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IN SILICO EVALUATION OF ANTICANCER ACTIVITY, DRUG-LIKENESS, PHARMACOKINETIC PROPERTIES, AND TOXICITY PROFILES OF ALKALOID COMPOUNDS FROM *Toddalia asiatica* Lamk

VALUASI *IN SILICO* AKTIVITAS ANTIKANKER, KEMIRIPAN OBAT (*DRUG-LIKENESS*), SIFAT FARMAKOKINETIK, DAN PROFIL TOKSISITAS SENYAWA ALKALOID DARI *Toddalia asiatica* Lamk

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Abstract

Toddalia asiatica Lamk is a medicinal plant known to contain various bioactive secondary metabolites, particularly alkaloids, with potential pharmacological activities. This study aimed to evaluate the anticancer potential, drug-likeness, pharmacokinetic properties, medicinal chemistry characteristics, and toxicity profiles of bioactive compounds from *Toddalia asiatica* using in silico approaches, including PASS prediction, SwissADME, and pkCSM analysis. PASS prediction results demonstrated that all studied compounds exhibited potential antineoplastic and apoptosis-inducing activities with varying probabilities. Norchelerythrine showed the highest antineoplastic activity, while several other alkaloids, such as dihydrochelerythrine and dihydroavicine, also demonstrated strong anticancer potential. SwissADME analysis revealed that all compounds met Lipinski's Rule of Five, the Ghose Filter, the Veber rule, the Egan rule, and the Muegge rule, indicating favorable drug-likeness and oral bioavailability potential. The compounds also showed moderate water solubility, high gastrointestinal absorption, and good membrane permeability, supporting their potential as oral drug candidates. Pharmacokinetic evaluation indicated relatively good distribution profiles, moderate tissue distribution, and low-to-moderate blood-brain barrier permeability, which may reduce the risk of neurological side effects. Medicinal chemistry analysis showed no PAINS or BRENDA alerts, suggesting that the compounds are free of problematic chemical fragments and exhibit good lead-likeness characteristics. Synthetic accessibility values ranged from moderate to relatively easy, indicating feasibility for chemical synthesis and optimization. Toxicity prediction revealed moderate toxicity profiles with acceptable maximum tolerated doses, relatively low acute toxicity, and manageable chronic toxicity risks. Although several compounds showed potential aquatic toxicity, the overall safety profiles remained acceptable for early-stage drug development. Among all compounds, norchelerythrine and dihydroavicine demonstrated the most promising combination of biological activity and pharmacokinetic properties. Overall, the findings suggest that alkaloid compounds from *Toddalia asiatica* possess strong potential as natural product-based anticancer drug candidates. However, further in vitro and in vivo studies are required to validate their biological activity, pharmacokinetics, and safety before clinical application.

Keywords: Toddalia asiatica, *alkaloids, anticancer, SwissADME, pkCSM, drug-likeness*

