INTRODUCTION

Insomnia is a prevalent sleep disorder characterized by difficulty falling asleep, staying asleep, or experiencing nonrestorative sleep. Its epidemiology reveals a widespread issue, with estimates suggesting that approximately 10%–30% of adults experience chronic insomnia, while up to 50% report occasional insomnia symptoms [1]. Compared with men, women are more susceptible to this sleeping disorder [2]. Insomnia becomes increasingly common with age, affecting up to 48% of older adults [3]. Insomnia is often comorbid with various conditions, such as depression, anxiety disorders, and chronic pain [4]. The etiology of insomnia is multifactorial and involves physiological, psychological, and environmental factors. Medical conditions such as chronic pain, respiratory disorders, neurological disorders, and endocrine disorders can contribute to sleep disturbances [5]. Psychiatric disorders,
particularly mood disorders such as depression and anxiety, are strongly linked to insomnia [6]. Certain medications, such as antidepressants, beta-blockers, and corticosteroids, can interfere with sleep patterns and cause insomnia as a side effect [7,8]. Stress, anxiety, and worrying thoughts are significant psychological factors, with up to 20% of insomnia cases attributed to stress and psychophysiological factors [9]. Circadian rhythm disruptions such as jet lag and shift work can disrupt the body’s internal clock and lead to insomnia [10]. Sleep environment factors, such as noise, light, temperature, and an uncomfortable sleeping environment, can also contribute to sleep disturbances [11]. Lifestyle factors, including poor sleep habits, excessive caffeine or alcohol consumption, lack of physical activity, and irregular sleep schedules, are associated with an increased risk of insomnia [12].

The gamma-aminobutyric acid A (GABA-A) receptor plays a pivotal role in regulating sleep and wakefulness, and its dysfunction has been implicated in the pathophysiology of insomnia [13]. The GABA-A receptor is a ligand-gated ion channel receptor that belongs to the Cys-loop family of receptors. It is the major inhibitory receptor in the central nervous system and is activated by the neurotransmitter gamma-aminobutyric acid (GABA) [14]. It is a heteropentameric protein composed of five subunits arranged around a central pore, with multiple subunit isoforms (α1-6, β1-3, γ1-3, δ, ε, π, θ) that determine its functional properties [15]. The most common subunits are two α subunits, two β subunits, and one γ subunit (α1β2γ2 is prevalent in the brain). When GABA binds, it triggers a conformational change allowing chloride ions to flow through the central pore, causing neuronal hyperpolarization and inhibition of firing, decreasing excitability and promoting sleep [16]. In individuals with insomnia, there is evidence of altered GABA-A receptor function and decreased GABA levels in brain regions involved in sleep regulation. Neuroimaging studies have shown reduced GABA-A receptor binding in cortical areas such as the frontal, temporal, and occipital lobes, as well as in the thalamus [17]. Proton magnetic resonance spectroscopy studies have demonstrated decreased GABA levels in the occipital cortex and anterior cingulate cortex [18,19]. This dysregulation of GABA-A receptors and reduced GABA contribute to hyperarousal, cortical excitability, and disrupted sleep-wake regulation in insomnia [20]. Targeting this receptor system is crucial for developing effective insomnia treatments.

*Withania somnifera*, commonly known as ashwagandha or Indian ginseng, is a medicinal herb traditionally used in Ayurvedic medicine for various purposes, including promoting better sleep [21-23]. This herb contains several bioactive compounds, such as withanolides, alkaloids, flavonoids, and withanolides such as withaferin A and withanolide D, which are believed to contribute to its therapeutic properties [24]. Ashwagandha may help alleviate insomnia through multiple mechanisms. First, its extracts and constituents exhibit GABA-mimetic activity, which can promote sleep by increasing inhibitory neurotransmission in the brain [25]. Second, ashwagandha possesses anxiolytic (anti-anxiety) properties, potentially reducing the stress and anxiety that often contribute to insomnia [26]. Additionally, its antioxidant and neuroprotective effects may protect the brain from oxidative stress and promote healthy neuronal function, thereby improving sleep quality [27].

