiol	66.8858	3	−8.61849	2	2.5
8D6	49.6896	4	−4.65541	3	3.5	Montanine	55.9324	4	−8.41181	3	3.5
Montanine	49.6702	5	1.56761	5	5	Lycorine	48.7987	5	−6.76123	5	5
Docking EGFR						**Docking dCK**					
Name	ChemPLP[a]	Rank CPLP	Vina	Rank Vina	Consensus	Name	ChemPLP[a]	Rank CPLP	Vina	Rank Vina	Consensus
03P	113.402	1	−9.41204	1	1	Yousef2	67.3063	3	−9.42304	1	2
634	76.6067	3	−8.58713	3	3	Yousef4	64.9072	4	−9.38764	2	3
3W2S	96.2395	2	−7.79536	5	3.5	Yousef6	64.4129	5	−8.72299	3	4
8AM	73.8263	5	−9.0758	2	3.5	1UX	90.4495	1	−7.25404	9	5
AQ4	75.6943	4	−7.33325	6	5	Yousef3	63.8796	7	−8.68237	4	5.5
Lycorine	49.9288	8	−8.16688	4	6	Yousef5	64.2004	6	−8.6799	5	5.5
ANP	65.5774	6	−6.84039	7	6.5	2XZ	87.3847	2	−6.23712	10	6
Montanine	51.6193	7	−6.45302	8	7.5	Lycorine	60.9012	9	−8.0102	6	7.5
						Montanine	61.3297	8	−7.4249	8	8
						Yousef1	56.3052	10	−7.58967	7	8.5
						DCZ	54.4967	11	−6.18603	11	11

b: Autodock vina score (kcal/mol).
[a] : ChemPLP score is dimensionless.

activities including anxiolytic, antidepressive, and anticonvulsive effects [Silva et al., 2006]. Furthermore, researchers have highlighted montanine's potential contribution to the exploration of alternative treatments for neurological dis-orders, attributed to its capability as an acetylcholinesterase inhibitor [Pagliosa et al., 2010]. In a recent report, a combination of montanine and benznidazole presented a potent synergistic effect, which could be an interesting combination for future studies to treat Chagas disease [Piñeiro et al., 2023].

3.2. Computational experiments

3.2.1. Target prediction and enrichment analysis

In order to investigate potential targets for the anticancer activity of the alkaloids, several computational experiments were accomplished. The compounds had their putative targets predicted by three web servers and the results were concatenated and re-ported as a Venn diagram (Fig. 2A). As we may notice a total of 143 targets were predicted as common to the two active alkaloids.

The second step involved a keyword search of disease-linked genes in the DisGeNET and GeneCards databases regarding breast cancer and colorectal adenocarcinoma. A total of 7769 and 1372 genes were retrieved from the databases for breast cancer and colorectal adenocarcinoma, respectively. The resultant gene list was then merged with the 143 compound-related targets predicted previously into a Venn diagram (Fig. 2B). A total of 42 targets were found common to both diseases and to the alkaloids (Supporting information Tab. s3).

The next step was conducted through STRING web server in order to build a PPI network within the 42 predicted targets (Fig. 3). As we may see, two targets (MARK4, CNR2) were found to be a single node; therefore it was predicted as not being closely related to the others in the built network. Considering the topological analysis we highlighted ten genes that showed the most important topological values (degree, betweenness, closeness, and shortest path length) suggesting their relevant roles regarding the network (EGFR, HIF1A, TLR4, MAPK1, NOS3, NFKB1, MAPK8, APEX1, PIK3R1 and MAP2K1) (Supporting information Tab. 3).

The enrichment analyses were performed at ShinyGO web server regarding the enriched pathways of the predicted targets based on the KEGG database and considering an FDR cutoff value of 0.05 (Fig. 4A). Among the top 20 selected pathways, nine were directly related to cancer: PD-L1 expression and PD-1 checkpoint pathway in cancer (hsa05235), pathways in cancer (hsa05200), pancreatic cancer (hsa05212), central carbon metabolism in cancer (hsa05230), chronic myeloid leukemia (hsa05220), chemical carcinogenesis (hsa05208),

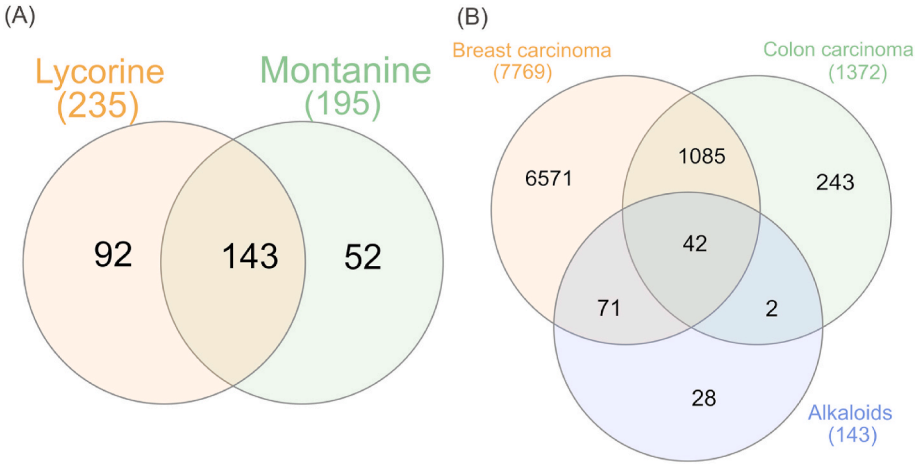

Fig. 2. Venn diagrams for the alkaloids. (**A**) Number of common targets predicted by ligand similarity; (**B**) Number of common targets related to the disease and to the alkaloids.

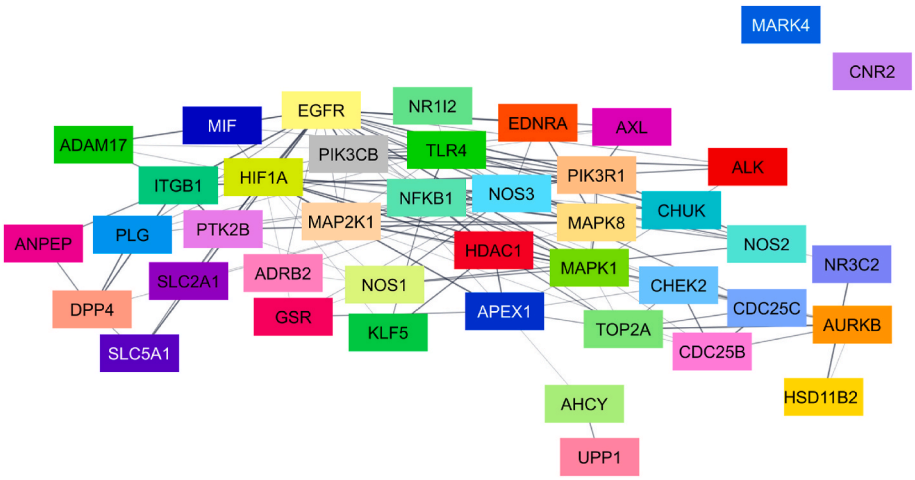

Fig. 3. Protein-protein Interaction Network built by STRING based on the 42 disease-compound common targets.