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Abstrak

Toddalia asiatica Lamk. merupakan tanaman obat yang dikenal mengandung berbagai metabolit sekunder bioaktif, khususnya alkaloid, yang memiliki potensi aktivitas farmakologis. Penelitian ini bertujuan untuk mengevaluasi potensi antikanker, kemiripan sifat obat (*drug-likeness*), sifat farmakokinetik, karakteristik kimia medisinal, dan profil toksisitas senyawa bioaktif dari *Toddalia asiatica* menggunakan pendekatan *in silico*, termasuk prediksi PASS, SwissADME, dan analisis pkCSM.Hasil prediksi PASS menunjukkan bahwa semua senyawa yang diteliti memiliki potensi aktivitas antineoplastik (antikanker) dan pemicu apoptosis dengan probabilitas yang bervariasi. Norchelerythrine menunjukkan aktivitas antineoplastik tertinggi, sementara beberapa alkaloid lain, seperti dihydrochelerythrine dan dihydroavicine, juga menunjukkan potensi antikanker yang kuat. Analisis SwissADME mengungkapkan bahwa semua senyawa memenuhi Aturan Lima Lipinski (*Lipinski's Rule of Five*), Filter Ghose, aturan Veber, aturan Egan, dan aturan Muegge, yang menunjukkan sifat *drug-likeness* yang baik serta potensi bioavailabilitas oral. Senyawa-senyawa tersebut juga menunjukkan kelarutan dalam air yang sedang, penyerapan gastrointestinal (pencernaan) yang tinggi, serta permeabilitas membran yang baik, sehingga mendukung potensinya sebagai kandidat obat oral. Evaluasi farmakokinetik menunjukkan profil distribusi yang relatif baik, distribusi jaringan yang sedang, serta permeabilitas sawar darah otak yang rendah hingga sedang, sehingga dapat mengurangi risiko efek samping neurologis (saraf). Analisis kimia medisinal menunjukkan tidak adanya peringatan PAINS atau Brenk. Hal ini mengindikasikan bahwa senyawa-senyawa tersebut bebas dari fragmen kimia yang bermasalah dan memiliki karakteristik *leadlikeness* yang baik. Nilai aksesibilitas sintesis (*synthetic accessibility*) berkisar dari sedang hingga relatif mudah, menunjukkan kelayakan untuk sintesis kimia dan optimasi. Prediksi toksisitas menunjukkan profil toksisitas sedang dengan dosis toleransi maksimum yang dapat diterima, toksisitas akut yang relatif rendah, serta risiko toksisitas kronis yang dapat dikelola. Meskipun beberapa senyawa menunjukkan potensi toksisitas akuatik (terhadap lingkungan air), profil keamanan secara keseluruhan tetap dapat diterima untuk pengembangan obat pada tahap awal.Di antara semua senyawa, norchelerythrine dan dihydroavicine menunjukkan kombinasi aktivitas biologis dan sifat farmakokinetik yang paling menjanjikan. Secara keseluruhan, temuan ini menunjukkan bahwa senyawa alkaloid dari *Toddalia asiatica* memiliki potensi kuat sebagai kandidat obat antikanker berbasis produk alam. Namun, penelitian *in vitro* dan *in vivo* lebih lanjut tetap diperlukan untuk memvalidasi aktivitas biologis, farmakokinetik, dan keamanan sebelum diaplikasikan secara klinis.

Kata Kunci: *Toddalia asiatica*, alkaloid, antikanker, SwissADME, pkCSM, *drug-likeness*

INTRODUCTION

Cancer remains one of the leading causes of death worldwide and continues to represent a major global health challenge. The disease is characterized by uncontrolled cell proliferation resulting from genetic mutations and abnormalities in cellular regulatory mechanisms. Despite significant advances in modern medicine, the incidence and mortality rates of cancer continue to increase annually, particularly in developing countries. Conventional cancer therapies such as chemotherapy, radiotherapy, and surgery are often associated with severe side effects, drug resistance, high treatment costs, and damage to healthy tissues. Therefore, the discovery and development of safer, more effective, and selective anticancer agents remain a major focus in pharmaceutical and biomedical research (Ioele *et al*., 2022).

Natural products derived from medicinal plants have long been recognized as valuable sources of therapeutic agents. More than half of the currently available anticancer drugs are

directly or indirectly derived from natural compounds. Plant secondary metabolites, especially alkaloids, flavonoids, terpenoids, and phenolic compounds, possess diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Among these groups, alkaloids have attracted significant scientific attention for their potent cytotoxic activity against various cancer cell lines and their ability to interfere with multiple molecular pathways involved in tumor progression (Chidananda *et al*., 2025). Alkaloid compounds can induce apoptosis, inhibit angiogenesis, suppress metastasis, disrupt DNA replication, and interfere with cell cycle progression in cancer cells. These mechanisms make alkaloids promising candidates for the development of novel anticancer drugs (Chidananda *et al*., 2025).

One medicinal plant that has gained increasing interest in pharmacological studies is *Toddalia asiatica* Lamk, commonly known as the orange climber plant. This plant belongs to the Rutaceae family and is widely distributed in tropical and subtropical regions of Asia and Africa. Traditionally, *Toddalia asiatica* has been used in folk medicine to treat fever, malaria, cough, inflammation, stomach disorders, rheumatism, and microbial infections. Previ henanthridine compounds, and essential oils with significant pharmacological activities. Phytochemical investigations have revealed that this plant contains various bioactive secondary metabolites, including alkaloids, coumarins, quinoline derivatives, benzop (Sharifi-Rad *et al*., 2021). Several studies have also reported that alkaloid compounds isolated from *Toddalia asiatica* exhibit antimicrobial, antioxidant, anti-inflammatory, and cytotoxic properties, indicating their potential as therapeutic agents for cancer treatment (Hu *et al*., 2014).

