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Investigation of the effects of lycorine and galanthamine extracted from *Galanthus elwesii* on viral and parasitic targets: An *in-silico* analysis and DFT Study

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Abstract: In this study, Density Functional Theory (DFT), ADME property analysis, and molecular docking simulations were employed to evaluate the electronic structure, antiviral potential, and antiparasitic effects of lycorine and galanthamine, two alkaloids extracted from Galanthus elwesii. We conducted a comprehensive study to assess the antiviral and antiparasitic potential of lycorine and galanthamine, two alkaloids whose biosynthetic production was significantly increased by zinc supplementation. DFT calculations revealed that lycorine has a lower E_{gap} than galanthamine, suggesting higher reactivity and lower stability, enhancing its potential as a drug candidate. Pharmacokinetic profiling indicated that galanthamine (TPSA: 41.93 Å², logP: 0.797) has a lower total polar surface area (TPSA) and higher lipophilicity (logP) compared to lycorine (TPSA: 62.16 Å², logP: -0.268), indicating that galanthamine may possess superior absorption and permeability characteristics. ADME analysis also identified galanthamine with a lower AMES toxicity score, implying reduced mutagenic risk. A total of nine target proteins, representing viral and parasitic diseases Zika virus, malaria, leishmaniasis, and dengue, were chosen for molecular docking. Molecular docking studies demonstrated that lycorine exhibited superior binding interactions (-8.76 kcal/mol), particularly against Leishmania, and displayed stronger binding affinity across all selected target proteins. Despite galanthamine's lower toxicity profile, lycorine's enhanced reactivity and stronger binding properties suggest its higher efficacy as a therapeutic candidate based on DFT and molecular docking results, while galanthamine shows potential based on its favorable ADME profile.

Keywords: Lycorine, Galanthamine, Zinc Supplementation, ADMET, Antiviral, Molecular Docking

1. Introduction

Turkey occupies a significant position in the global context of plant diversity and richness, serving as a critical reservoir of various plant species. Among the diverse flora, bulbous tuberous plants, commonly called geophytes, represent a vital component of this biodiversity. According to the TUBIVES database, Turkey is home to an impressive array of 816 geophyte taxa, distributed across 73 genera and 11 families. The geographical distribution of these plants is notably concentrated in regions such as the Southwestern Aegean, the Black Sea Region, the Mediterranean Region, and the Taurus Mountains (Anonim 2015; Şekeroğlu et al. 2012).

The species of the Amaryllidaceae family, in which the snowdrop is included, contain alkaloids such as nivalin, galanthamine, tazettin and lycorenin, which number up to 150 and are called Amaryllidaceae alkaloids (Ay et al. 2018; Bozkurt et al. 2021). Galanthamine and lycorine exhibit anti-inflammatory (Kang et al. 2012), anti-cancer (Cimmino et al. 2017; Ying et al. 2017), anti-bacterial, anti-malarial, acetylcholinesterase and butyrylcholinesterase inhibitor (Pesaresi et al. 2022) properties. Lycorine is also known for its potential to affect SARS-CoV-2 infection due to its antiviral activity (Jin et al. 2021), while galanthamine is used in the treatment of Alzheimer's disease and other neurological disorders (Kaur et al. 2022; Pesaresi et al. 2022).

To enhance alkaloid production in selected plant species, zinc was utilized as a nutritional supplement during the growth phase, aiming to stimulate alkaloid biosynthesis. Research indicates that zinc plays a crucial role in plant metabolism and can influence the accumulation of secondary metabolites, including alkaloids. For instance, studies have shown that the presence of zinc can enhance the expression of genes involved in alkaloid biosynthesis pathways, thereby increasing the overall alkaloid content in plants (Sun et al. 2018; Zhang et al. 2020).

Viral and parasitic diseases are significant public health concerns worldwide, as they can lead to severe morbidity and mortality. Zika virus, malaria, leishmaniasis, and dengue are significant infectious diseases that pose substantial public health challenges, particularly in tropical and subtropical regions (Waggoner et al. 2016). Zika virus, primarily transmitted by Aedes mosquitoes, is notorious for its association with severe birth defects, such as microcephaly, when contracted during pregnancy. Malaria, caused by Plasmodium parasites and transmitted through Anopheles mosquitoes, remains a leading cause of morbidity and mortality (Fernando et al. 2013), particularly in sub-Saharan Africa, with the World Health Organization (WHO) estimating approximately 627.000 deaths globally in 2020 (Girarad et al. 2020; Subhadra, et al. 2021). Leishmaniasis, transmitted by sandflies and caused by protozoan parasites of the genus Leishmania, is prevalent in the Middle East, South Asia, and South America, manifesting in forms that can be fatal if untreated. Dengue fever, caused by the dengue virus and also transmitted by Aedes mosquitoes, has seen a dramatic rise in incidence, with an estimated 390 million infections annually and approximately 20,000 deaths each year from severe forms (Ramirez-Jimenez et al. 2013). Addressing these diseases is crucial for their direct health impacts and socio-economic consequences, as they strain healthcare systems and hinder economic development. Dengue fever, classified as the most significant mosquito-borne viral disease, has increased tenfold in incidence over the past three decades, affecting over 100 countries (Ravilala et al. 2018). Similarly, malaria continues to contribute to high rates of child mortality in endemic regions. The emergence and re-emergence of these diseases are linked to climate change, urbanization, and increased human-animal interactions, necessitating comprehensive strategies for prevention, control, and treatment to mitigate their impact on global health.

This study aims to enhance the biosynthesis of galanthamine and lycorine alkaloids in Galanthus elwesii Hook through zinc supplementation. This approach aims to increase the production of these bioactive compounds, which have demonstrated antiviral and antiparasitic properties. To further investigate their potential therapeutic applications, molecular docking studies and assessments of physicochemical and pharmacokinetic properties (ADMET) will be conducted.

2. Materials and Method

A field experiment was carried out in Suluova, Amasya, Turkey, during the 2018-2019 growing seasons on a previously cultivated field. Bulbs of G. elwesii with a diameter exceeding 4 cm, procured from commercial sources, were used as planting material. The experimental design was a randomized complete block design with three replications. Three zinc sulfate ($ZnSO_4$) rates (2.5, 5, and 10 kg/da) were applied.