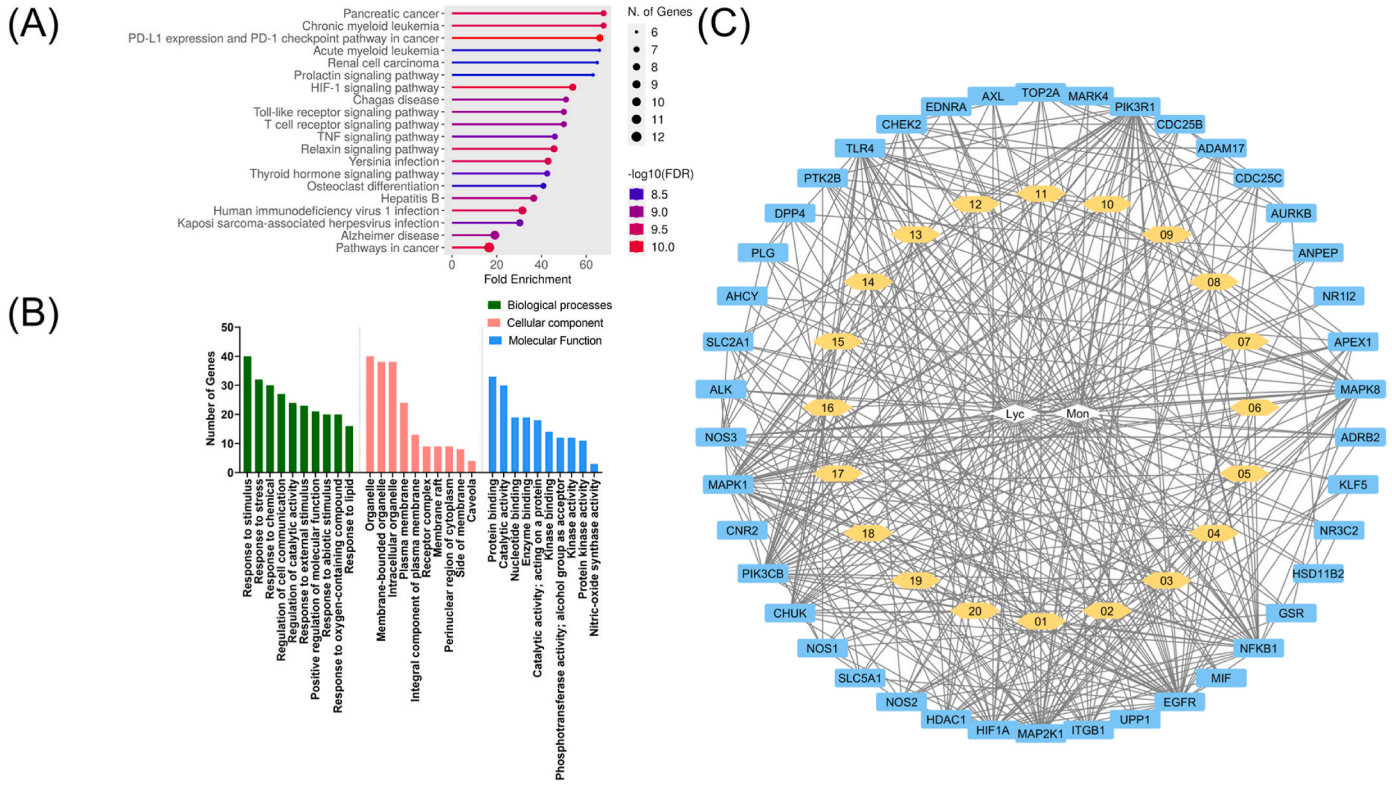

Fig. 4. ShinyGO enrichment analyses were performed for the common targets. (A) KEGG enrichment for the 42 key targets; (B) GO annotations for the common targets. (C) Compound-pathway-target: The two alkaloids were represented as orange ellipses, the pathway numbers were represented as beige hexagons, and the key targets were represented as blue rectangle. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

microRNAs in cancer (hsa05206), prostate cancer (hsa05215) and choline metabolism in cancer (hsa0523); moreover, four of them were closely related to cancer: HIF-1 signaling pathway (hsa04066), relaxin signaling pathway (hsa04926) and toll-like receptor signaling pathway (hsa04620) and PI3K-Akt signaling pathway (hsa04151). The highlighted pathways (e.g: pancreatic cancer) are related and contain genes that are expressed in those diseases according to the KEGG database and were common to both compounds and breast cancer and colon carcinoma. The top ten GO annotations within the established FDR cut-off (<0.5) are depicted in Fig. 4B. The biological processes (BPs) showed that most of the enriched common targets were related to responsive processes, which included: response to stress, response to stimulus, response to abiotic stimulus, response to chemical, response to lipid, response to oxygen-containing compounds and response to external stimulus. Furthermore, other BPs commonly found were: regulation of catalytic activity, positive regulation of molecular functions and regulation of cell communication. The CC indicated that most of the targets are related to receptor complexes and membrane raft location, whereas their MF were related to kinase, nucleotide and protein binding and related to both catalytic activity such as: protein kinase, phosphotransferase activity and nitric-oxide synthase. Considering the enriched pathways, a pharmacological network (Fig. 4C) was built to link: (a) lycorine and montanine, (b) 20 predicted pathways, and (c) 42 related targets. The analyses of the protein-protein interaction and

the pharmacological networks highlighted the PI3K-Akt, signaling as a potential pathway related to breast and colorectal cancer. The main predicted targets of this pathway include the following: PIK3R1, PIK3CB, EGFR, NFKB1, TLR4, ITGB1, NOS3 and CHUK. Su and co-workers identified 10 targets associated to GBM and to lycorine, through a similar network pharmacology combined to molecular docking approach: AKT1, SRC, HSP90AA1, HRAS, MMP9, BCL2L1, IGF1, MAPK14, STAT1, and KDR. The authors proposed that lycorine would act by multiple pathways, especially through apoptosis induction and reactive oxygen species generation, which corroborated their experimental findings [Su et al., 2023a, 2023b].