Recent advances in computational biology and bioinformatics have accelerated the drug discovery process through the application of in silico methods. Computational approaches allow researchers to predict biological activity, pharmacokinetic properties, toxicity, and drug-likeness of compounds before conducting laboratory experiments. These methods reduce research costs, shorten development time, and improve the efficiency of selecting promising drug candidates. In silico analysis has become an essential strategy in early-stage pharmaceutical research, particularly for natural product-based compounds that possess large structural diversity and complex molecular characteristics (Chang *et al*., 2022).

One widely used computational tool for predicting biological activity is the Prediction of Activity Spectra for Substances (PASS) online server. PASS predicts the probability of biological activity based on the structural similarity of compounds with known bioactive molecules. The platform has been extensively used to identify compounds with anticancer, anti-inflammatory, antimicrobial, and enzyme-inhibitory activities. Compounds with high probability of activity (Pa) values are considered to possess greater pharmacological potential and are therefore prioritized for further investigation (Druzhilovskii *et al*., 2017). In addition to biological activity prediction, evaluation of drug-likeness is also essential in determining whether a compound possesses suitable physicochemical characteristics for drug development. Lipinski's Rule of Five, Ghose Filter, Veber rule, Egan rule, and Muegge rule are commonly used parameters to assess oral bioavailability, membrane permeability, molecular stability, and overall suitability as drug candidates (Lipinski, 2004).

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Pharmacokinetic and toxicity evaluations are equally important in the drug development process. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties determine the behavior of compounds within the human body and influence their therapeutic effectiveness and safety. Computational tools such as SwissADME and pkCSM are widely used to predict these parameters accurately and efficiently. SwissADME provides information regarding physicochemical properties, medicinal chemistry parameters, and pharmacokinetic profiles, whereas pkCSM predicts absorption characteristics, tissue distribution, clearance, and potential toxicity based on graph-based molecular signatures (Daina *et al*., 2017). Early prediction of ADMET properties is highly important because many drug candidates fail during clinical trials due to poor pharmacokinetic performance or unacceptable toxicity profiles (Purwaningsih *et al*., 2025).

Although *Toddalia asiatica* has been traditionally used as a medicinal plant and several of its bioactive compounds have demonstrated promising pharmacological activities, comprehensive in silico evaluation of its alkaloid compounds as anticancer drug candidates remains limited. Therefore, this study aimed to investigate the biological activity, drug-likeness, pharmacokinetic characteristics, medicinal chemistry properties, and toxicity profiles of bioactive compounds from *Toddalia asiatica* using PASS, SwissADME, and pkCSM analyses. The findings of this study are expected to provide scientific insight into the therapeutic potential of *Toddalia asiatica* metabolites and support the development of natural product-based anticancer agents for future pharmaceutical applications (Zeng *et al*., 2021).

RESEARCH METHODS

This study employed an in silico approach to evaluate the biological activity, drug-likeness, pharmacokinetic properties, medicinal chemistry characteristics, and toxicity profiles of bioactive compounds derived from the orange climber plant (*Toddalia asiatica* Lamk). The analysis focused on alkaloid compounds reported as major secondary metabolites with potential pharmacological activity, particularly as anticancer agents. Computational methods were selected because they provide rapid, cost-effective, and reliable preliminary screening during the early stages of drug discovery and development (Sliwoski *et al*., 2014).

The chemical structures of the selected compounds, including norchelerythrine, dihydrochelerythrine, dihydroavicine, dihydronitidine, arnottianamide, and 6-methoxy dihydrochelerythrine, were obtained from publicly available chemical databases in Structure Data File (SDF) format. The structures were subsequently converted into canonical SMILES format using cheminformatics software for further computational analysis. SMILES notation was used because it allows efficient molecular representation and compatibility with various bioinformatics platforms (Han *et al*., 2025).