2.1. Extraction method

At the end of the drying process, the roots and bulbs were macerated. The methanol was then evaporated by rotary evaporation and the extracts were obtained. After the methanol was evaporated from the rotary evaporator, the crude extract obtained was acidified with 10% CH_3COOH (pH 2-3). The resulting extract was extracted with chloroform to remove oils, waxes, etc. other than alkaloids. The remaining extract was basicised with 25% NH_3 until pH 8-9 to release the alkaloids in the extract and the resulting phase was extracted with chloroform.

2.2. Determination of Alkaloid Components by HPLC

HPLC working conditions and gradient elution program were used to quantitatively determine the alkaloid compounds of the extracted plants. The components in the samples were determined according to the retention times of the components in the system. For the quantification, firstly, the standards were taken as single readings and their retention times were determined. Then, mix. solutions of the standards at different concentrations were prepared and it was determined whether galantamine and lycorine caused a change in the retention times. Calibration curves were generated from the mix. solutions, and the amount was calculated.

High-Pressure Liquid Chromatography (HPLC) analysis conditions are given below:

Instrument: Shimadzu Prominence Modular LC20A HPLC; Column oven CTO-10AS VP; Column Used Intersil ODS 3, 5um 4,6x250 mm; Mobile phase: 95% TFA-water / 5% acetonitrile; Detector: SPD-M2OA.

2.3. Statistical Analysis

The data obtained were evaluated using the JUMP statistical package program. The Duncan test was used to check the significance of the differences between the means.

2.4. Theoretical Details and In-silico Methods

The theoretical investigations of galanthamine and lycorine were conducted using the Gaussian 09 software package and the GaussView 5.0 molecular visualization tool (Frisch et al., 2009). Gaussian 09 is a widely recognized computational chemistry software that facilitates the modeling of molecular systems using quantum mechanical principles. In our study, geometric optimization of the compounds was achieved through Density Functional Theory (DFT), employing Becke's (1988) Three-Parameter Hybrid Functional in conjunction with the Lee, Yang, and Parr (1988) correlation (B3LYP) method. This hybrid functional is well-regarded for its balance of accuracy and computational efficiency in predicting molecular geometries and electronic properties. We utilized the 6-311G(d,p) basis set for our calculations, which is commonly employed in DFT studies as it provides a good compromise between computational cost and accuracy. Including polarization functions (the 'd' in 6-311G(d,p)) is particularly important for accurately describing the electronic environment of the molecules under investigation, ensuring that our results are robust and reliable under ground state conditions in the gas phase.

For the assessment of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties, we utilized the ADMET Lab platform, which is a user-friendly web-based tool that provides predictions of ADMET properties using a multitask graph attention framework. This platform covers various endpoints, including physicochemical properties, ADME characteristics, and toxicity assessments (Xiong et al., 2021). ADMET Lab employs various predictive models that have been validated against experimental data, enhancing the reliability of the predictions. The specific models used in our analysis include those for predicting solubility, permeability, and metabolic stability, which are critical for evaluating the pharmacokinetic profiles of the compounds. The integrated databases within ADMET Lab ensure that the predictions are based on reliable and precise data. Furthermore, the tool utilizes machine learning algorithms trained on extensive datasets, which significantly enhances the accuracy of the predictions. We ensured that the parameters and settings were appropriately configured to reflect the chemical nature of the compounds we were investigating. The tool is accessible online at: https://admetlab3.scbdd.com/.

The 3D structures of the malaria food vacuole function (PDB:1YVB), apicoplast maintenance (PDB:2QG8), mitochondrial activity (PDB:4PLT), and lipid biosynthesis (PDB:4ZCR), zika virus, proteins like the Zika virus protease (PDB:5H6V) and NS5 protein (PDB:5TFR), dengue virus NS2B/NS3 Protease (PDB: 2FOM), for leishmania Trypanothione Reductase from Leishmania infantum (PDB:2JK6), and Leishmania mexicana arginase (PDB: 4ITY) were obtained from the RCSB PDB database. Using AutoDock Tools 1.5.6, water molecules were removed, proteins were isolated, nonpolar hydrogens were added, Gasteiger charges were computed, and the structures were saved in PDBQT format. The PubChem database provided the 2D structures of alkaloid candidate compounds named lycorine and galanthamine, which were converted to PDBQT format via Chem3D for docking.

Table 1 Molecular docking active site coordinates of target proteins

	Dimension Grid Box	Grid Box
1YVB	70.21*-38.10*-84.35	40*60*40
2FOM	5.56*-22.04*2.45	40*40*40
2JK6	19.83*42.98*-2.05	60*60*60
2QG8	5.38*38.00*15.02	40*40*40
4ITY	13.66*14.59*-5.85	60*60*60
4PLT	-1.47*48.30*42.11	40*40*40
4ZCR	13.04*-55.22*255.38	40*40*40
5H6V	-8.20*10.59*-17.25	40*40*40
5TFR	30.03*-40.47*-10.19	40*40*40

The binding site was defined by the ligand coordinates in the target protein complex and tabulated in Table 1. Ligands were modeled as flexible, while the receptor remained rigid. AutoDock 1.1.2 generated 20 conformations per ligand-receptor complex, and the conformation with the highest binding affinity was selected for further analysis. Discovery Studio was used to visualize the docking results.

3. Results

The predominant family of bulbous plants in Turkey are Amaryllidaceae, Liliaceae, and Iridaceae, esteemed for their ecological roles and ornamental value (Arslan et al., 2008). In Turkey, the bulbs and tubers of geophytes are harvested from their natural environments and subsequently exported as ornamental plants, thereby playing a significant role in both local and international horticultural markets. However, this practice has raised critical conservation issues, as certain species are increasingly threatened with extinction due to over-collection and habitat degradation (Batı Ay, 2019). Particularly concerning are several species within the genus Galanthus, commonly referred to as snowdrops, which have been classified as endangered or vulnerable. These species hold specific conservation statuses, including Critically Endangered (CR), Endangered (EN), and Vulnerable (VU), as documented in the Red Book of Plants of Turkey.