According to the literature, the PI3K pathway, including its membrane receptor EGFR, has been identified as an important target in breast and colorectal cancers, suggesting that the inhibition of this pathway may be effective for the treatment of these tumors, while the proteins CDK-8 and ER-alpha have been used as targets for human colorectal carcinoma cancer and breast adenocarcinoma cancer cell lines, respectively [Baselga et al., 2011; Yu and Grady, 2012; Shawky et al., 2018; Shawky et al., 2018; Tahlan et al., 2019]. Moreover, the enzyme dCK is an interesting target in cancer therapy due to its potential for DNA repair

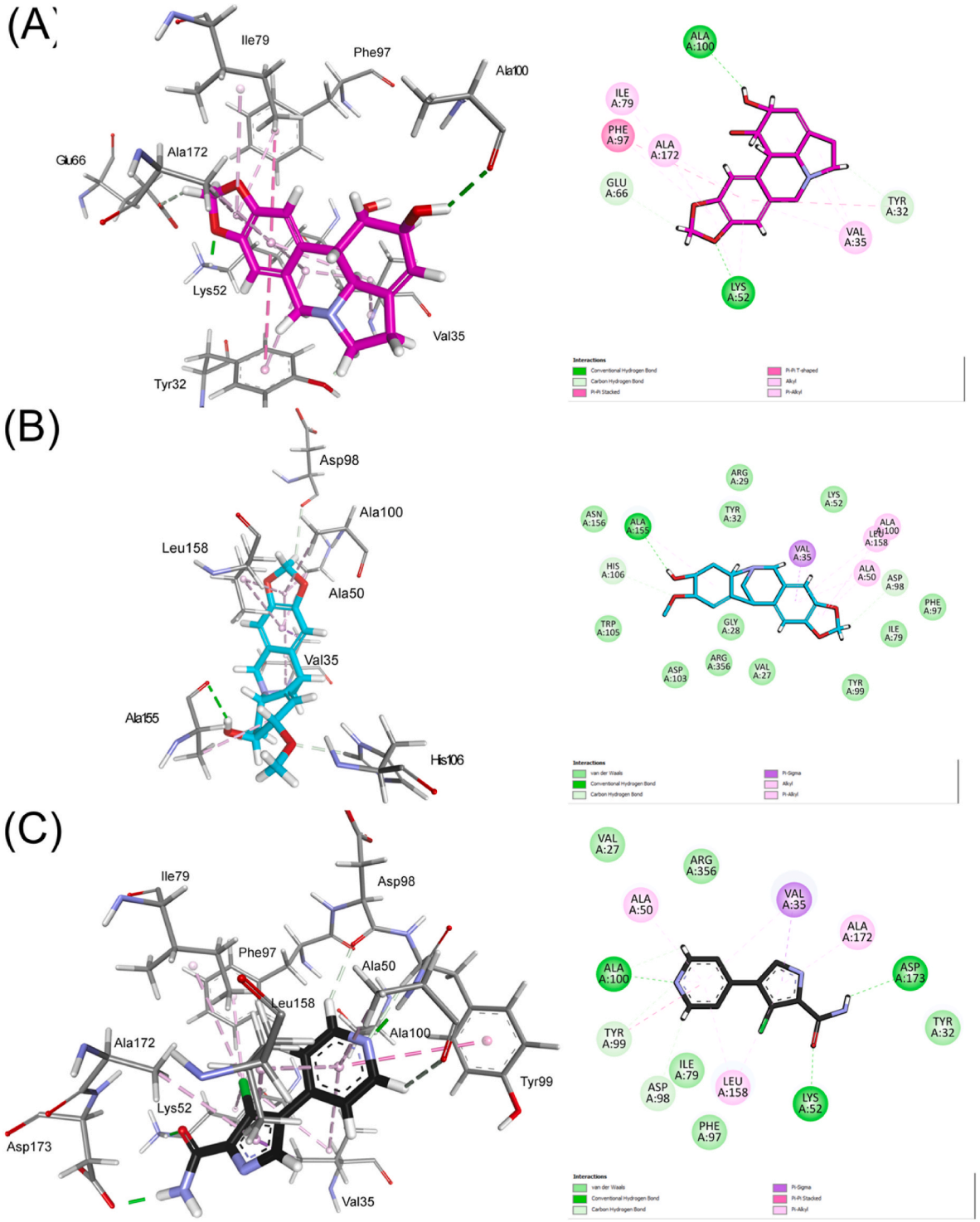

Fig. 5. Best found poses for the molecular docking of the alkaloids in CDK8 (5XS2) A: Lycorine; B: Montanine; C: Co-crystallized ligand – 8D6.

and apoptosis [Murphy et al., 2013; Nomme et al., 2014; Yousef et al., 2018]. Different narciclasine-type structures presented good interactions toward dCK suggesting that the cytotoxic activity of these Amaryllidaceae alkaloids could be mediated through dCK inhibition [Yousef et al., 2018]. Therefore, these targets were selected for molecular docking analyses in order to investigate their binding mode.

3.2.2. Molecular docking

The docking validation was performed through a redocking procedure for the three PI3K pathway-related targets (CDK-8, ER-alpha, and EGFR) and for dCK. The RMSD values found in the protocols for each target were found suiTab. with at least one pose below the cut-off of 2.0 Å (Supporting information Table 2).

The analysis of the obtained scores for the docked alkaloids suggested that the compounds may interact preferentially with CDK-8, and in a minor proportion with dCK, compared to the other targets (Table 2), according to ChemPLP scoring function, whereas Vinascore also suggests potential interactions with EGFR. This examination relies on the comparison of the docking scores obtained for the alkaloids compared to the reference compounds (co-crystallized and literature reported). As we