Prediction of biological activities was performed using the Prediction of Activity Spectra for Substances (PASS) online server. PASS analysis was conducted to assess the probability that compounds exhibit antineoplastic and apoptosis-inducing activities. The prediction results were expressed as probability-of-activity (Pa) values, where higher Pa values indicate greater potential

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biological activity. Compounds with Pa values greater than 0.3 were considered to possess promising pharmacological potential (Pogodin *et al*., 2018).

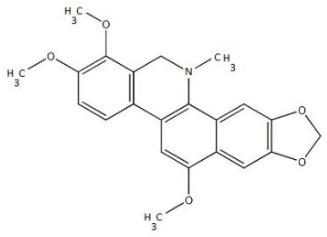
Drug-likeness, physicochemical properties, medicinal chemistry parameters, and pharmacokinetic profiles were analyzed using the SwissADME web server. The evaluated parameters included Lipinski's Rule of Five, Ghose Filter, Veber rule, Egan rule, and Muegge rule, as well as gastrointestinal absorption, blood-brain barrier permeability, water solubility, bioavailability score, PAINS alert, Brenk alert, leadlikeness, and synthetic accessibility. These parameters are widely used to predict oral bioavailability, membrane permeability, molecular stability, and suitability of compounds as drug candidates (Purwaningsih *et al*., 2025).

Pharmacokinetic and toxicity predictions were further analyzed using the pkCSM online platform. The evaluated pharmacokinetic parameters included absorption, distribution, metabolism, excretion, and toxicity (ADMET). Absorption analysis included water solubility, Caco-2 permeability, intestinal absorption, and skin permeability. Distribution parameters included volume of distribution steady state (VDss), fraction unbound (Fu), blood-brain barrier permeability (Log BB), and central nervous system permeability (Log PS). Excretion analysis evaluated total clearance and renal OCT2 substrate status. Toxicity prediction included maximum tolerated dose (MTD), oral rat acute toxicity (LD50), chronic toxicity (LOAEL), Tetrahymena pyriformis toxicity, and minnow toxicity. PKCSM analysis is commonly used in modern computational pharmacology to predict the safety and pharmacokinetic behavior of candidate compounds before experimental validation (Pires *et al*., 2015).

All data obtained were analyzed descriptively by comparing the predicted values for each compound against standard criteria commonly used in pharmaceutical development. The interpretation focused on identifying compounds with the most favorable biological activity, pharmacokinetic profile, medicinal chemistry properties, and safety characteristics as potential natural anticancer drug candidates from *Toddalia asiatica* (Oresanya *et al*., 2026).

RESULT AND DISCUSSION

Table 1. Structural Images of Compounds from the Orange Climber Plant (*Toddalia asiatica* LAMK)

No	Compound	Chemical Structure Image
1	6-Methoxydihydrochelerythrine	

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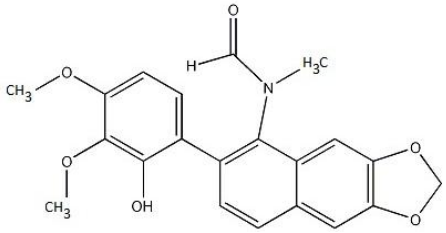
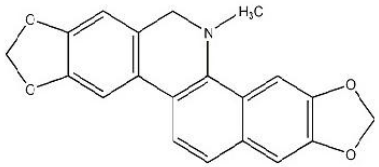
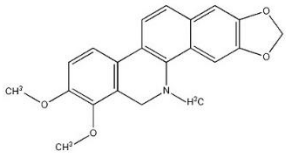
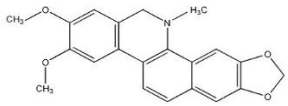
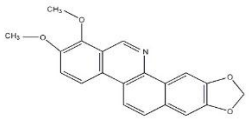
No	Compound	Chemical Structure Image
2	Arnottianamide	
3	Dihydroavicine	
4	Dihydrochelerythrine	
5	Dihydronitidine	
6	Norchelerythrine	

Table 2. SMILES (Simplified Molecular Input Entry System) Akar Kucing (*Todddalia Asiatica LAMK*)