The results of the HPLC study on the effects of zinc supplementation on lycorine and galanthamine reveal significant differences in their flowering and ripening periods. For lycorine, increasing zinc doses resulted in a flowering period of 7.74 kg/da at 2.5 kg/da, which decreased to 2.65 kg/da at 5 kg/da, and then increased to 4.56 kg/da at 10 kg/da, indicating a complex response to zinc levels. In contrast, galanthamine showed a more consistent increase in flowering period with zinc doses, starting at 11.53 kg/da for 2.5 kg/da and peaking at 21.61 kg/da for 5 kg/da, before dropping to 5.76 kg/da at 10 kg/da.

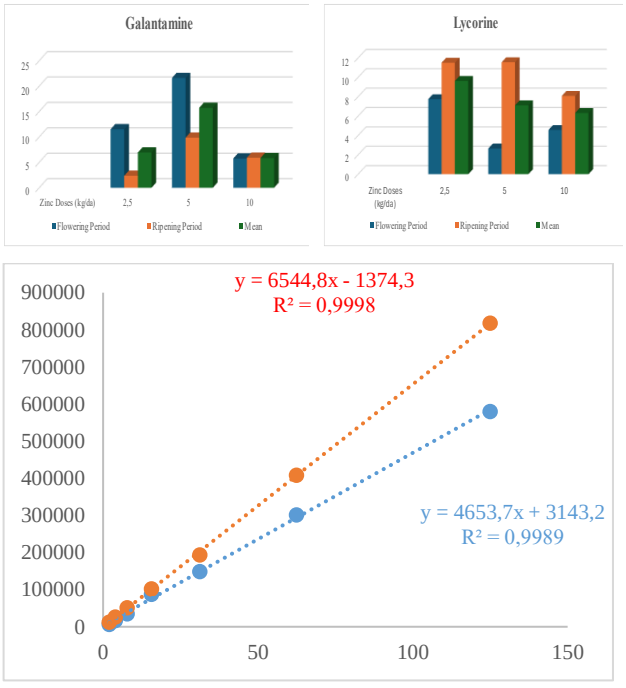


Fig. 1 Effects of zinc supplementation on the flowering and ripening periods of lycorine (blue), and galanthamine (red)

These findings suggest that zinc plays a crucial role in the biosynthesis of alkaloids in both plant species, potentially influencing their growth and development, which may have implications for their therapeutic efficacy and yield in medicinal applications (Figure 1).

Zinc was added as a nutritional supplement during the growth phase to increase the concentration of alkaloids in the selected plant species. The addition of zinc supports plant growth and promotes the activity of specific transcription factors that regulate alkaloid biosynthesis (Deng et al. 2018). Furthermore, the interaction between zinc and other nutrients can lead to synergistic effects that further enhance alkaloid production (Kamran et al. 2017). This approach underscores the importance of optimizing nutrient management in agricultural practice secondary metabolites, such as alkaloids, which have significant pharmacological properties and economic value.

Density Functional Theory (DFT)-based calculations play a crucial role in drug design by offering a detailed understanding of the electronic properties and reactivity of pharmacophoric groups. This knowledge is essential for optimizing the efficacy and safety of therapeutic agents. These calculations allow researchers to predict how potential drug candidates interact with biological targets, facilitating the identification of compounds with desirable pharmacokinetic and pharmacodynamic properties.

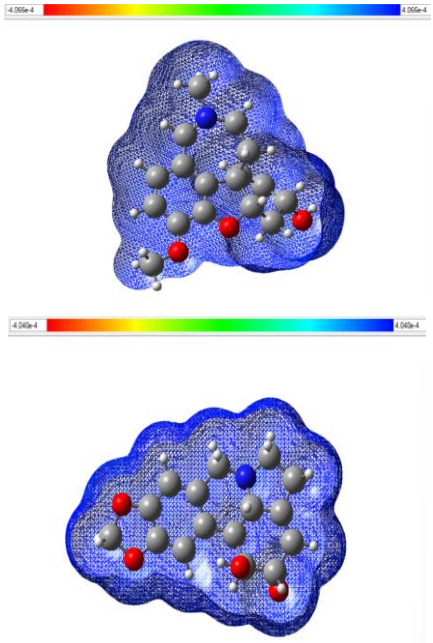


Fig. 2 Electron density map results of the galanthamine and lycorine

For instance, studies have demonstrated that DFT can be employed to analyze the molecular interactions and binding affinities of alkaloids, which are known for their diverse biological activities, including antiviral and antiparasitic effects (Painter et al. 2013, Flores-Holgúın et al. 2019). Moreover, integrating DFT calculations with molecular docking studies enhances the understanding of how specific structural features of alkaloids contribute to their biological activity. This approach enables the identification of key pharmacophoric elements that can be further optimized to improve drug-like properties, such as solubility, permeability, and metabolic stability, as assessed through ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis (Frau & Flores-Holgúın, 2019; Flores-Holgúın et al. 2019). Consequently, DFT-based methodologies are invaluable in rationalizing novel therapeutic agents targeting viral and parasitic diseases, ultimately leading to the development of more effective and safer pharmaceuticals.