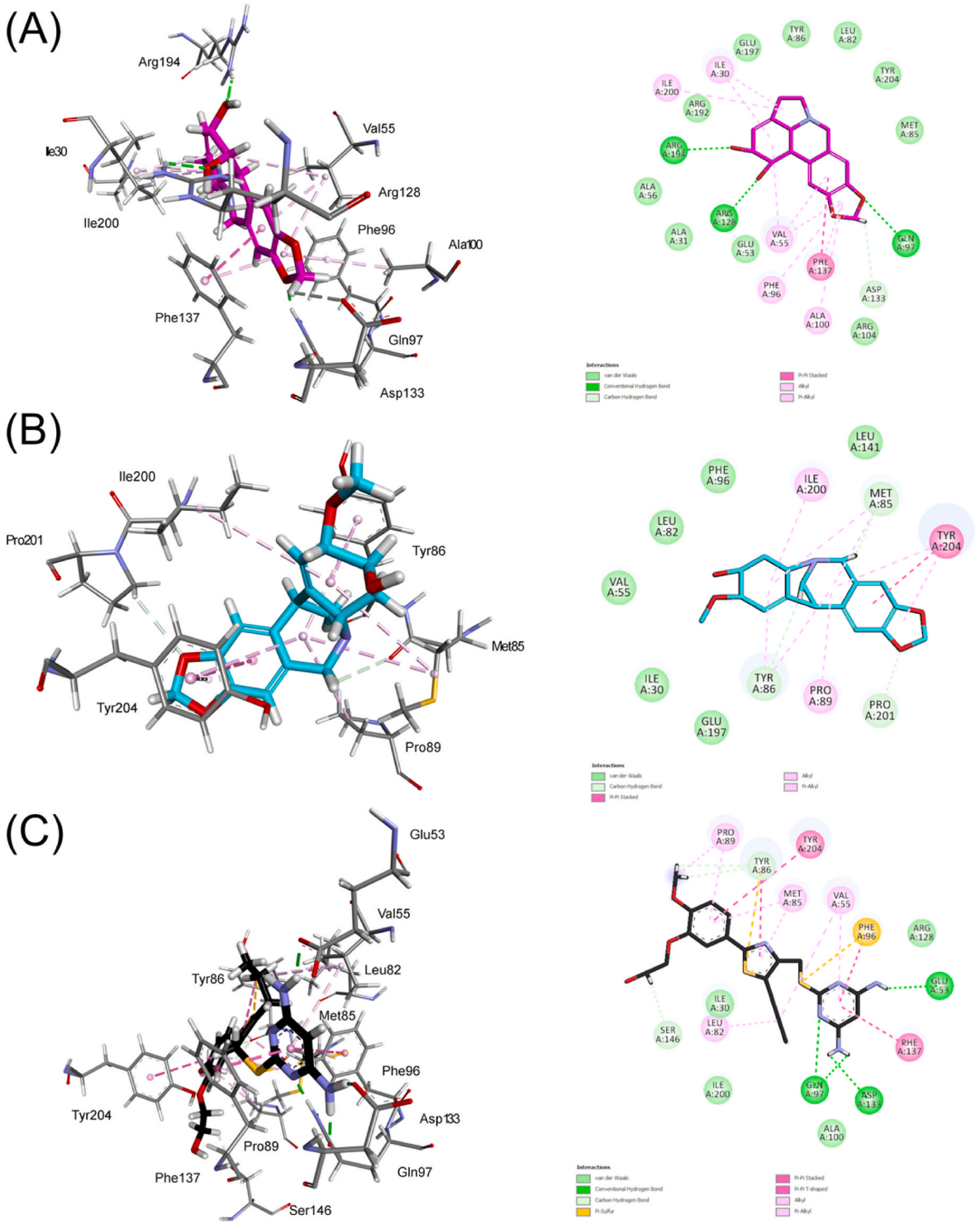

Fig. 6. Best found poses for the molecular docking of the alkaloids in dCK (4Q1A) A: Lycorine; B: Montanine; C: Co-crystallized ligand – 2XZ.

may observe, in all targets the best-docked ligands were the co-crystallized inhibitors, which, according to the literature, possess binding affinity values in the nanomolar range. On the other hand, the best-docked poses obtained for the alkaloids were found to be at the last positions. Nevertheless, the alkaloids displayed docking score values in the range of two CDK8 inhibitors (50R and 8D6), whose binding affinity values reported in BindingDB and in the literature were 5.3 and 7 nM, respectively (Supporting information Table 2). Furthermore, regarding the dCK all the alkaloids displayed docking score values close to the substrate DCZ and to the narciclasine-type compounds predicted by Yousef and collaborators [Yousef et al., 2018].

The residue interaction pattern observed for both targets may be further examined in Supporting information Table 2. The co-crystallized ligands regarding the CDK8 point out common interactions with the following amino acid residues: (a) van der Waals interactions with Val35, Ala50, Asp98, Ala100 and Leu158; (b) hydrogen bonds with Ala100. The alkaloids were capable to interact with some of the high-lighted residues, explaining the low scores observed. However, they

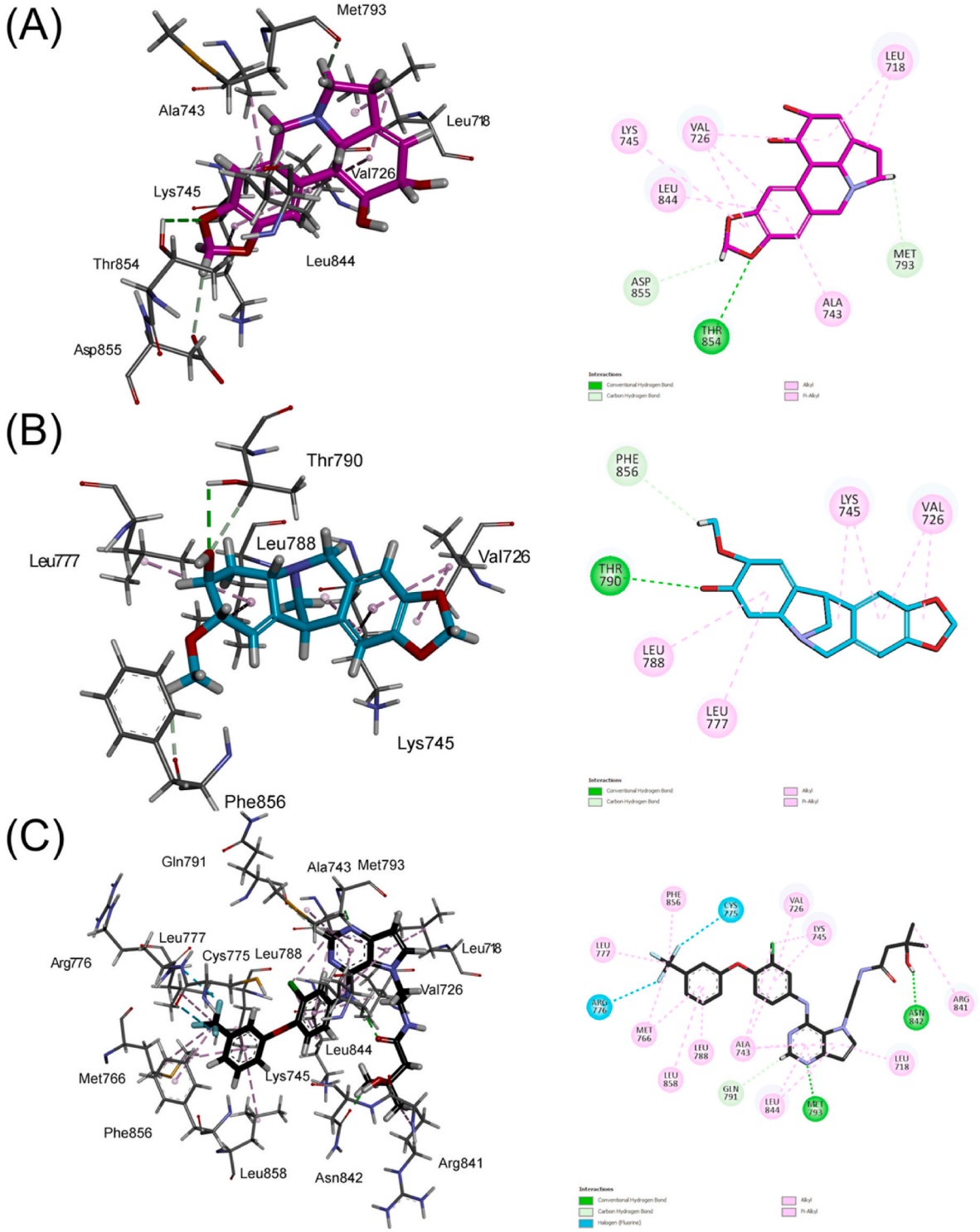

Fig. 7. Best found poses for the molecular docking of the alkaloids in EGFR (3POZ) A: Lycorine; B: Montanine; C: Co-crystallized ligand – 03P.