In this study, we conducted computational examination to identify the phytochemicals in *W. somnifera* that possess GABA-A stimulating properties. The DISPEL (Diseases Plants Eliminate; https://compbio.iitr.ac.in/dispel/) server deemed *W. somnifera* as an appropriate plant source for phytochemicals in relation to the disease under study. Moreover, we carried out molecular docking, molecular docking simulation, Prediction of Activity Spectra for Substances (PASS) analysis, and absorption, distribution, metabolism, excretion, and toxicity (ADME/T) analysis to pinpoint potential phytochemicals. These identified compounds could serve as prospective drug candidates for the treatment of insomnia.

### METHODS

**Prediction of suitable plant species**

The DISPEL server was used to identify suitable plant species for evaluating their phytochemicals against a specific target protein. This platform is a comprehensive database that contains more than 60,000 links between medicinal plants and diseases, covering approximately 5,500 plants and 1,000 diseases worldwide. The DISPEL database can be used for various purposes, such as investigating specific plants or diseases and performing comparative studies of multiple plants and diseases. The platform provides interactive visualizations such as network graphs, which help in easily understanding the connections between medicinal plants and diseases [28]. Users can search the database by entering the disease name or the scientific/common name of the plant. Importantly, this platform allows users to identify the most effective plants for treating their chosen diseases. In the context of the study, a search query for “insomnia” suggested *W. somnifera* (ashwagandha, Indian ginseng) as the recommended plant for therapeutic use in insomnia.

**Assessment of plant compounds in *W. somnifera***

The phytochemical constituents of *W. somnifera* were investigated using the Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0 (IMPPAT 2.0; https://cb.imsc.res.in/imppat/) server. This database is meticulously curated, drawing from 100 traditional Indian medicine texts and more than 7,000 published research articles, along with other credible sources. IMPPAT 2.0 represents the most comprehensive compilation of phytochemical information from Indian medicinal plants to date, surpassing its earlier version, IMPPAT 1.0, with notable enhancements and expansions [29]. The selected phytochemicals phytochemicals, structure-data files (SDF) were subsequently obtained from the PubChem database.

**Ligand retrieval and preparation**

The ligands (phytochemicals) were sourced from the PubChem database.
database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. PubChem functions as an extensive repository for chemical compounds, documenting their interactions with biological assays. Oversight of PubChem lies with the National Center for Biotechnology Information (NCBI), a division of the National Library of Medicine (NLM) under the United States National Institutes of Health (NIH). Subsequently, the ligands were prepared using UCSF Chimera version 1.15 [30], versatile software tailored for interactive exploration and analysis of molecular structures and associated data. UCSF Chimera facilitates the visualization and examination of diverse components, including density maps, supramolecular assemblies, sequence alignments, docking outcomes, trajectories, and collections of distinct molecular conformations. Ligand structure optimization was executed via the steepest descent and conjugate gradient energy minimization method within the UCSF Chimera software. The selected ligands for virtual screening, along with their corresponding PubChem IDs and molecular weights (MWs), are detailed in Table 1.

**Protein retrieval and preparation**

The 3D structure of the human GABA-A receptor in combination with GABA (with the PDB ID: 7PBD) was obtained from the Protein Data Bank (https://www.rcsb.org/). This site functions as the U.S. data center for the worldwide Protein Data Bank (PDB), which stores 3D atomic coordinate data for biologically important molecules such as proteins and nucleotides (DNA and RNA). The protein was prepared using UCSF chimera 1.15 [31]. The docked structure of GABA, heteroatoms not included in the primary protein structure, and water molecules were eliminated. Finally, polar hydrogen was added to the protein structure.

**Virtual screening of phytochemicals**

The phytochemicals were subjected to virtual screening using AutoDock Vina 1.2.0 [32], which was subsequently integrated into PyRx 0.8 [33] for molecular docking studies. PyRx is a widely used computational tool in drug discovery that facilitates virtual screening, allowing the examination of compound libraries against potential drug targets. AutoDock Vina is known for its high performance and extensive use as a free docking software. This method offers a simplified computational docking process, employing a basic scoring system and efficient gradient-optimized conformational exploration.