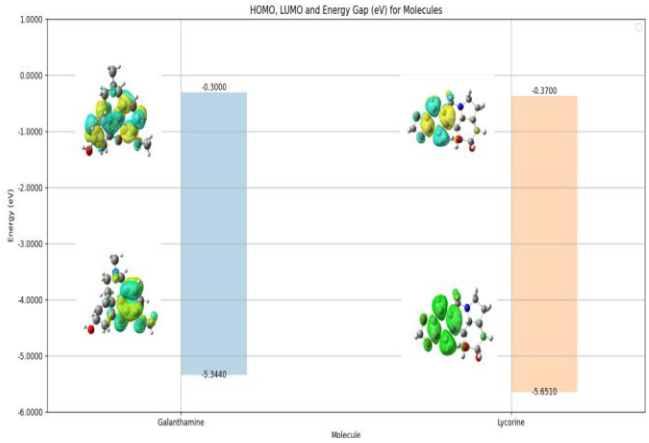


Fig. 3 FMO of galanthamine, and lycorine

To calculate the global reactivity values for galanthamine and lycorine based on their frontier molecular orbital (FMO) energies, we can utilize the energy gap (E_{gap}) derived from density functional theory (DFT) calculations via with the Gaussian 09 W package and GaussView 5.0 programmes (Dennington et al. 2009; Frisch et al. 2009). The energy gap is defined as the difference between the lowest unoccupied molecular orbital (LUMO) energy and the highest occupied molecular orbital (HOMO) energy (Khaled et al., 2022). This energy gap can be used to derive important global reactivity parameters such as electron affinity (EA), electronegativity (χ), chemical hardness (η), and chemical softness (S), which provide insights into the stability and reactivity of these alkaloids (Khelfaoui et al. 2020). The calculated HOMO and LUMO energies for galanthamine and lycorine (Figure 3) offer valuable information about their reactivity profiles, with the data indicating that lycorine has a smaller energy gap (Egap) of 5.282 eV compared to galanthamine's Egap of 5.314 eV. This smaller E_{gap} for lycorine suggests higher reactivity and lower stability, which may enhance its potential as a drug candidate.

The ADMET profiles obtained for galanthamine and lycorine reveal important pharmacokinetic and toxicity characteristics critical for their potential therapeutic applications (Figure 4). In the radar chart, the green color represents the minimum values a drug candidate can have, while the blue color indicates the possible maximum values. The yellow color represents the values for the selected alkaloids. The TPSA, logP, logS, hydrogen bond donor and acceptor numbers, and ring numbers for AAAS and AAAC alkaloids fall within the maximum and minimum ranges. However, the logD (octanol/water distribution) values for these alkaloids are lower than the minimum threshold.

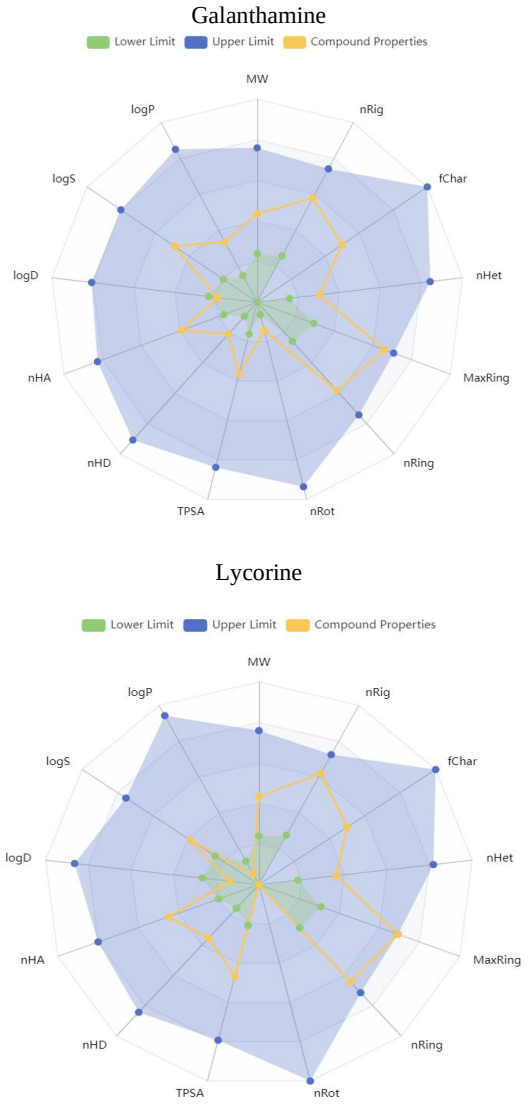


Fig. 4 Radar view of the pharmacokinetic properties

Galanthamine, with a TPSA of 41.93 Å² and a logP of 0.797, demonstrates favorable absorption and permeability, while its CLplasma of 6.176 ml/min/kg indicates moderate clearance. In contrast, lycorine exhibits a higher TPSA of 62.16 Å² and a lower logP of -0.268, suggesting differences in their distribution and solubility profiles. In terms of metabolic interactions, galanthamine is identified as a CYP2C19 substrate, whereas lycorine acts as a CYP2D6 inhibitor, which could influence their pharmacokinetic behavior and drug-drug interactions.

The half-life (T1/2) of galanthamine is significantly longer at 5.088 hours compared to lycorine's 2.987 hours, suggesting that galanthamine may provide more sustained therapeutic effects. Additionally, while both compounds show potential for drug-induced liver injury (DILI) and carcinogenicity, lycorine has a higher AMES toxicity score, indicating a greater risk of mutagenicity compared to galanthamine (Table 2).

In the molecular docking analysis for malaria, the binding affinities of galanthamine and lycorine were evaluated against the food vacuole protein (PDB: 1YVB; Wang, et al., 2006), with galanthamine exhibiting a binding energy of -6.09 kcal/mol and lycorine showing a stronger binding energy of -6.54 kcal/mol.

Both compounds formed hydrogen bonds with the amino acid residues MET(oa) and TYR(0C) for galanthamine, while lycorine interacted with SER111 and MET(0A), highlighting their distinct binding profiles; additionally, both compounds demonstrated π-alkyl interactions with PRO113, suggesting a favorable interaction with the target protein that may enhance their potential as therapeutic agents against malaria.

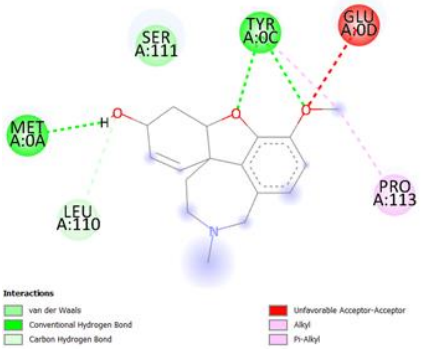
Table 2 Some ADMET parameters of the galanthamine (GAL) and lycorine (LYC)