were able to compensate for the binding with other residue interactions (Fig. 5). The dCK key interactions based on the co-crystallized ligands were: (a) hydrogen bonds with Glu53, Gln97, and Asp133; (b) π-stacking with Tyr86, Phe96 and Phe137. The evaluation of the docked poses indicated interactions with key residues such as Gln97 and Phe137. Notwithstanding, the compounds assumed a similar situation as for the CDK8 regarding the number of key residues and type of interactions since some of them were compensated for others (Fig. 6). Saez-Ayala and collaborators pointed out that the hydrogen bonds with Glu53, Gln97 and Asp133 were important for the development of new dCK inhibitors [Saez-Ayala et al., 2023]. Whereas Yousef and collaborators reported that the narciclasine derivatives may interact with Arg128 through hydrogen bonds, which may act as a major anchor for the binding, as well as may interact with Glu53 [Yousef et al., 2018]. The rescoring protocol using Autodock Vina score was applied on the poses found by GOLD, which highlighted lycorine as a potential ligand of EGFR. As we may observe, lycorine interacted through hydrogen bonds with Thr854 and several VdW interactions with the following residues: Leu718, Val726, Ala743, Met793 and Leu844 (Fig. 7A). On the other hand, montanine did not exhibit the same interaction pattern, which resulted on low scoring. Shen and coworkers reported that lycorine inhibited GBM damaging phosphorylation of EGFR and AKT and would act on EGFR signaling pathway. The authors proposed that lycorine interacts with EGFR through direct inhibition in ATP binding pocket by hydrogen bonds with three amino acids residues: lycorine's hydroxyls with Asn842 and Thr854 and lycorine's oxygen from methylenedioxy moiety with Arg745 [Shen et al., 2018]. The difference relies mostly on the observed orientation between the reported poses, whereas both findings are associated to hydrogen bonds with Thr854.

The docking scores for both alkaloids in ER-alpha (PDBID: 7KBS) were lower than the reference ligands. Raloxifene an ER inhibitor showed scores of 111.61/-10.4936 kcal/mol (ChemPLP/Vinascore) and the ER substrate, estradiol, 66.8858/-8.61849 kcal/mol (ChemPLP/Vinascore). On the other hand of, the alkaloids showed the following values for ChemPLP and Vinascore, respectively: (a) montanine: 55.9324 and −8.41181 kcal/mol; (b) lycorine: 48.7987 and −6.76123 kcal/mol. The interaction profile observed for both alkaloids were also distinct from raloxifene and estradiol. Both compounds showed a low number of interactions and mainly through Van der Waals, whereas the references interacted in different ways with a greater number of amino acids residues (Supporting information Fig. 1 and Supporting information Table 2).

4. Conclusions

This study unveils the antitumoral potential of three Amaryllidaceae alkaloids. Among them, montanine exhibited the most favorable outcomes against colorectal (HCT-116) and breast carcinoma (MDAMB231 and Hs578T) cell lines, while lycorine demonstrated the best activity against MCF-7 cells, also associated with breast carcinoma. However, the alkaloid 1-*O*-acetylcaranine exhibited no discernible *in vitro* activity against these cancer cell lines, and this could have two possible explanations: the acetylation of C1 hydroxyl group and/or the absence of a hydroxyl at C2. To enhance the comprehension of the mechanisms underlying these molecules' growth-inhibitory effects on tumors, computational experiments were undertaken.

The target prediction studies revealed 28 common targets between the alkaloids and the diseases (breast and colorectal cancer). The topological analyses of the predicted STRING protein-protein interaction network accomplished at Cytoscape highlighted EGFR, HIF-1, NOS3, TLR4, and PIK3R1 as possessing relevant roles in this network. The enrichment analyses performed at ShinyGO suggested that the PI3K-Akt signaling could be potentially linked with these cancers. Based on these results and on the literature, four potential targets were chosen for a molecular docking analysis: CDK8, EGFR, ER-alpha, and dCK. The adopted protocols were suiTab. regarding the redocking validation cut-off. The docking scores revealed three potential targets for the alkaloids with scores similar to co-crystallized inhibitors and substrates: CDK8, dCK and EGFR. The visual inspection of the best-docked poses indicates that the alkaloids may interact with some key residues compared to other docked compounds, suggesting that they may bind to these targets. Nevertheless, molecular dynamics simulations and *in vitro* studies should be performed to further validate these findings. In summary, the therapeutic potential of Amaryllidaceae alkaloids has been taken into account in drug discovery, nevertheless different experiments must be carried out to determine the safety and efficacy of these alkaloids before drawing any conclusions about their therapeutic potential.

Supporting information

Tab. S1: Crystallography models of the proteins and grid settings used in the molecular docking simulations; Tab. S2: Docking information; Tab. S3: Target prediction information.

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CRediT authorship contribution statement

Luciana R. Tallini: Writing – original draft, Investigation, Conceptualization. **Gustavo Machado das Neves:** Writing – original draft, Validation, Software, Methodology, Investigation. **Maria Helena Vendruscolo:** Visualization. **Paula Rezende-Teixeira:** Validation, Methodology, Investigation. **Warley Borges:** Visualization. **Jaume Bastida:** Writing – review & editing. **Letícia V. Costa-Lotufo:** Writing – review & editing, Supervision. **Vera Lucia Eifler-Lima:** Supervision, Visualization. **José Angelo S. Zuanazzi:** Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2024.118154.

References

Barman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The protein data bank. Nucleic Acids Res. 28 (1), 235–242. https://doi.org/10.1093/nar/28.1.235.

Baselga, J., 2011. Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. Oncol. 16 (1), 12–19. https://doi.org/10.1634/theoncologist.2011-S1-12.

Bastida, J., Lavilla, R., Viladomat, F., 2006. Chemical and biological aspects of *Narcissus* alkaloids. In: Cordell, G.A. (Ed.), The Alkaloids: Chemistry and Physiology, 63. Elsevier, Amsterdam, pp. 87–179.