**Prediction of biological activity**

The PASS web server (http://www.pass.expert.ru/passonline) [34] was used to predict the biological behaviors of the chosen molecules. With the ability to predict over 4,000 different types of biological activities, PASS Online covers a wide spectrum, including pharmacological impacts, action mechanisms, toxic and adverse effects, interactions with metabolic enzymes and transporters, and influences on gene expression, among others. The PASS system uses detailed descriptors of neighboring atoms to help understand a drug's effects based solely on its molecular structure, shedding light on the intricate relationship between its chemical composition and biological role.

**Visualization of docking results**

The best docked poses of the hit compounds were visualized using Discovery Studio 4.5 [35]. This software enabled a thorough analysis of ligand-receptor interactions through both 2D and 3D representations, providing insights into the binding mode.

**Drug-likeness and physiochemical property prediction**

The SwissADME webservice (http://www.swissadme.ch/) [36] was utilized to assess the physicochemical properties and drug-likeness of the compounds. This tool computes descriptors and predicts ADME parameters, pharmacokinetic properties, and suitability for medicinal chemistry. Key parameters, including topological polar surface area (TPSA), lipophilicity (logP), solubility (logS), hydrogen acceptors, hydrogen donors, and molecular weight, were examined. Additionally, compounds violating Lipinski's rule of five were identified [37]. This rule outlines essential criteria for orally active drugs to demonstrate pharmacological efficacy.

**ADME/T analysis**

The study employed ADME analysis conducted using the ADMETLab 2.0 webservice (https://admetlab.scbdd.com/) for the absorption, distribution, metabolism, and excretion profile of the ligands [38]. This platform provides predictions for the pharmacokinetics and toxicity properties of chemicals. Notably, it offers a significant expansion in supported ADME/T-related endpoints compared to its previous version, nearly doubling the number. This encompasses 17 physicochemical properties, 13 medicinal chemical properties, 23 ADME properties, 27 toxicity endpoints, and 8 toxicophore rules comprising 751 substructures. Toxicity (T) prediction was performed using the ProTox 3.0 server (https://tox.charite.de/protox3/) [39]. ProTox 3.0 is a comprehensive tool that integrates various elements such as molecular resemblance, fragment tendencies, commonly occurring features, and machine

<table>
<thead>
<tr>
<th>Ligands</th>
<th>PubChem ID</th>
<th>MW (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somniferine</td>
<td>14106343</td>
<td>608.7</td>
</tr>
<tr>
<td>Hygrine</td>
<td>440933</td>
<td>141.21</td>
</tr>
<tr>
<td>Withanolide D</td>
<td>161671</td>
<td>470.6</td>
</tr>
<tr>
<td>Withaferin A</td>
<td>265237</td>
<td>470.6</td>
</tr>
<tr>
<td>Withanolide E</td>
<td>301751</td>
<td>486.6</td>
</tr>
<tr>
<td>Withasomnine</td>
<td>442877</td>
<td>184.24</td>
</tr>
<tr>
<td>Withanone</td>
<td>21679027</td>
<td>470.6</td>
</tr>
<tr>
<td>Sitoindoside IX</td>
<td>189586</td>
<td>632.7</td>
</tr>
<tr>
<td>Tropine</td>
<td>449293</td>
<td>141.21</td>
</tr>
<tr>
<td>Physagulin-d</td>
<td>10100412</td>
<td>620.8</td>
</tr>
<tr>
<td>Withanolide C</td>
<td>101559583</td>
<td>523.1</td>
</tr>
<tr>
<td>Withanolide B</td>
<td>14236711</td>
<td>454.6</td>
</tr>
</tbody>
</table>

Table 1. Ligands chosen for the study with their PubChem ID and molecular weight (MW)
learning based on fragment similarity and cluster cross-validation. It utilizes a total of 61 models to predict various toxicity endpoints. These include acute toxicity, organ toxicity, toxicological endpoints, molecular initiating events, metabolism, adverse outcomes (Tox21) pathways, and toxicity targets.