	GAL	**LYC**	**Limit value**
TPSA	41.93	62.16	Opt:0-140
logP	0.797	-0.268	0-3
logS	-1.855	-2.767	-4
QED	0.801	0.69	Drug-likeness score >0.67
Caco-2 Permeability	-4.313	-5.148	<-5.15
F50%	0.1	0.1	50% bioavalibity
PPB	21.3%	64.15	Plasma protein binding <90%
BBB	0.9	0.9	Blood brain barrier
CYP2C19 substrate	0.7-0.9	-	-
CYP2D6 inhibitor	-	0.9-1	-
CLplasma	6.176	6.257	5-15 ml/min/kg moderate
T1/2	5.088	2.987	>4 short halflife drug 4-8 intermediate
AMES Toxicity	0.559	0.778	0 (-) 1(+)
DILI	0.461	0.426	Drug induced liver injury 0 (-) 1(+)
Carcino-genicity	0.726	0.817	0 (-) 1(+)
hERG Blockers	0.358	0.209	IC50<10μM

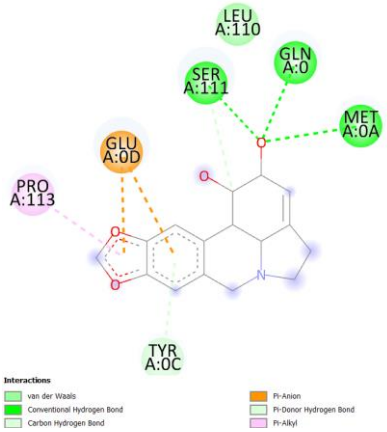
The interaction of galanthamine and lycorine with the acyl carrier protein synthase, which is essential (Figure 5) for the function of acyl carrier proteins in covalently binding nascent fatty acids during biosynthesis, was assessed using molecular docking studies. The Protein Data Bank entry PDB 2QG8 was utilized for this analysis (Nguyen, et al. 2022). The binding energies obtained were -5.29 kcal/mol for galanthamine and -4.98 kcal/mol for lycorine, indicating relatively low binding affinities. Given these values, it can be concluded that the interaction between these alkaloids and the acyl carrier protein synthase is insufficient to suggest an active role, thereby indicating that galanthamine and lycorine are unlikely to be effective in targeting this protein for therapeutic purposes.

The molecular docking analysis of dihydroorotate dehydrogenase, a crucial druggable target in the mitochondria (PDB: 4PLT, Boucher, et al., 2014), highlights its significant role in malaria pathogenesis, particularly in electron transport and protein synthesis.

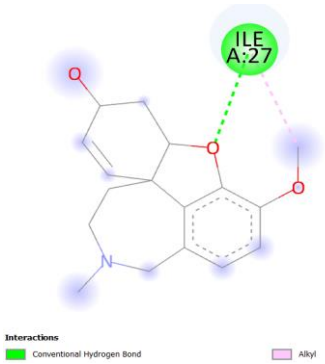
1YVB-Gal



1YVB-Lyc



2QG8-Gal



2QG8-Lyc

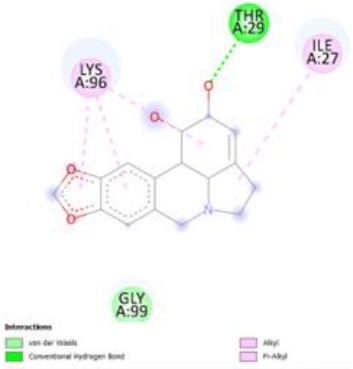
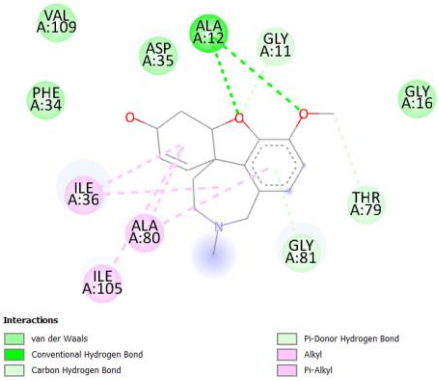
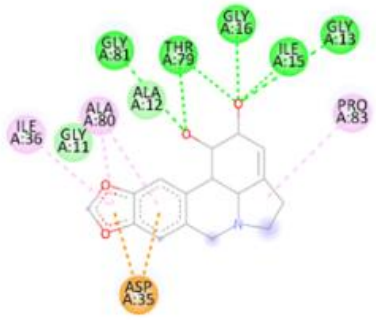


Fig. 5 2D visualization of the related Malaria disease proteins with the galanthamine (Gal), and lycorine (Lyc) compounds

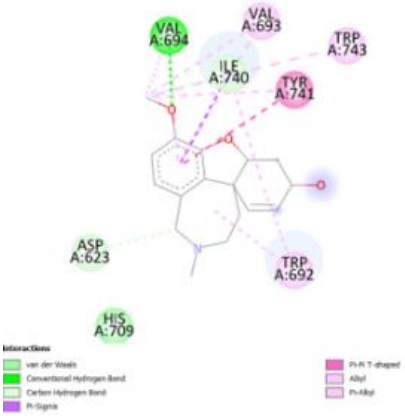
4PLT-Gal



4PLT-Lyc



4ZCR-Gal



4ZCR-Lyc

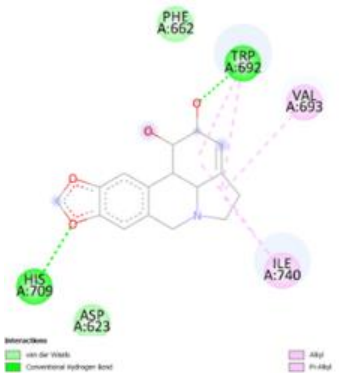


Fig. 6 2D visualization of the related Malaria disease proteins as PDB code 4PLT and 4ZCR with the galanthamine (Gal), and lycorine (Lyc) compounds

Lycorine exhibits a notably stronger binding affinity to this target, suggesting its potential as an effective therapeutic agent. This enhanced binding is supported by the electron density map from Gaussian calculations, confirming the structural compatibility of lycorine with the target protein. Additionally, key hydrogen bonds between lycorine and the residues Asn14, Ile15, and Asn127 were identified, indicating specific interactions that may enhance its efficacy in inhibiting the enzyme and disrupting malaria pathogenesis. The molecular docking analysis of dihydroorotate dehydrogenase, a crucial druggable target in the mitochondria (PDB: 4PLT, Boucher, et al., 2014), highlights its significant role in malaria pathogenesis, particularly in electron transport and protein synthesis. Lycorine exhibits a notably stronger binding affinity to this target, suggesting its potential as an effective therapeutic agent. This enhanced binding is supported by the electron density map from Gaussian calculations, confirming the structural compatibility of lycorine with the target protein. Additionally, key hydrogen bonds between lycorine and the residues Asn14, Ile15, and Asn127 were identified, indicating specific interactions that may enhance its efficacy in inhibiting the enzyme and disrupting malaria pathogenesis (Figure 6).