No	Compound	SMILES
1	6-Methoxydihydrochelerythrine	COc1ccc2c(c1OC)CN(C)c1c-2cc(OC)c2cc3c(cc12)OCO3
2	Arnottianamide	COc1ccc(-c2ccc3cc4c(cc3c2N(C)C=O)OCO4)c(O)c1OC
3	Dihydroavicine	CN1Cc2cc3c(cc2-c2ccc4cc5c(cc4c21)OCO5)OCO3
4	Dihydrochelerythrine	COc1ccc2c(c1OC)CN(C)c1c-2ccc2cc3c(cc12)OCO3
5	Dihydronitidine	COc1cc2c(cc1OC)-c1ccc3cc4c(cc3c1N(C)C2)OCO4
6	Norchelerythrine	COc1ccc2c(cnc3c4cc5c(cc4ccc23)OCO5)c1OC

Table 3. Antineoplastic and Apoptosis of the Orange Climber Plant (*Toddalia asiatica* LAMK) using Online *PAST*

Nu	Compound	Activity (% probabilitasaktif)	
		Antineoplastic	Apoptosis
1	6-Methoxydihydrochelerythrine	0,307	0,465
2	Arnottianamide	0,493	0,465
3	Dihydroavicine	0,52 (alkaloid)	0,313
4	Dihydrochelerythrine	0,527 (alkaloid)	0,44

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Nu	Compound	Activity (% probabilitasaktif)	
		Antineoplastic	Apoptosis
5	Dihydronitidine	0,515 (alkaloid)	0,386
6	Norchelerythrine	0,626 (colorectal cancer)	0,462

Table 4. Lipinski's Rule of Five Drug-Likeness of the Orange Climber Plant (*Toddalia asiatica* LAMK) Using SwissADME

Nu	Compound	Molecular Weight (MW)	LogP	Hydrogen Bond Donor (HBD)	Rogen Bobd Acceptor (HBA)	Topological Polar Surface (TPSA)	Rottable Bonds
		≤ 500 Da	≤ 5	≤ 5	≤ 10	≤ 140 Å²	≤ 10
1	6-Methoxydihydrochelerythrine	379.14	4.309	0.0	6.0	49.39	3.0
2	Arnottianamide	381.12	3.125	1.0	7.0	77.46	5.0
3	Dihydroavicine	333.1	4.594	0.0	5.0	40.16	0.0
4	Dihydrochelerythrine	349.13	4.221	0.0	5.0	40.16	2.0
5	Dihydronitidine	349.13	4.171	0.0	5.0	40.16	2.0
6	Norchelerythrine	333.1	3.908	0.0	5.0	49.81	2.0

Table 5. Drug-Likeness (Ghose Filter, Veber, Egan, and Muegge Rules) of the Orange Climber Plant (*Toddalia asiatica* LAMK) Using SwissADME

Nu	Compound	Ghose Filter	Veber rule	Egan Ryule	Muegge rule	Bioavinbility
1	6-Methoxydihydrochelerythrine	yes	yes	yes	yes	0.55
2	Arnottianamide	yes	yes	yes	yes	0.55
3	Dihydroavicine	yes	yes	yes	yes	0.55
4	Dihydrochelerythrine	yes	yes	yes	yes	0.55
5	Dihydronitidine	yes	yes	yes	yes	0.55
6	Norchelerythrine	yes	yes	yes	yes	0.55

Table 6. Water Solubility and Pharmacokinetics of the Orange Climber Plant (*Toddalia asiatica* LAMK) Using SwissADME

Nu	Compound	Water Solubility	Pharmacokinetics	Egan Rule	Muegge Rule
		Log. *S*(ESOL)	GI Absorption	BBB Permeant	P-gp Substrate
1	6-Methoxydihydrocheleryth rine	-4.12 (Moderately soluble)	High	No	Yes
2	Arnottianamide	-3.45 (Moderately soluble)	High	No	Yes
3	Dihydroavicine	-3.89 (Moderately soluble)	High	No	Yes
4	Dihydrochelerythrine	-3.97 (Moderately soluble)	High	No	Yes
5	Dihydronitidine	-4.20 (Moderately soluble)	High	No	Yes
6	Norchelerythrine	-3.78 (Moderately	High	No	Yes

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soluble)

Table 7. Medicinal Chemistry of the Orange Climber Plant (*Toddalia asiatica* LAMK) Using SwissADME