**Molecular dynamic simulation**

Molecular dynamics (MD) simulations were conducted on the structures of the GABA-A receptor and selected phytochemicals. These simulations were executed using the WEBGRO Macromolecular Simulations server (https://simlab.uams.edu/), a public service provided by the University of Arkansas for Medical Sciences (UAMS) through the GRACE High-Performance Computing Facility. Before the MD simulations, molecular topologies for the chosen compounds were created using the GlycoBioChem PRODRG2 server. The GORMOS96 43A1 force field was used for MD simulations of the GABA-A receptor with the identified compounds, using the simple point charge (SPC) water model in a triclinic system with sodium chloride. Subsequently, energy minimization of the resulting complexes was performed using a steepest descent integrator with steps taken at 5,000 intervals. Equilibration followed under NVT/NPT (NVT: number of particles, system volume, and temperature; NPT: number of particles, system pressure, and temperature) conditions at 300 K and 1 bar pressure. The MD simulations were carried out with a Leap-Frog integrator over a simulation time of 50 ns, limited by the resources available. A set frame count of 1,000 frames was determined. The trajectories from the MD simulations included the root-mean-square deviation (RMSD), radius of gyration (Rg), and solvent-accessible surface area (SASA). These parameters were analyzed at 300 K to understand the dynamics of complex formation [40-42]. The application of these simulation techniques improves our understanding of the interactions between the GABA-A receptor and selected phytochemicals, offering valuable insights for further research in this field.

**RESULTS**

**Virtual screening of phytochemicals**

The docking study was conducted using the structure of the GABA-A receptor, with AutoDock Vina, accessed via PyRx 0.8, serving as the tool for analysis. The protein was prepared for docking using the DockPrep feature of UCSF Chimera, transforming it into a macromolecule. The selected compounds were initially minimized using the mmff94 force field, and polar hydrogens were added. These compounds were then converted to pdbqt format using Open Babel within PyRx. For the blind docking process, a grid box with dimensions of 69.95 Å×86.02 Å×122.94 Å centered at coordinates (178.79, 176.19, and 155.92 Å) was used. The exhaustiveness level was set to the default value of 8. Figure 1 provides specific details about the ligands and their respective docking scores (kcal/mol). Docking scores indicate the binding affinity of a ligand for its target protein, providing insights into the potential effectiveness of the ligand as a therapeutic agent. Lower docking scores generally indicate stronger binding affinity and greater potential for drug effectiveness. Phytochemicals with docking scores of -7 or lower were chosen for further analysis.

**Biological activity of ligands**

The PASS webservice was utilized to predict the biological activity of the identified phytochemicals, with a focus on GABA-A receptor agonist activity. The probability of being active (Pa) for being a GABA-A receptor agonist varied from 0.167 to 0.377, while the probability of being inactive (Pi) ranged from 0.016 to 0.104. The summarized results are displayed in Table 2. Ligands that demonstrated GABA-A receptor agonist activity were selected for further analysis, while the remaining ligands were discarded.

**Physiochemical and drug-likeliness analysis of selected phytochemicals**

The SwissADME webservice was utilized to analyze the physicochemical properties of the sorted phytochemicals from previous steps (Table 3). Various parameters including solubility (logS), lipophilicity (logP), hydrogen bond acceptors (Accept H), hydrogen bond donors (Donor H), TPSA, and gastrointestinal (GI) absorption were assessed. The drug-likeliness properties of the phytochemicals were evaluated based on Lipinski’s rule of five, where adherence to the rule suggests favorable drug-like properties. Hygrine, tropine, and withasomnine are three ligands that have been evaluated for their drug-like properties. Each of these

### Table 2. Biological activity prediction of GABA-A agonist for selected compounds

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pa</td>
</tr>
<tr>
<td>Hygrine</td>
<td>0.167</td>
</tr>
<tr>
<td>Tropine</td>
<td>0.225</td>
</tr>
<tr>
<td>Withasomnine</td>
<td>0.377</td>
</tr>
</tbody>
</table>

GABA-A, gamma-aminobutyric acid A; Pa, probability of being active; Pi, probability of being inactive
compounds has a molecular weight within the range typically observed for drugs, suggesting good ADME properties. Hygrine has a logS value of -0.88, indicating moderate solubility in water, which could be beneficial for bioavailability. It also has a logo/w value of 0.47, indicating a balance between water and oil solubility, which is important for transport in the blood and crossing cell membranes. Tropine, on the other hand, has a slightly lower water solubility (logS: -1.19) and a slightly higher oil preference (logo/w: 0.75). Withasomnine has moderate water solubility (logS: -2.90) and the highest oil preference (logo/w: 2.22) among the three compounds. The number of hydrogen bond acceptors and donors, as well as the TPSA, of the selected compounds are within an acceptable range. All three compounds have high GI absorption, which is beneficial for orally administered drugs and are considered drug-like, indicating that they could be promising candidates for further study.

