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IN SILICO TOXICITY EVALUATION OF NOVEL COMPOUNDS ISOLATED FROM WITHANIA SOMNIFERA

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ABSTRACT

This study validates and expands upon previous findings regarding two novel compounds isolated from *Withania somnifera*, emphasizing their potential therapeutic applications. The significance of this work lies in its comprehensive *in silico* toxicity analysis, which assesses the safety and efficacy of these compounds. Using ACD/ChemSketch and OpenBabel software, the chemical structures of the compounds were prepared and converted into SMILES format for further analysis. The PASS prediction software indicated that Compound 1 exhibits significant biological activities, including strong potential as a growth hormone agonist and antineoplastic agent, while Compound 2 also shows promising activity as an antineoplastic and CYP3A substrate. Drug-likeness assessments using SwissADME revealed that both compounds conform to multiple drug-likeness rules, with Compound 1 demonstrating slightly better properties than Compound 2. Furthermore, ADMET analysis indicated favorable pharmacokinetic profiles for both compounds, with distinct advantages in absorption and distribution characteristics. These findings suggest that both compounds possess significant therapeutic potential, warranting further investigation for their development as drug candidates.

Key words: *W. somnifera, In silico*, SMILES, PASS, Chem Sketch, Swiss ADME.

1. INTRODUCTION

In silico toxicity evaluation of novel compounds isolated from *Withania somnifera* reflects the growing interest in the therapeutic potential of this medicinal plant. *W. somnifera*, or ashwagandha, is celebrated for its diverse pharmacological activities, attributed to its bioactive compounds, including alkaloids and withanolides (Narinderpal et al., 2013; Gujjeti et al., 2013). Recent research has highlighted these compounds' roles in mitigating stress, enhancing cognitive function, and exhibiting anti-inflammatory and anticancer properties (Burgula et al., 2024; Swapna et al., 2024). Understanding the mechanisms underlying these effects is vital for their application in modern medicine (Kushwaha et al., 2012; Gujjeti et al., 2014). The increasing demand for natural therapeutics underscores the importance of evaluating the safety and efficacy of these compounds through robust scientific methodologies.

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In silico toxicity evaluation has emerged as a crucial component of drug design, allowing researchers to predict the safety profiles of novel compounds before advancing to costly clinical trials. Tools such as PASS and SwissADME facilitate the assessment of various toxicity parameters, including AMES toxicity, carcinogenic potential, and pharmacokinetic properties (Singh et al, 2022; Jagannathan et al., 2019; Daina et al., 2017). These computational methods provide insights into the drug-likeness of compounds, ensuring they meet established pharmacological criteria, such as Lipinski's Rule of Five (Cheng et al., 2012). The integration of *in silico* approaches streamlines the drug discovery process, enhancing the safety and efficacy profiles of new therapeutics derived from natural sources.

This study aims to conduct a comprehensive *in silico* toxicity evaluation of novel compounds isolated from *W. somnifera*, validating previous findings while exploring their therapeutic potential. The novelty of this work lies in its systematic approach to assessing both biological activity and drug-likeness using advanced computational tools. By employing PASS for biological activity prediction and SwissADME for drug-likeness assessment, we seek to elucidate the pharmacokinetic profiles of these isolated compounds (Kandagatla et al., 2024; Lucas et al., 2019). The objective is to provide a thorough evaluation framework that identifies promising candidates for further development in drug design, ultimately contributing to the advancement of herbal medicine and pharmacology.

2. MATERIAL AND METHODS

2.1 Preparation of Novel Compounds

Base on our previous studies (Swapna et al., 2024), wherein two compounds isolated from *W. somnifera* plant crude extracts and characterized these compounds. The current study aims to validate and complement these findings through *in silico* toxicity analysis. The structures of compounds 1 and 2 were drawn in ACD/ChemSketch software (Figure-1 and 2) and saved in .mol format. The .mol files of the two compounds were converted into .smile format with using OpenBabel Software.