The molecular docking analysis targeting Plasmodium CCT, a crucial enzyme involved in malaria lipid biosynthesis, was conducted using the C-terminal catalytic domain of Plasmodium falciparum CTP:phosphocholine cytidylyltransferase (PDB: 4ZCR, Guca, et al. 2018).The results indicated that lycorine engages in π-alkyl interactions with the phenanthridine moiety of its structure, while also forming hydrogen bonds with the oxygen atoms present in the diol and dioxolo regions. In contrast, galanthamine establishes hydrogen bonds with the substituted oxygen atoms in its benzofuran framework, alongside π-alkyl interactions with the azepine component of its structure. These distinct binding interactions suggest that both alkaloids may effectively inhibit Plasmodium CCT, thereby contributing to their potential as therapeutic agents against malaria.

The molecular docking results targeting Zika virus protease (PDB: 5H6V) (Li, et al. 2017), and NS5 protein (PDB: 5TFR) (Upadhyay, 2017), indicate that lycorine exhibits a significantly stronger binding affinity compared to galanthamine. Specifically, the active binding site for lycorine is identified as the phenanthridine-1,2-diol moiety, which facilitates effective interactions with the target proteins. In contrast, galanthamine's binding involves the nitrogen atom in the azepine ring, which participates in hydrogen bond formation (Figure 7). These findings suggest that lycorine may serve as a more promising candidate for the development of therapeutic agents against Zika virus, given its enhanced binding efficacy and the critical role of these proteins in the viral lifecycle.

We thoroughly examined the interactions of alkaloids with the Dengue Virus NS2B/NS3 protease (PDB: 2FOM), a critical target for dengue viral disease.

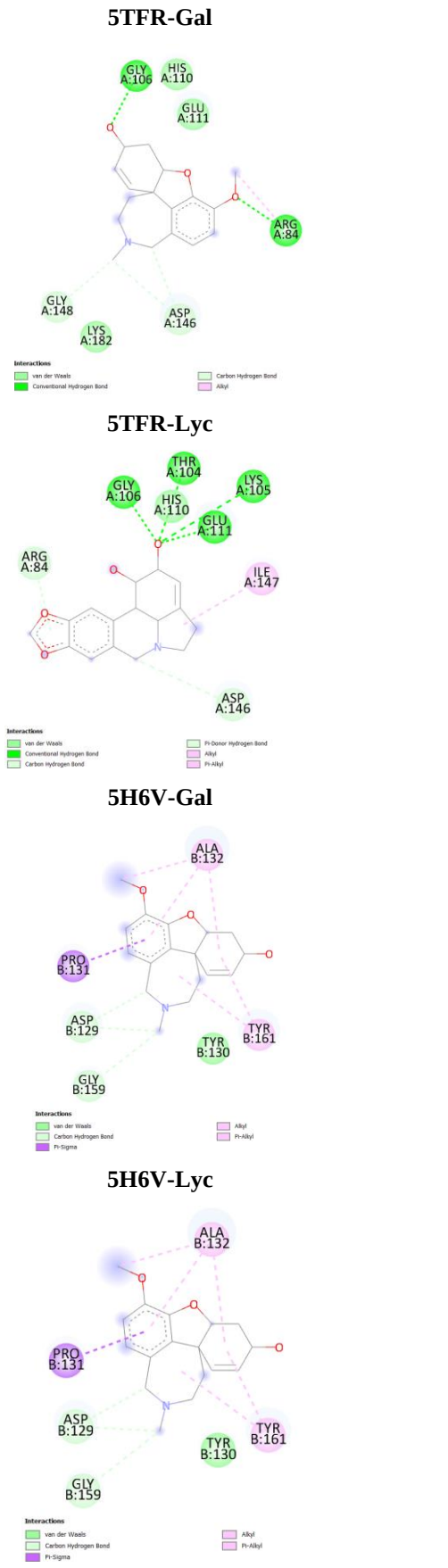


Fig. 7 Binding interaction visualization of the Zika virus protease and NS5 protein with the galanthamine (left), and lycorine (right) compounds

Lycorine exhibited a binding energy of -7.76 kcal/mol, indicating a stronger affinity than galanthamine, which had a binding energy of -6.45 kcal/mol (Figure 8). Galanthamine formed hydrogen bonds with Lys74 through its benzofuran ring and Ala164 and Asn152 via its hydroxy substituent. In contrast, lycorine established hydrogen bonds with several residues, including Val146, Gly87, Trp83, Leu149, and Asn152, in addition to interacting with the oxygen atoms present in its molecular structure (Erbel, et al. 2006).

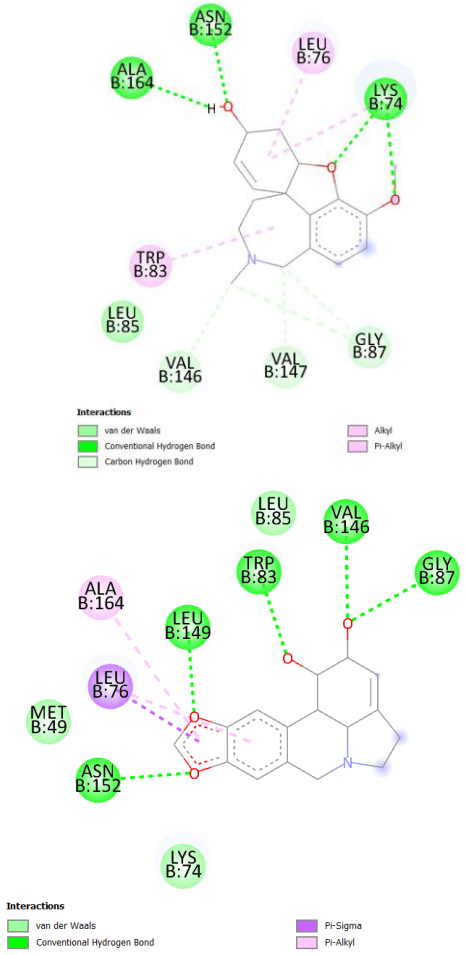


Fig. 8 Binding interaction visualization of target protein 2FOM with the galanthamine (top), and lycorine (bottom) compounds