**ADME/T analysis of selected compounds**

The ADMETlab 2.0 server was used to analyze the ADME properties while toxicity (T) prediction was performed using Protox 3.0. The various properties considered and their respective observed values are given in Tables 4 and 5, respectively. Hygrine exhibited a Caco-2 permeability value of -4.47, suggesting a moderate ability to cross the human intestinal barrier. It is not a substrate for P-glycoprotein, indicating that it may not be actively pumped out of cells, potentially enhancing its bioavailability. Hygrine is not an inhibitor of P-glycoprotein, suggesting that it will not interfere with the absorption of other drugs. It has a volume distribution of 1.581, indicating a good distribution throughout the body. It is a substrate for CYP2C9 and CYP2D6, suggesting it may be metabolized by this enzyme. It is not an inhibitor of any of the listed CYP enzymes, indicating it may not interfere with the metabolism of other drugs. Its clearance value is 9.251, suggesting that it is efficiently eliminated from the body. Tropine has a Caco-2 permeability value of -4.94, indicating that tropine has a slightly lower ability to cross the human intestinal barrier than does hygrine. It is a substrate for P-glycoprotein, which may affect its intracellular concentration and bioavailability. It is not an inhibitor of P-glycoprotein, suggesting that it will not interfere with the absorption of other drugs. It has a volume distribution of 2.975, indicating that it is more widely distributed throughout the body than is hygrine. Tropine is a substrate for both CY-

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**Table 3. Physiochemical properties and drug-likeness properties of phytochemicals according to the SwissADME server**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>MW (g/mol)</th>
<th>logS</th>
<th>logo/w</th>
<th>Accept H</th>
<th>Donor H</th>
<th>TPSA (Å)</th>
<th>GI absorption</th>
<th>Drug-likeness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hygrine</td>
<td>141.21</td>
<td>-0.88</td>
<td>0.47</td>
<td>2</td>
<td>0</td>
<td>20.31</td>
<td>High</td>
<td>Yes</td>
</tr>
<tr>
<td>Tropine</td>
<td>141.21</td>
<td>-1.19</td>
<td>0.75</td>
<td>2</td>
<td>1</td>
<td>23.47</td>
<td>High</td>
<td>Yes</td>
</tr>
<tr>
<td>Withasomnine</td>
<td>184.24</td>
<td>-2.90</td>
<td>2.22</td>
<td>1</td>
<td>0</td>
<td>17.82</td>
<td>High</td>
<td>Yes</td>
</tr>
</tbody>
</table>

MW, molecular weight; TPSA, topological polar surface area; GI, gastrointestinal

**Table 4. ADME (absorption, distribution, metabolism, and excretion) properties of the ligands as predicted by ADMETlab 2.0 server**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hygrine</th>
<th>Tropine</th>
<th>Withasomnine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caco-2 permeability</td>
<td>-4.47</td>
<td>-4.94</td>
<td>-4.51</td>
</tr>
<tr>
<td>P-glycoprotein substrate</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>P-glycoprotein inhibitor</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume distribution (mg/L)</td>
<td>1.581</td>
<td>2.975</td>
<td>1.872</td>
</tr>
<tr>
<td>Metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9 substrate</td>
<td>Substrate</td>
<td>Non-substrate</td>
<td>Substrate</td>
</tr>
<tr>
<td>CYP2D6 substrate</td>
<td>Substrate</td>
<td>Substrate</td>
<td>Non-substrate</td>
</tr>
<tr>
<td>CYP3A4 substrate</td>
<td>Non-substrate</td>
<td>Substrate</td>
<td>Non-substrate</td>
</tr>
<tr>
<td>CYP1A2 inhibition</td>
<td>Non-inhibitor</td>
<td>Non-inhibitor</td>
<td>Non-inhibitor</td>
</tr>
<tr>
<td>CYP2C9 inhibition</td>
<td>Non-inhibitor</td>
<td>Non-inhibitor</td>
<td>Non-inhibitor</td>
</tr>
<tr>
<td>CYP2D6 inhibition</td>
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<td>Non-inhibitor</td>
</tr>
<tr>
<td>CYP2C19 inhibition</td>
<td>Non-inhibitor</td>
<td>Non-inhibitor</td>
<td>Non-inhibitor</td>
</tr>
<tr>
<td>Excretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearance</td>
<td>9.251</td>
<td>2.37</td>
<td>6.93</td>
</tr>
</tbody>
</table>