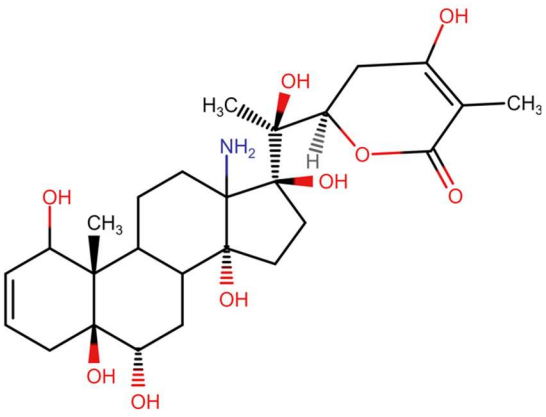

Figure-1. 2D structure of compound-1

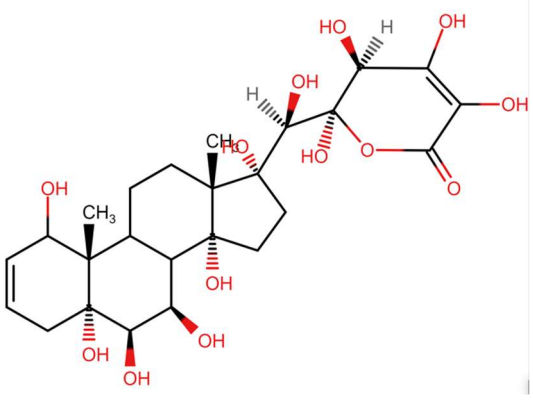

Figure-2. 2D structure of compound-2

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2.2 *In silico* toxicity Evaluation:

PASS prediction:
PASS (Prediction of Activity Spectra for Substances) is a software product designed as a tool for evaluating the general biological potential of an organic drug-like molecule (https://www.way2drug.com/passonline/). PASS provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. PASS Pa= Pi value is chosen as a threshold, therefore all compounds with Pa>Pi are suggested to be active. Another criterion for selection is the compounds' novelty. If Pa value is high, sometimes one may find close analogues of known biologically active substances among the tested compounds. The smile format of the two compounds were uploaded into PASS server and submitted for the results for their biological activity prediction.

Drug likeness Property:
The drug-likeness properties of two novel compounds isolated from Withania somnifera were assessed using the SwissADME web tool (http://www.swissadme.ch/). The chemical structures of the compounds were converted into SMILES format and uploaded to the SwissADME server, which evaluated key parameters including Lipinski's Rule of Five, Ghose Filter, Veber's Rule, Egan's Rule, Muegge's Rule, and the Bioavailability Score. These parameters provide insights into molecular weight, lipophilicity (logP), hydrogen bond donors and acceptors, number of rotatable bonds, topological polar surface area (TPSA), molar refractivity, and other structural features important for oral bioavailability and drug-likeness. The analysis revealed that the compounds conformed to multiple drug-likeness rules, indicating their potential as orally active therapeutic agents with favorable bioavailability profiles. The values obtained for each parameter were analyzed to determine the overall drug-likeness and potential bioavailability of the two novel compounds. Compounds that meet the criteria for multiple rules are considered to have better drug-likeness properties.

Pharmacokinetic Properties
The pharmacokinetic properties (Absorption, Distribution, Metabolism, Excretion, and Toxicity - ADMET) of two novel compounds isolated from Withania somnifera were assessed using the pkCSM web tool (https://biosig.lab.uq.edu.au/pkcsm/prediction). The SMILES formats of the compounds were uploaded into the web tool and submitted for analysis. Key parameters were evaluated, including absorption (ability to cross the blood-brain barrier and human intestinal absorption), distribution (subcellular localization), metabolism (substrate potential for CYP450 2C9 and CYP450 2D6 enzymes), excretion (total clearance rate in log ml/min/kg), and toxicity (AMES toxicity, carcinogenic potential, and acute oral toxicity). This comprehensive ADMET analysis provides crucial insights into the compounds' pharmacokinetic profiles and potential safety for therapeutic use.

3. RESULTS AND DISCUSSION

3.1 PASS Prediction Analysis:

The PASS prediction results for Compound 1 indicate several promising biological activities, with high Pa values suggesting strong potential efficacy (Table-1). The compound shows significant activity as a growth hormone agonist (Pa = 0.705, Pi = 0.002), a CYP3A2 substrate (Pa = 0.673, Pi = 0.014), and an antineoplastic agent (Pa = 0.672, Pi = 0.031). Additionally, it demonstrates potential

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immunosuppressant activity (Pa = 0.631, Pi = 0.025) and gluconate 2-dehydrogenase (acceptor) inhibitor activity (Pa = 0.672,

Pi = 0.073). Compound 1 also exhibits anti-inflammatory properties (Pa = 0.573, Pi = 0.038), testosterone 17beta-dehydrogenase (NADP+) inhibitor activity (Pa = 0.596, Pi = 0.092), and phosphatase inhibitor activity (Pa = 0.562, Pi = 0.068). These activities, supported by relatively low Pi values, suggest that Compound 1 could be a versatile candidate for multiple therapeutic applications.