Berkov, S., Osorio, E., Viladomat, F., Bastida, J., 2020. Chemodiversity, chemotaxonomy and chemoecology of AA. In: Knölker, H.-J. (Ed.), The Alkaloids: Chemistry and Biology, 83. Elsevier, Amsterdam, pp. 113–185. https://doi.org/10.1016/bs.alkal.2019.10.002.

Caroli, A., Ballante, F., Wickersham, R.B., Corelli, F., Ragno, R., 2014. Hsp90 inhibitors, Part 2: combining ligand-based and structure-based approaches for virtual screening application. J. Chem. Inf. Model. 54 (3), 970–977. https://doi.org/10.1021/ci400760a.

Castilhos, T.S., Giorgani, R.B., Henriques, A.T., Menezes, F.S., Zuanazzi, J.A.S., 2007. Avaliação *in vitro* das atividades antiinflamatória, antioxidante e antimicrobiana do alcalóide montanina. Rev. Bras. Farmacogn. 17 (2), 209–214. https://doi.org/10.1590/S0102-695X2007000200013.

ChemSketch, version 2022.1.2, Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada, www.acdlabs.com.

Daina, A., Michielin, O., Zoete, V., 2019. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 47 (W1), W357–W364. https://doi.org/10.1093/nar/gkz382.

DiMassi, J.A., Grabowski, H.G., Hansen, R.W., 2016. Innovation in the pharmaceutical industry: new estimates of R&D costs. J. Health Econ. 47, 20–33. https://doi.org/10.1016/j.jhealeco.2016.01.012.

Duke, J.A., Duke, P.K., 1983. Medicinal Plants of the Bible. Trad-Medic Books, New York, p. 98.

Eberhardt, J., Santos-Martins, D., Tillack, A.F., Forli, S., 2021. AutoDock vina 1.2.0: new docking methods, expanded Force Field, and Python bindings. J. Chem. Inf. Model. 61 (8), 3891–3898. https://doi.org/10.1021/acs.jcim.1c00203.

Farinon, M., Clarimundo, V.S., Pedrazza, G.P.R., Gulko, P.S., Zuanazzi, J.A.S., Xavier, R. M., Oliveira, P.G. de, 2017. Disease modifying antirheumatic activity of the alkaloid montanine on experimental arthritis and fibroblastlike synoviocytes. Eur. J. Pharmacol. 799, 180–187. https://doi.org/10.1016/j.ejphar.2017.02.013.

Feher, M., Schmidt, J.M., 2003. Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. J. Chem. Inf. Comput. Sci. 43, 218–227. https://doi.org/10.1021/ci0200467.

Ge, S.X., Jung, D., Yao, R., 2020. ShinyGO: a graphical gene set enrichment tool for animals and plants. Bioinformatics 36 (8), 2628–2629. https://doi.org/10.1093/bioinformatics/btz931.

Goietsenoven, G. van, Mathieu, V., Lefranc, F., Kornienko, A., Evidente, A., Kiss, R., 2013. Narciclasine as well as other Amaryllidaceae isocarbostyrils are promising GTP-ase targeting agents against brain cancers. Med. Res. Rev. 33 (2), 439–455. https://doi.org/10.1002/med.21253.

Govindaraju, K., Masi, M., Colin, M., Mathieu, V., Evidente, A., Hudnall, T.W., Kornienko, A., 2018. Novel topologically complex scaffold derived from alkaloid haemanthamine. Molecules 23, 255. https://doi.org/10.3390/molecules23020255.

Heberle, H., Meirelles, G.V., Silva, F.R. da, Telles, G.P., Minghim, R., 2015. InteractiVenn: a web based tool for the analysis of sets through Venn diagrams. BCM Bioinformatics 16 (1), 169. https://doi.org/10.1186/s12859-015-0611-3.

Heinrich, M., Teoh, H.L., 2004. Galanthamine from snowdrop - the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. J. Ethnopharmacol. 92, 147–162. https://doi.org/10.1016/j.jep.2004.02.012.

Jones, G., Willett, P., Glen, R.C., Leach, A.R., Taylor, R., 1997. Development and validation of a genetic algorithm for flexible docking. J. Mol. Biol. 267 (3), 727–748. https://doi.org/10.1006/jmbi.1996.0897.

Kekre, N., Griffin, C., McNulty, J., Pandey, S., 2005. Pancratistatin causes early activation of caspase-3 and the flipping of phosphtidyl serine followed by rapid apoptosis specifically in human lymphoma cells. Cancer Chemother. Pharmacol. 56, 29–38. https://doi.org/10.1007/s00280-004-0941-8.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E.E., 2018. PubChem 2019 update: improved access to chemical data. Nucleic Acids Res. 47 (D1), D1102–D1109. https://doi.org/10.1093/nar/gky1033.

Korlyukov, A.A., Stash, A.I., Romanenko, A.R., Trzybinski, D., Wozniak, K., Vologzhanina, A.V., 2023. Ligand-receptor interactions of *Lamivudine*: a view from charge density study and QM/MM calculations. Biomedicines 11 (3), 743. https://doi.org/10.3390/biomedicines11030743.

Kornienko, A., Evidente, A., 2008. Chemistry, biology and medicinal potential of narciclasine and its congeners. Chem. Rev. 108, 1982–2014. https://doi.org/10.1021/cr078198u.

Koutova, D., Maafi, N., Muthna, D., Kralovec, K., Kroustkova, J., Pidany, F., Timbilla, A. A., Cermakova, E., Cahlikova, L., Rezacova, M., Havelek, R., 2023. Antiproliferative activity and apoptosis inducing mechanism of Amaryllidaceae alkaloid montanine on A549 and MOLT-4 human cancer cells. Biomed. Pharmacother. 166, 115295 https://doi.org/10.1016/j.biopha.2023.115295.

Kuhn, M., Mering, C. von, Campillos, M., Jensen, L.J., Bork, P., 2008. STITCH: interaction networks of chemicals and proteins. Nucleic Acids Res. 36 (1), D684–D688. https://doi.org/10.1093/nar/gkm795.

Lim, C.P., Yan, M.F., Asmawi, M.Z., Chin, V.K., Khairruddin, N.H., Yong, Y.K., Hassan, H., Basir, R., 2019. Cytostatic and antiproliferative activities of F5 fraction of *Crinum amabile* leaf chloroform extract showed its potential as cancer chemotherapeutic agent. Evid-Based. Compl. Alt., 7521504 https://doi.org/10.1155/2019/7521504, 2019.

Lu, T., Li, T., Wu, M.-K., Zheng, C.-C., He, X.-M., Zhu, H.-L., Li, L., Man, R.-J., 2023. Molecular simulations required to target novel and potent inhibitors of cancer invasion. Expet Opin. Drug Discov. 18 (12), 1367–1377. https://doi.org/10.1080/17460441.2023.2254695.