**Table 5. Toxicity prediction of selected compounds according to the Protox 3.0 server**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hepatotoxicity</th>
<th>Clinical toxicity</th>
<th>Cytotoxicity</th>
<th>Immunotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hygrine</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>Tropine</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>Withasomnine</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
</tbody>
</table>
Withania Compounds as GABA-A Modulators

P2D6 and CYP3A4, suggesting that it may be metabolized by these enzymes. It is not an inhibitor of any of the listed CYP enzymes, indicating that it may not interfere with the metabolism of other drugs. Its clearance value is 2.37, suggesting that it is less efficiently eliminated from the body compared to hygrine. Furthermore, withasomnine has a Caco-2 permeability value of -4.51, suggesting that a moderate ability to cross the human intestinal barrier. It is not a substrate of P-glycoprotein, indicating that it may not be actively pumped out of cells, potentially enhancing its bioavailability. It is not an inhibitor of P-glycoprotein, suggesting it will not interfere with the absorption of other drugs. It has a volume of distribution of 1.872, indicating a good distribution throughout the body. It is not a substrate of any of the listed CYP enzymes except CYP2C9, suggesting that it may be metabolized by the substrate enzyme. It is not an inhibitor of any of the listed CYP enzymes, indicating that it may not interfere with the metabolism of other drugs. Its clearance value is 6.93, suggesting it is efficiently eliminated from the body.

The parameters that were evaluated for toxicity prediction were hepatotoxicity (toxicity to the liver), clinical toxicity (harmful effects in humans), cytotoxicity (toxicity to cells), and immunotoxicity (toxicity to the immune system). For all three compounds and across all four parameters, the status is listed as “inactive” suggesting that these compounds are not expected to exhibit toxic effects in these categories. In the context of drug likeness and safety, this is a positive indication. A compound with low toxicity is generally safer and more likely to be successful in the later stages of drug development.

Molecular dynamic simulation

This study explored receptor-inhibitor complexes in details using MD analysis. The objective was to discern critical parameters, including the RMSD, SASA, and Rg. By conducting MD simulations spanning 50 ns, we obtained insights into the dynamic interactions between the receptor and the inhibitor, with a specific focus on the GABA-A receptor agonist. The RMSD trajectories obtained from the MD simulations corroborated the data acquired from the docking studies. These trajectories offer a temporal perspective on the interaction between small molecules and the receptor throughout the 50 ns simulation period. The RMSD values serve as indicators of GABA-A receptor stability, particularly in the context of insomnia and the presence of selected docked phytochemical molecules. By monitoring conformational changes in the receptor, we can determine the average distance between specific atoms. This reflection of the ligand’s influence on distance informs us about any alterations in protein structure conformation. A decrease in distance signifies effective binding and stable complex formation. Figure 2 shows the RMSD profiles for the interactions of hygrine, tropine, and withasomnine with the GABA-A receptor binding site. The withasomnine exhibited high fluctuations at approximately 23 ns but subsequently decreased. Conversely, the RMSD value of hygrine increased significantly as the simulation approached 50 ns. In contrast, tropine demonstrated the lowest RMSD fluctuation compared to the other ligands. Importantly, all selected ligands maintained RMSD values within an acceptable range, suggesting effective stability of the receptor-ligand complexes. Consequently, these compounds hold promise as potent agonists of the GABA-A receptor.

The Rg serves as a metric to evaluate the compactness of the GABA-A receptor structure in the presence of specific ligands. Rg provides insights into the spatial arrangement of the protein’s secondary structure in 3D space. In this context, compactness refers to the ratio of the accessible surface area to the surface area of an ideal sphere with an equivalent volume. Lower Rg values correspond to a more tightly packed protein structure. By analyzing the trajectory, we can investigate how the selected molecules influence changes in the compactness of the GABA-A receptor. Notably, the GABA-A receptor-hygrine complex exhibited maximum compactness, as evidenced by its minimal Rg values (Figure 3). Conversely, the trajectory of withasomnine revealed a high Rg
value, indicating a decrease in structural compactness and implying reduced stability of the complex. Moreover, both tropine and hygrine exhibited low Rg values, signifying the presence of stable protein-ligand complexes (Figure 3).