Table-1. PASS prediction analysis of compounds from *W. somnifera*

Compound 1			**Compound 2**		
Pa	Pi	Activity	Pa	Pi	Activity
0.705	0.002	Growth hormone agonist	0.660	0.033	Antineoplastic
0.673	0.014	CYP3A2 substrate	0.651	0.032	CYP3A substrate
0.672	0.031	Antineoplastic	0.621	0.023	CYP3A2 substrate
0.631	0.025	Immunosuppressant	0.581	0.022	UDP-glucuronosyltransferase substrate
0.672	0.073	Gluconate 2-dehydrogenase (acceptor) inhibitor	0.583	0.076	CYP2H substrate
0.563	0.018	Polarisation stimulant	0.538	0.046	HIF1A expression inhibitor
0.573	0.038	Antiinflammatory	0.487	0.001	Interleukin 10 antagonist
0.512	0.001	Interleukin 10 antagonist	0.567	0.095	CDP-glycerol glycerophosphotransferase inhibitor
0.596	0.092	Testosterone 17beta-dehydrogenase (NADP+) inhibitor	0.524	0.057	CYP3A4 substrate
0.562	0.068	Phosphatase inhibitor	0.483	0.017	P-glycoprotein substrate
0.507	0.014	P-glycoprotein substrate	0.554	0.108	Antieczematic
0.512	0.021	Antiviral (Rhinovirus)	0.472	0.026	Antimetastatic
0.505	0.017	Gestagen antagonist	0.468	0.047	Apoptosis agonist
0.513	0.038	Apoptosis agonist	0.515	0.099	Phosphatase inhibitor
0.522	0.055	CYP3A substrate	0.437	0.029	H±transporting two-sector ATPase inhibitor

Compound 2 also demonstrates a variety of biological activities with notable efficacy. It shows significant potential as an antineoplastic agent (Pa = 0.660, Pi = 0.033), a CYP3A substrate (Pa = 0.651, Pi = 0.032), and a CYP3A2 substrate (Pa = 0.621, Pi = 0.023). The compound is predicted to be a UDP-glucuronosyltransferase substrate (Pa = 0.581, Pi = 0.022) and exhibits activity as a CYP2H substrate (Pa = 0.583, Pi = 0.076). Additionally, it shows potential as an HIF1A expression inhibitor (Pa = 0.538, Pi = 0.046) and an interleukin 10 antagonist (Pa = 0.487, Pi = 0.001). Compound 2 is also predicted to

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be a P-glycoprotein substrate (Pa = 0.483, Pi = 0.017), a CDP-glycerol glycerophosphotransferase inhibitor (Pa = 0.567, Pi = 0.095), and an apoptosis agonist (Pa = 0.468, Pi = 0.047).

These results suggest that Compound 2 has diverse therapeutic potential, especially in cancer treatment and metabolic regulation.

3.2 Drug likeness Property Analysis:

The drug-likeness properties of the two novel compounds isolated from Withania somnifera were evaluated using several parameters. Compound 1 had no violations of Lipinski's rule. It had one violation of the Ghose filter (number of atoms > 70), one violation of Veber's rule (TPSA > 140), no violations of Egan's rule, and two violations of Muegge's rule (TPSA > 150 and hydrogen donors > 5). It had a bioavailability score of 0.17 (Table-2).

Compound 2 also had no violations of Lipinski's rule. It had one violation of the Ghose filter (number of atoms > 70), one violation of Veber's rule (TPSA > 140), one violation of Egan's rule (TPSA > 131.6), and one violation of Muegge's rule (hydrogen donors > 5). It had a bioavailability score of 0.11. These results indicate that while both compounds show some violations across different drug-likeness criteria, they also have potential as bioavailable drug candidates with relatively few major issues.

Compound 1 exhibits slightly better drug-likeness properties and a higher bioavailability score than Compound 2, suggesting it may be the more promising candidate for further development.

Table-2. Drug likeness property analysis of compounds of *W. somnifera*

Sl.No	Drug likeness Parameters	Compound 1	Compound 2
1	Lipinski	No; 0 violations	No; 0 violations
2	Ghose	No; 1 violation: #atoms>70	No; 1 violations, #atoms>70
3	Veber	No; 1 violation: TPSA>140	No; 1 violation: TPSA>140
4	Egan	No; 0 violation	No; 1 violation: TPSA>131.6
5	Muegge	No; 2 violations: TPSA>150, H-don>5	No; 1 violations: H-don>5
6	Bioavailability Score	0.17	0.11

3.3. Analysis of Pharmacokinetic Properties

The pharmacokinetic (ADMET) properties of the two novel compounds were evaluated, revealing several key findings. For absorption, both compounds showed a positive probability for crossing the