Mak, K.K., Pichika, M.R., 2019. Artificial intelligence in drug development: present status and future prospects. Drug Discov. Today 24, 773–780. https://doi.org/10.1016/j.drudis.2018.11.014.

Masi, M., Slambrouck, S. van, Gunawardana, S., Rensburg, M.J. van, James, P.C., Mochel, J.G., Heliso, P.S., Albalawi, A.S., Cimmino, A., Otterlo, W.A.L. van, Kornienko, A., Green, I.R., Evidente, A., 2019. Alkaloids isolated from *Haemanthus humilis* Jacq., an indigenous South African Amaryllidaceae: anticancer activity of coccinine and montanine. South Afr. J. Bot. 126, 277–281. https://doi.org/10.1016/j.sajb.2019.01.036.

McLachlan, A., Kekre, N., McNulty, J., Pandey, S., 2005. Pancratistatin: a natural anticancer compound that targets mitochondria specifically in cancer cells to induce mitosis. Apoptosis 10, 619–630. https://doi.org/10.1007/s10495-005-1896-x.

Meerow, A.W., Snijman, D.A., 1998. Amaryllidaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants, 3. Springer, Berlin, pp. 83–110.

Mohan, K., Jeyachandran, R.Deepa, 2012. Alkaloids as anticancer agents. Annals of Phytomecidine: Int. J. 1 (1), 46–53.

Murphy, J.M., Armijo, A.L., Nomme, J., Lee, C.H., Smith, Q.A., Li, Z., Campbell, D.O., Liao, H.-I., Nathanson, D.A., Austin, W.R., Lee, J.T., Darvish, R., Wei, L., Wang, J., Su, Y., Damoiseaux, R., Sadeghi, S., Phelps, M.E., Herschman, H.R., Czernin, J., Alexandrova, A.N., Jung, M.E., Lavie, A., Radu, C.G., 2013. Development of new deoxycytidine kinase inhibitors and noninvasive in vivo evaluation using Positron Emission Tomography. J. Med. Chem. 56 (17), 6696–6708. https://doi.org/10.1021/jm400457y.

Nair, J.J., Staden, J. Van, 2022. Insights to the tribe Haemantheae of the South African Amaryllidaceae. J. Ethnopharmacol. 292, 115177 https://doi.org/10.1016/j.jep.2022.115177.

Nair, J.J., Staden, J. Van, Bastida, J., 2016. Cytotoxic alkaloids constituents of the Amaryllidaceae. In: Rahman, A.-U. (Ed.), Studies in Natural Products Chemistry, 49. Elsevier, Amsterdam, The Netherlands, pp. 107–156.

Napo, K.V., Mokoena, L.E., Mangoejane, C., Mfengwana, H., Mashele, S., Sekhoacha, M. P., 2020. *In vitro* antiproliferative activity of Amaryllidaceae species against the K562 human leukaemia cell line. South Afr. J. Bot. 135, 429–436. https://doi.org/10.1016/j.sajb.2020.07.012.

Newman, D.J., Cragg, G.M., 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J. Nat. Prod. 83, 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285.

Nickel, J., Gohlke, B.-O., Erehman, J., Banerjee, P., Rong, W.W., Goede, A., Dunkel, M., Preissner, R., 2014. SuperPred: update on drug classification and target prediction. Nucleic Acids Res. 42 (W1), W26–W31. https://doi.org/10.1093/nar/gku477.

Nomme, J., Li, Z., Gipson, R.M., Wang, J., Armijo, A.L., Le, T., Poddar, S., Smith, T., Santarsiero, B.D., Nguyen, H.-A., Czernin, J., Alexandrova, A.N., Jung, M.E., Radu, C.G., Lavie, A., 2014. Structure guided development of deoxycytidine kinase inhibitors with nanomolar affinity and improved metabolic stability. J. Med. Chem. 57 (22), 9480–9494. https://doi.org/10.1021/jm501124j.

O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R., 2011. Open Babel: an open chemical toolbox. J. Cheminf. 3, 33. https://doi.org/10.1186/1758-2946-3-33.

Pagliosa, L.B., Monteiro, S.C., Silva, K.B., Andrade, J.A. de, Dutilh, J., Bastida, J., Cammarota, M., Zuanazzi, J.A.S., 2010. Effect of isoquinoline alkaloids from two *Hippeastrum* species on *in vitro* acetylcholinesterase activity. Phytomedicine 17, 698–701. https://doi.org/10.1016/j.phymed.2009.10.003.

Pandey, S., Kekre, N., Naderi, J., McNulty, J., 2012. Induction of apoptotic cell death specifically in rat and human cancer cells by pancratistatin. Artif. Cell Blood Sub 33, 1–17. https://doi.org/10.1081/bio-200066621, 2005.

Petró-Silveira, B., Rigo, G.V., Trentin, D. da S., Macedo, A.J., Sauer, E., Alves, E. de O., Tallini, L.R., Garcia, S.C., Borges, W. de S., Zuanazzi, J.A.S., Tasca, T., 2020. *Trichomonas vaginalis* NTPDase inhibited by lycorine modulates the parasite neutrophil interaction. Parasitol. Res. 119 (8), 2587–2595. https://doi.org/10.1007/s00436-020-06739-8.

Pettit, G.R., Gaddamidi, V., Goswami, A., Cragg, G.M., 1984a. Antineoplastic agents, 99. Amaryllis belladonna. J. Nat. Prod. 47 (5), 796–801.

Pettit, G.R., Gaddamidi, V., Cragg, G.M., Herald, D.L., Sagawa, Y., 1984b. Isolation and structure of pancratistatin. J. Chem. Soc. Chem. Commun. 24, 1693–1694.

Piñeiro, M., Ortiz, J.E., Zapata, R.M.S., Barrera, P.A., Sosa, M.A., Roitman, G., Bastida, J., Feresin, G.E., 2023. Antiparasitic activity of *Hippeastrum* species and synergistic interaction between montanine and benznidazole against *Trypanosoma cruzi*. Microorganisms 11, 144. https://doi.org/10.3390/microorganisms11010144.

Piñero, J., Ramírez-Anguita, J.M., Saüch-Pitarch, J., Ronzano, F., Centeno, E., Sanz, F., Furlong, L.I., 2020. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res. 48 (D1), D845–D855. https://doi.org/10.1093/nar/gkz1021.

Pushpakom, S., Iorio, F., Eyers, P.A., Escott, K.J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C., Norris, A., Sanseau, P., Cavalla, D., Pirmohamed, M., 2019. Drug repurposing: progress, challenges and recommendations. Nat. Rev. Drug Discov. 18, 41–58. https://doi.org/10.1038/nrd.2018.168.