SASA calculations were conducted for the protein-ligand complexes to discern alterations in the protein surface area. Higher SASA values signify surface expansion, whereas lower values indicate volume truncation of the protein. The tropine–GABA-A receptor and withasomnine–GABA-A receptor complexes exhibited the lowest SASA values, indicating their compactness and stability. In contrast, the SASA of the hygrine–GABA-A receptor complex fluctuated the most, as shown in Figure 4.

Ligand-protein interactions

Table 6 lists the amino acids and corresponding bond lengths that are involved in the interactions between the GABA-A receptor and three ligands: hygrine, tropine, and withasomnine. Different ligand-protein interaction patterns are displayed for each ligand. In particular, hygrine forms an alkyl bond with Ala300 with a bond length of 5.08 Å. Hygrine primarily interacts via Van der Waals interactions with the amino acids Leu297, Phe301, Tyr304, and Val611. Tropine interacts with a wide range of amino acids, such as Lys173, Val175, Thr176, Gly177, Glu179, His191, Ser446, Tyr447, and Tyr494, through Van der Waals interactions. Moreover, tropine and Val178 form an alkyl bond with a bond length of 4.81 Å. Withasomnine possesses Van der Waals interactions with Glu198, Phe301, Tyr304, Val611, and Trp614, and an alkyl bond with Leu297 at 4.37 Å. Figure 5 shows the interactions between the ligands and the binding site of the target. Understanding these interactions is critical for molecular docking research and drug design. These interactions, as shown in the table, play an important role in defining ligand binding affinity and specificity for the protein receptor. By determining the individual amino acids involved in ligand-protein interactions, as well as the related bond lengths, researchers may optimize ligand structures to increase effectiveness and selectivity, eventually enhancing drug discovery and development.

DISCUSSION

Through systematic virtual screening, this computational study identified hygrine, tropine, and withasomnine from *W. somnifera* as promising GABA-A receptor agonists for insomnia treatment. These compounds exhibited favorable binding affinities and desirable molecular characteristics, aligning with Lipinski’s rule of five. Moreover, comprehensive ADME/T analysis revealed favorable pharmacokinetic profiles, and toxicity predictions confirmed their safety. MD simulations provided insights into the stable interactions of these compounds with the GABA-A receptor, suggesting their potential efficacy in modulating receptor activity.

The identification of hygrine, tropine, and withasomnine as lead compounds holds significant implications for insomnia treatment. These phytochemicals offer promising avenues for the development of novel therapeutics targeting the GABA-A receptor, a key player in sleep regulation. By using natural compounds from *W. somnifera*, this study presents a potential alternative to synthetic drugs, leveraging traditional medicinal knowledge for modern therapeutic advancements. The favorable pharmacokinetic profiles and safety profiles of these compounds further support their potential clinical translation, offering a safer and more accessible option for individuals suffering from insomnia.

While this computational study provides valuable insights into the potential of hygrine, tropine, and withasomnine as GABA-A receptor agonists, several limitations must be acknowledged. Firstly, the findings are based on in silico predictions and molecular simulations, which necessitate experimental validation to confirm the actual efficacy and safety of these compounds. Additionally, the study focused solely on the GABA-A receptor, overlooking potential interactions with other molecular targets involved...
Figure 5. Representation of the interactions between the ligands and the target proteins hygrine (A), tropine (B), and withasomnine (C). Light green bubbles signify Van der Waals interaction while pink colored bubbles show alkyl interaction between the ligand and active site of the protein.
in insomnia pathophysiology. Further research is warranted to explore the broader pharmacological effects and mechanisms of action of these phytochemicals.

Conflicts of Interest
The authors have no potential conflicts of interest to disclose.

Availability of Data and Material
The dataset generated or analysed during the study are available from the corresponding author on reasonable request.

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Funding Statement
None

Acknowledgments
Authors would like to acknowledge Department of Zoology and J.N. Medical College, Aligarh Muslim University for providing required facilities.

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