The molecular docking analysis targeting arginase from the parasitic protozoa Leishmania, specifically Leishmania mexicana (PDB: 4ITY), (D'Antonio, et al., 2013), highlights its potential as a drug target for the treatment of leishmaniasis. The results indicate that lycorine exhibits a binding energy of -7.45 kcal/mol, demonstrating a stronger affinity than galanthamine, which has a binding energy of -6.10 kcal/mol. Both compounds interact with the active site of the enzyme, with lycorine forming hydrogen bonds with key residues such as Asn14, Ile15, and Asn127.

In contrast, galanthamine's binding involves hydrogen bonding with the nitrogen atom in the azepine ring (Figure 9).

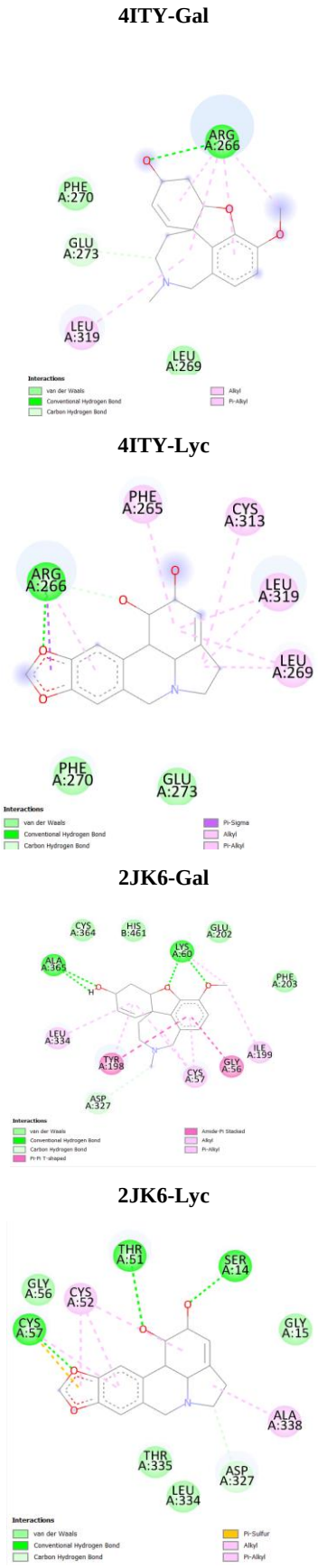


Fig. 9 Binding interaction visualization of target protein 4ITY and 2JK6 with the galanthamine (left), and lycorine (right) compounds

These findings suggest that lycorine may be a more effective inhibitor of Leishmania arginase, potentially disrupting polyamine biosynthesis essential for the parasite's growth and survival. The final docking results for the structure of Trypanothione Reductase from Leishmania infantum, a human protozoan parasite belonging to the Trypanosomatidae family, indicate significant binding affinities for the selected compounds. The protein structure analyzed is PDB: 2JK6 (Baiocco, et al. 2009), which exhibited the highest binding energy values among the target proteins evaluated. Specifically, lycorine demonstrated a binding energy of -8.76 kcal/mol, while galanthamine showed a binding energy of -8.49 kcal/mol. These findings suggest that both compounds have strong potential as inhibitors of Trypanothione Reductase, which may contribute to their efficacy in treating leishmaniasis.

4. Discussion

These results indicate that zinc as a nutritional supplement significantly enhances the concentration of alkaloids in selected plant species. This finding aligns with previous research demonstrating the role of zinc in promoting plant growth and its involvement in activating transcription factors that regulate alkaloid biosynthesis (Onyeonagu 2012). The synergistic effects observed when zinc is combined with other nutrients further emphasize the need to optimize nutrient management in agricultural practices to maximize the production of secondary metabolites, such as alkaloids, known for their pharmacological properties and economic significance (Helander et al. 2016).

Table 3 Some global reactivity parameters of the alkaloids

	Galanthamine	Lycorine
HOMO(eV)	-5.344	-5.651
LUMO(eV)	-0.30	-0.370
E_{gap}	5.314	5.282
(EA)*	0.30	0.370
(χ)*	2.687	3.010
(η)*	2.657	2.641
(S)*	0.188	0.189

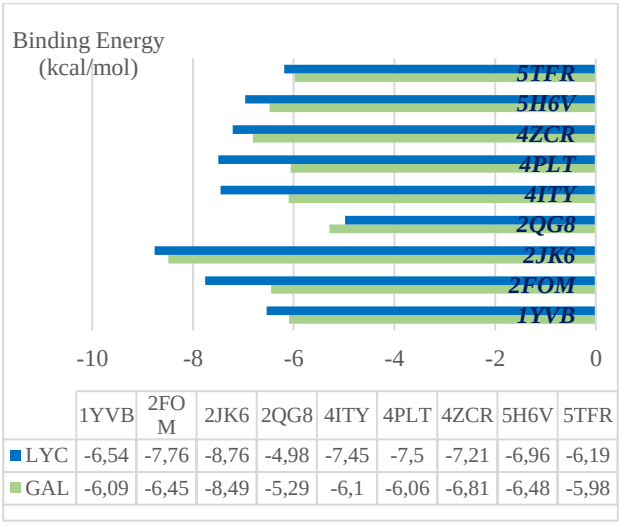
* I; ionization potential ($I = -E_{HOMO}$), A; electron affinity ($A = -E_{LUMO}$), χ;electronegativity ($\chi = (I + A)/2$), η;chemical hardness ($\eta = (I-A)/2$), S; chemical softness ($S = 1/2\eta$).