Nu	Compound	PAINS	Brenk	Leadlikeness	Synthetic Accebility
1	6-Methoxydihydrochelerythrine	0 alert	0 alert	Yes (1 violation)	3.85
2	Arnottianamide	0 alert	0 alert	Yes (1 violation)	3.42
3	Dihydroavicine	0 alert	0 alert	Yes (1 violation)	3.67
4	Dihydrochelerythrine	0 alert	0 alert	Yes (1 violation)	3.71
5	Dihydronitidine	0 alert	0 alert	Yes (1 violation)	3.90
6	Norchelerythrine	0 alert	0 alert	Yes (1 violation)	3.55

Table 8. Absorption of the Orange Climber Plant (*Toddalia asiatica* LAMK) using pkCSM

Nu	Compound	Water Solubility (Log mol/L)	Caco-2 Permeability(log Papp in 10^{-6} cm/s)	Intestinal Absorbtion(Human) (% Absorbed)	Skin Permeability (log Kp)
1	6-Methoxydihydrochelerythrine	-5.203	1.052	99.92	-2.714
2	Arnottianamide	-4.857	1.028	95.983	-2.757
3	Dihydroavicine	-5.792	2.037	98.084	-2.69
4	Dihydrochelerythrine	-5.465	1.028	99.229	-2.673
5	Dihydronitidine	-5.507	1.046	98.342	-2.663
6	Norchelerythrine	-5.201	1.338	98.884	-2.726

Table 9. Distribution of the Orange Climber Plant (*Toddalia asiatica* LAMK) using pkCSM

Nu	Compound	VDss (Log L/Kg)	Fraction unbound (Human)(Fu)	BBB Permeability (Log BB)	CNS Permeability (Log PS)
1	6-Methoxydihydrochelerythrine	-0.237	0.152	-0.278	-2.035
2	Arnottianamide	-0.389	0.034	-0.765	-2.984
3	Dihydroavicine	0.178	0.159	-0.033	-0.033
4	Dihydrochelerythrine	0.069	0.144	-0.012	-1.511
5	Dihydronitidine	0.089	0.104	-0.07	-1.451
6	Norchelerythrine	-0.341	0.258	0.252	-1.884

Table 10. Excretion of the Orange Climber Plant (*Toddalia asiatica* LAMK) Using pkCSM

No	Compound	Total Clearance (Log ml/min/kg)	Renal OCT2 Subrate
1	6-Methoxydihydrochelerythrine	0.342	No
2	Arnottianamide	0.181	No
3	Dihydroavicine	0.02	No

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No	Compound	Total Clearance (Log ml/min/kg)	Renal OCT2 Subrate
4	Dihydrochelerythrine	0.263	No
5	Dihydronitidine	0.201	No
6	Norchelerythrine	0.186	No

Table 11. Toxicity of the Orange Climber Plant (*Toddalia asiatica* LAMK) Using pkCSM

Nu	Compound	Max. Tolerated dose (human) (log mg/kg/day)	Oral Rat Acute Toxicity (LD50) (mol/kg)	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	T. Pyriforms Toxicity (log ug/L)	Minnow toxicity (log mM)
1	6-Methoxydihydrochelerythrine	-0.018	2.919	0.697	0.316	-0.713
2	Arnottianamide	0.272	2.085	0.811	0.333	-1.051
3	Dihydroavicine	0.138	2.377	1.567	0.341	0.242
4	Dihydrochelerythrine	-0.045	2.633	0.625	0.35	-1.091
5	Dihydronitidine	0.036	2.699	0.756	0.363	-1.252
6	Norchelerythrine	0.462	2.704	0.452	0.289	-1.311

The results of biological activity prediction using PASS Online indicate that all secondary metabolites from Toddalia asiatica Lamk. have potential as anticancer agents through antineoplastic and apoptosis-inducing activities, with varying probabilities. Most of the compounds belong to the alkaloid group, which is known to inhibit DNA synthesis, disrupt the cell cycle, increase reactive oxygen species (ROS) formation, and activate the apoptotic pathway in cancer cells. Among all compounds, norchelerythrine showed the highest antineoplastic activity (Pa = 0.626), followed by dihydrochelerythrine (Pa = 0.527) and dihydroavicine (Pa = 0.520). These compounds are thought to work by inhibiting cancer cell proliferation, activating caspases, and inducing the intrinsic apoptotic pathway. Meanwhile, arnottianamide and 6-methoxydihydrochelerythrine showed relatively high apoptotic activity, suggesting they have the potential to be more dominant at triggering cancer cell death than at inhibiting proliferation. These findings indicate that *T. asiatica* alkaloids have potential as natural anticancer drug candidates, although validation through in vitro and in vivo testing is still required (Seca & Pinto, 2018; Gaziano *et al*., 2016; Mendoza-Calderón *et al*., 2026; Chunarkar-Patil *et al*., 2024).