Saez-Ayala, M., Hoffer, L., Abel, S., Yaala, K.B., Sicard, B., Andrieu, G.P., Latiri, M., Davison, E.K., Ciufolini, M.A., Brémond, P., Rebuffet, E., Roche, P., Derviaux, C., Voisset, E., Montersino, C., Castellano, R., Collette, Y., Asnafi, V., Betzi, S., Dubreuil, P., Combes, S., Morelli, X., 2023. From a drug repositioning to a structure based drug design approach to tackle acute lymphoblastic leukemia. Nat. Commun. 14 (1), 3079. https://doi.org/10.1038/s41467-023-38668-2.

Schenone, M., Dancik, V., Wagner, B.K., Clemons, P.A., 2013. Target identification and mechanism of action in chemical biology and drug discovery. Nat. Chem. Biol. 9, 232–240. https://doi.org/10.1038/nchembio.1199.

Selvaraj, C., Chandra, I., Singh, S.K., 2022. Artificial intelligence and machine learning approaches for drug design: challenges and opportunities for the pharmaceutical industries. Mol. Divers. 26, 1893–1913. https://doi.org/10.1007/s11030-021-10326-z.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13 (11), 2498–2504. https://doi.org/10.1101/gr.1239303.

Shawky, E., Takla, S.S., Hammoda, H.M., Darwish, F.A., 2018. Evaluation of the influence of green extraction solvents on the cytotoxic activities of *Crinum* (Amaryllidaeae) alkaloid extracts using in-vitro-in-silico approach. J. Ethnopharmacol. 227, 139–149. https://doi.org/10.1016/j.jep.2018.08.040.

Shen, J., Zhang, T., Cheng, Z., Zhu, N., Wang, H., Lin, L., Wang, Z., Yi, H., Hu, M., 2018. Lycorine inhibits glioblastoma multiforme growth through EGFR suppression. J. Exp. Clin. Cancer Res. 37 (1), 1–19. https://doi.org/10.1186/s13046-018-0785-4.

Silva, A.F.S. da, Andrade, J.P. de, Bevilaqua, L.R.M., Souza, M.M. de, Izquierdo, I., Henriques, A.T., Zuanazzi, J.A.S., 2006. Anxiolytic, antidepressant- and anticonvulsant-like effects of the alkaloid montanine isolated from *Hippeastrum vittatum*. Pharmacol., Biochem. Behav. 85, 148–154. https://doi.org/10.1016/j.pbb.2006.07.027.

Silva, A.F.S., Andrade, J.P. de, Machado, K.R.B., Rocha, A.B., Apel, M.A., Sobral, M.E.G., Henriques, A.T., Zuanazzi, J.A.S., 2008. Screening for cytotoxic activity of extracts and isolated alkaloids from bulbs of Hippeastrum vittatum. Phytomedicine 15, 882–885. https://doi.org/10.1016/j.phymed.2007.12.001.

Singh, R.K., Lee, J.K., Selvaraj, C., Singh, R., Li, J., Kim, S.-Y., Kalia, V.C., 2018. Protein engineering approaches in the postgenomic era. Curr. Protein Pept. Science 19, 1–15. https://doi.org/10.2174/1389203718666161117114243.

Spartan'14, Wavefunction, Inc., 18401, Von karman avenue, Suite 370, Irvine CA 92612 USA.

Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Stein, T.I., Nudel, R., Lieder, I., Mazor, Y., Kaplan, S., Dahary, D., Warshawsky, D., Guan-Golan, Y., Kohn, A., Rappaport, N., Safran, M., Lancet, D., 2016. The gene cards suite: from gene data mining to disease genome sequence analyses. Curr. Protoc. Bioinformatics 54 (1), 1.30.1–1.30.33. https://doi.org/10.1002/cpbi.5.

Su, J., Yin, W., Huo, M., Yao, Q., Ding, L., 2023a. Induction of apoptosis in glioma cells by lycorine via reactive oxygen species generation and regulation of NF-κB pathways. N-S Arch Pharmacol 396 (6), 1247–1255. https://doi.org/10.1007/s00210-023-02384-x.

Su, J., Huo, M., Xu, F., Ding, L., 2023b. Molecular mechanism of lycorine in the treatment of glioblastoma based on network pharmacology and molecular docking. N-S Arch Pharmacol 2023. https://doi.org/10.1007/s00210-023-02702-3.

Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N., Legeay, M., Fang, T., Bork, P., Jensen, L.J., Mering, C. von, 2020. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 49 (D1), D605–D612. https://doi.org/10.1093/nar/gkaa1074.

Tahlan, S., Kumar, S., Ramasamy, K., Lim, S.M., Shah, S.A.A., Mani, V., Narasimhan, B., 2019. *In silico* molecular design of heterocyclic benzimidazole scaffolds as prospective anticancer agents. BMC Chemistry 13, 90. https://doi.org/10.1186/s13065-019-0608-5.

Tallini, L.R., Andrade, J.P. de, Kaiser, M., Viladomat, F., Nair, J.J., Zuanazzi, J.A.S., Bastida, J., 2017. Alkaloid constituents of the Amaryllidaceae plant *Amaryllis belladonna* L. Molecules 22, 1437. https://doi.org/10.3390/molecules22091437.

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31 (2), 455–461. https://doi.org/10.1002/jcc.21334.

UniProt Consortium, 2019. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 47 (D1), D506–D515. https://doi.org/10.1093/nar/gky1049.

Unni, S., Huang, Y., Hanson, R.M., Tobias, M., Krishnan, S., Li, W.W., Nielsen, J.E., Baker, N.A., 2011. Web servers and services for electrostatics calculations with APBS and PDB2PQR. J. Comput. Chem. 32 (7), 1488–1491. https://doi.org/10.1002/jcc.21720.

WHO, World Health Organization – Cancer. Available online: https://www.who.int/news-room/fact-sheets/detail/cancer (accessed on 24 March 2024).

Williams, G.H., Stoeber, K., 2012. The cell cycle and cancer. J. Pathol. 226, 352–364. https://doi.org/10.1002/path.3022.

Yousef, B.A., Dirar, A.I., Elbadawi, M.A.A., Awadalla, M.K., Mohamed, M.A., 2018. Potential deoxycytidine kinase inhibitory activity of Amaryllidaceae alkaloids: an *In silico* approach. J. Pharm. Bioall. Sci. 10, 137–143. https://doi.org/10.4103/jpbs.JPBS_44_18.

Yu, M., Grady, W.M., 2012. Therapeutic targeting of the phosphatidylinositol 3-kinase signaling pathway: novel targeted therapies and advances in the treatment of colorectal cancer. Ther. Adv. Gastroenterol. 5 (5), 319–337. https://doi.org/10.1177/1756283X12448456.

Yu, Z., Wu, Z., Wang, Z., Wang, Y., Zhou, M., Li, W., Liu, G., Tang, Y., 2024. Network-based methods and their applications in drug discovery. J. Chem. Inf. Model. 64, 57–75. https://doi.org/10.1021/acs.jcim.3c01613.