The integration of Density Functional Theory (DFT) calculations in the analysis of alkaloids provides valuable understanding of their electronic properties and reactivity, which are crucial for drug design (Jain et al. 2023). Furthermore, lycorine exhibits higher electron affinity (0.370 eV) and chemical softness (0.189) relative to galanthamine, which has an electron affinity of 0.030 eV and a chemical softness of 0.188. These properties indicate that lycorine has a greater ability to accept electrons and a more favourable interaction profile with biological targets (Table 3). Consequently, these findings suggest that both compounds may be effective as drug candidates, with lycorine particularly well-positioned for further exploration in treating viral and parasitic diseases.

Additionally, the electrostatic potential maps generated from these calculations can elucidate the interaction sites and binding affinities of these alkaloids, enhancing our understanding of their potential therapeutic applications.

The ADMET profiles of galanthamine and lycorine reveal significant pharmacokinetic and toxicity characteristics that are crucial for their potential therapeutic applications. Galanthamine demonstrates favorable absorption and permeability, with a TPSA of 41.93 Å² and a logP of 0.797, indicating its suitability for central nervous system (CNS) targeting, which is essential for its role as an acetylcholinesterase inhibitor in Alzheimer's disease treatment (Sancha et al., 2023). In contrast, lycorine presents a higher TPSA of 62.16 Å² and a lower logP of -0.268, suggesting challenges in its distribution and solubility profiles, which may limit its bioavailability (Bui, 2022). The pharmacokinetic parameters further differentiate the two compounds; galanthamine has a longer half-life (T1/2) of approximately 5.088 hours compared to lycorine's 2.987 hours, potentially allowing for more sustained therapeutic effects in clinical settings (Zhang et al., 2021).

The low AMES toxicity score of galanthamine indicates a reduced risk of mutagenic effects, thereby establishing a favorable safety profile for long-term administration (Shahid et al., 2016). This is particularly significant given the chronic nature of diseases such as Alzheimer's, which often necessitate prolonged therapeutic interventions, especially in vulnerable populations like the elderly. Consequently, this finding strongly supports the continued development of galanthamine as a therapeutic agent. Conversely, while lycorine has demonstrated promising anticancer efficacy, its toxicity profile warrants a comprehensive investigation (Ali et al., 2013). A thorough understanding of the underlying mechanisms of toxicity and the establishment of safe dosage ranges is imperative to ensure that the therapeutic benefits outweigh the potential adverse effects. In-depth investigations into the toxicity of lycorine using various in vitro and in vivo models can provide valuable insights into its safety profile and guide its development as a viable therapeutic option (Han et al., 2013).



	1YVB	2FOM	2JK6	2QG8	4ITY	4PLT	4ZCR	5H6V	5TFR
LYC	-6,54	-7,76	-8,76	-4,98	-7,45	-7,5	-7,21	-6,96	-6,19
GAL	-6,09	-6,45	-8,49	-5,29	-6,1	-6,06	-6,81	-6,48	-5,98

Fig. 10 Comparison of binding affinities of GAL and LYC alkaloids across selected target proteins

In the context of drug discovery, computational methods such as molecular docking have become instrumental in predicting the binding interactions between potential inhibitors and their target enzymes. The results presented indicate the binding affinities of galanthamine and lycorine compounds to the title proteins related to Zika virus, malaria, leishmaniasis, and dengue as represented by the PDB code 1YVB, 2FOM, 2JK6, 2QG8, 4ITY, 4PLT, 4ZCR, 5H6V, 5TFR with negative values reflecting favourable binding interactions.

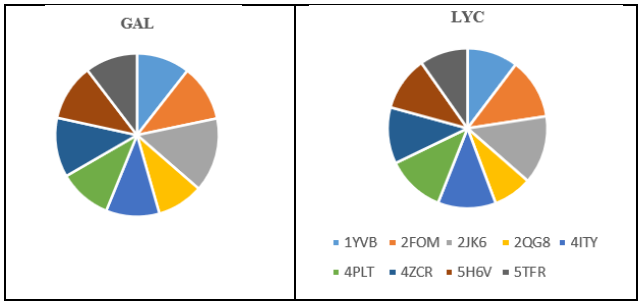


Fig. 11 General aspect of the GAL, LYC against target proteins

When evaluating the binding affinities of the selected alkaloids, galanthamine (GAL) and lycorine (LYC), against the target proteins, GAL demonstrated a more favorable binding profile, specifically against the target protein 2QG8, which is the apicoplast is a vital organelle in the malaria parasite *Plasmodium falciparum*, playing a crucial role in various metabolic processes essential for the parasite's survival and development. However, for all other proteins, LYC exhibited stronger binding affinities (Figure 10). As depicted in Figure 11, among the target proteins analyzed, the best binding was observed for the protein with the PDB code 2JK6.

5. Conclusion

This study demonstrates that zinc supplementation significantly enhances alkaloid biosynthesis in Galanthus elwesii, with optimal zinc doses of 2.5 kg/da for lycorine and 5 kg/da for galanthamine. The molecular docking studies conducted on various targets relevant to malaria, leishmania, and viral diseases such as Zika and Dengue provide significant information about the binding affinities and potential therapeutic applications of the alkaloids lycorine and galanthamine. The DFT analysis indicates that lycorine possesses a smaller energy gap (E_{gap}) of 5.282 eV compared to galanthamine's 5.314 eV, suggesting greater reactivity and potential as a therapeutic agent. While both alkaloids exhibit promising binding affinities, particularly to Trypanothione Reductase from Leishmania infantum (PDB: 2JK6), lycorine demonstrates superior binding energy of -8.76 kcal/mol, indicating its potential effectiveness against leishmaniasis. Conversely, galanthamine, with a longer half-life (T1/2) of 5.088 hours compared to lycorine's 2.987 hours, and favorable ADMET properties—such as a TPSA of 41.93 Å² and a logP of 0.797—remains a strong candidate for therapeutic applications, particularly in conditions requiring sustained effects. Overall, these findings underscore the therapeutic promise of both alkaloids, warranting further investigation into their roles in treating viral and parasitic diseases.

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Authors' contributions:

M.G: Writing – review & editing, Writing an original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. E.B.A: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation.

Conflict of interest disclosure:

The authors confirm that there are no financial or personal relationships that could have influenced the research or findings presented in this paper.

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