SwissADME analysis showed that all compounds met Lipinski's Rule of Five, with molecular weights below 500 Da, LogP values <5, TPSA <140 Å², and an appropriate number of hydrogen-bond donors and acceptors. These characteristics indicate that all compounds have good oral absorption, permeability, and potential for bioavailability. Furthermore, all compounds also met the Ghose Filter, Veber Rule, Egan Rule, and Muegge Rule, and had a bioavailability score of 0.55, indicating a high potential for development as oral drugs. The low TPSA value and low number of rotatable bonds support molecular stability and the ability to penetrate biological membranes. Therefore, T. asiatica alkaloid compounds possess physicochemical characteristics

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that meet the initial requirements for developing natural product-based drug candidates (Veber *et al*., 2002; Bickerton *et al*., 2012; Martin, 2005; Khalil *et al*., 2026; Matei & Visan, 2025).

Pharmacokinetic analysis indicates that all compounds exhibit moderate water solubility, high gastrointestinal (GI) absorption, and good Caco-2 permeability, thereby supporting their potential as oral agents. All compounds are predicted to be unable to penetrate the blood-brain barrier (BBB) and are substrates of P-glycoprotein (P-gp), resulting in more focused distribution in peripheral tissues and potentially reducing neurological side effects. Distribution analysis also showed that dihydroavicin, dihydrochelerythrine, and dihydronitidine had broader tissue distribution, while norchelerythrine had the highest free fraction, potentially providing optimal biological activity. In terms of excretion, all compounds exhibited low to moderate total clearance values and were not OCT2 substrates, indicating a relatively stable elimination profile with a lower risk of renal drug interactions (Dahan & Miller, 2012; Liu *et al*., 2010; Elmeliegy *et al*., 2020; Berry *et al*., 2011; Celestin & Musteata, 2021; Kido et al., 2011; Serrano *et al*., 2024).

Medicinal chemistry evaluation revealed that all compounds exhibited neither PAINS nor Brenk alerts, thus making it unlikely that they would produce spurious biological activity or pose toxicity issues due to their chemical structure. All compounds also met the leadlikeness criteria, with only one minor violation, and had synthetic accessibility values between 3.42 and 3.90, indicating that these compounds are still relatively easy to synthesize and modify during the drug optimization stage. These results further strengthen the prospects of T. asiatica alkaloids as lead compounds in the development of natural-based anticancer drugs (Capuzzi *et al*., 2017; Dzobo, 2022; Ertl & Schuffenhauer, 2009; Purwaningsih *et al*., 2025).

Toxicity prediction using pkCSM indicated that all compounds had moderate toxicity profiles and remained within tolerable limits. Norchelerythrine had the highest maximum tolerated dose (MTD) value, while 6-methoxydihydrochelerythrine showed the lowest acute toxicity based on the LD50 value. Dihydroavicine also had the best profiles for chronic and environmental toxicity compared with other compounds. Although some compounds were predicted to be toxic to aquatic organisms, the overall analysis results support the development of Toddalia asiatica secondary metabolites as safe natural anticancer drug candidates. However, further toxicity, pharmacokinetic, and efficacy testing through in vitro, in vivo, and preclinical studies are needed before they can be applied as modern drugs (Koshman *et al*., 2024; Denny & Stewart, 2024; Ponepal *et al*., 2024; Pastorino *et al*., 2024).

CONCLUSION

The in silico analyses demonstrated that bioactive alkaloid compounds from *Toddalia asiatica* possess promising anticancer potential, favorable drug-likeness, good pharmacokinetic properties, moderate toxicity, and acceptable medicinal chemistry characteristics. These findings support their potential development as natural oral anticancer drug candidates, although further in vitro and in vivo studies remain necessary.

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