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blood-brain barrier (BBB+), with Compound 1 at 0.8627 and Compound 2 at 0.9245, and human intestinal absorption (HIA+), with Compound 1 at 0.8407 and Compound 2 at 0.6419. In terms of distribution, both compounds are likely to localize in mitochondria, with Compound 1 at 0.6841 and Compound 2 at 0.8469. For metabolism, neither compound is a substrate for CYP450 2C9 or CYP450 2D6, with high non-substrate probabilities for both enzymes. Excretion analysis showed total clearance rates of 0.499 log ml/min/kg for Compound 1 and 0.642 for Compound 2. Regarding toxicity, both compounds were predicted to be non-AMES toxic (Compound 1 at 0.8403 and Compound 2 at 0.6821) and non-carcinogenic (Compound 1 at 0.9751 and Compound 2 at 0.9714), with acute oral toxicity classified as Category I (Compound 1 at 0.5085 and Compound 2 at 0.4721) (Table-3).

Based on the ADMET properties, Compound 1 shows higher human intestinal absorption and non-AMES toxicity, while Compound 2 has a higher probability for blood-brain barrier penetration and total clearance, indicating distinct advantages for each compound in different pharmacokinetic aspects.

Table-3. Pharmacokentic properties of compounds isolated from *W. somnifera*

Sl.No	Model	Result	Compound 1	Compound 2
			Probability Values	
1	**Absorption**			
	Blood-Brain Barrier	BBB+	0.8627	0.9245
	Human Intestinal Absorption	HIA+	0.8407	0.6419
2	**Distribution**			
	Subcellular localization	Mitochondria	0.6841	0.8469
3	**Metabolism**			
	CYP450 2C9 Substrate	Non-substrate	0.8162	0.8127
	CYP450 2D6 Substrate	Non-substrate	0.8867	0.8494
4	**Excretion**			
	Total Clearance (log ml/min/kg)		0.499	0.642
5	**Toxicity**			
	AMES Toxicity	Non AMES toxic	0.8403	0.6821
	Carcinogens	Non-carcinogens	0.9751	0.9714
	Acute Oral Toxicity	I	0.5085	0.4721

Compound 1 demonstrated significant activity as a growth hormone agonist, antineoplastic agent, and immunosuppressant, with high Pa values indicating strong efficacy. Similarly, Compound 2 showed notable potential as an antineoplastic agent, CYP3A substrate, and apoptosis agonist. These activities

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align with findings by Campillos et al., (2008), who reported that *Withania somnifera* extracts exhibit diverse biological activities including anticancer and anti-inflammatory properties .

The drug-likeness assessment revealed that Compound 1 had fewer violations in Egan and Muegge rules compared to Compound 2, suggesting a relatively better profile for oral bioavailability. Compound 1's higher bioavailability score (0.17) compared to Compound 2 (0.11) further supports this finding.

These results are consistent with the work of Belayneh et al, (2020) and Vijayagiri et al (2012), who emphasized the importance of minimal violations in drug-likeness rules for enhancing drug development prospects. The ADMET analysis showed that both compounds exhibit promising pharmacokinetic profiles with specific strengths. Compound 1 had higher human intestinal absorption and was non-AMES toxic, indicating its potential for oral administration and safety. Compound 2 had a higher probability for blood-brain barrier penetration and total clearance, suggesting its utility in targeting central nervous system disorders. This is in line with the study by Cragg et al. (2001); Venkanna et al., (2013), which highlighted the need for favorable ADMET properties to ensure effective drug delivery and minimal toxicity .

Comparing our findings with previous studies, the bioactive compounds from Withania somnifera have been widely recognized for their therapeutic potential. For instance, Mamidala et al. (2013) demonstrated the anticancer efficacy of withanolides derived from *W. somnifera*, supporting our PASS prediction results for antineoplastic activity in both compounds . Additionally, our drug-likeness and ADMET results align with the pharmacokinetic characteristics reported by Gurrapu et al. (2017), who underscored the importance of comprehensive in silico analyses in the early stages of drug discovery .

4. CONCLUSION

In silico toxicity evaluation of novel compounds isolated from *Withania somnifera* demonstrates their promising therapeutic potential and safety profiles. This study effectively utilized advanced computational tools, such as PASS and SwissADME, to assess biological activities and drug-likeness properties, revealing that both compounds adhere to key pharmacological criteria. The findings indicate significant biological activities for these compounds, alongside favorable pharmacokinetic properties, suggesting their viability as candidates for further development in drug design. By integrating *in silico* methods into the evaluation process, this research not only enhances the understanding of the therapeutic applications of *Withania somnifera* but also contributes to the broader field of herbal medicine. Future studies should focus on experimental validation to confirm these predictions and explore the full therapeutic potential of these compounds in clinical settings.

Conflicting Interests: The authors have declared that no conflicting interests exist.

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